

Markus Müller
Editor

Clinical Pharmacology: Current Topics and Case Studies



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**Clinical Pharmacology:
Current Topics
and Case Studies**

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SECTION 1

Introduction

CHAPTER 1

The discipline of Clinical Pharmacology

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In it's Chapter about "Principles of Clinical Pharmacology" Harrsion's Textbook of Internal Medicine 2008 states that "drugs are the cornerstone of modern therapeutics" and that "drug therapy varies widely among individuals" [1]. These two statements set the stage for the discipline of Clinical Pharmacology (CP) which pursues two main goals: (1) an empirical description of conditions under which drug actions vary in humans and (2) to determine and understand the molecular mechanisms underlying this variability [1]. Both goals can be pursued (a) scientifically, by studying drug action in humans, (b) clinically, by administering appropriate drug therapy to patients and (c) within a regulatory framework, to provide guidance on the risk/benefit ratio of drug candidates in drug development and drug reimbursement.

Historically, the discipline of CP was established in several countries as an academic discipline about 40 years ago. Whereas CP was established as a clinical sub-discipline of internal medicine in many countries, experimental pharmacology emerged as a second common trunk for the discipline in others. Hand in hand with its emergence in academia, a large number of CP centers were set up in pharmaceutical companies. In 1970 the WHO published an overall document on CP [2] to stimulate the development of CP and in several countries national and international Societies for Clinical Pharmacology and Therapeutics were established, e.g. the American Society of Clinical Pharmacology and Therapeutics (ASCPT) and the American College of Clinical Pharmacology (ACCP) in the US and the European Association of Clinical Pharmacology and Therapeutics (EACPT) in Europe (for an overview of Clinical Pharmacology departments worldwide see: <http://www.meduni-graz.at/pharma/pharma-www/>).

Today ASCPT states its vision as follows: "CP is recognized and serves as the premier discipline at the forefront of the discovery, development, regulation and use (DDRU) of safe and effective medications necessary for the prevention and treatment of illness" (<http://www.ascpt.org/about/obj.cfm>), whereas the ACCP states: "Promotion of rational use of medications in humans: innovative research,

Keywords: Clinical Pharmacology, ASCPT, EACPT, ACCP, WHO

development and regulation of medications and Education of health care professionals and patients on the optimal utilization of medications” (<http://accp1.org/>). EACPT was founded after a meeting in Verona in 1991, in an attempt to foster the emerging discipline of CP in the eastern European countries [3]. The aims of the Association are to develop Clinical Pharmacology and Therapeutics in Europe by promoting the utilization of Clinical Pharmacological services in health care delivery (<http://www.eacpt.org/?q=node/2>).

Among others, the development of CP is driven by various CP Journals, most notably *Clinical Pharmacology and Therapeutics* (current Impact Factor: 7.586, <http://www.nature.com/clpt/index.html>), *Journal of Clinical Pharmacology* (current Impact Factor: 3.134, <http://www.sagepub.com/>), *British Journal of Clinical Pharmacology* (current Impact Factor: 3.128, <http://www.bjcp-journal.com/>), *European Journal of Clinical Pharmacology* (current Impact Factor: 2.497, <http://www.springer.com/biomed/pharmaceutical+science/>), or the *International Journal of Clinical Pharmacology* (current Impact Factor: 1.28, <http://www.dustri.com/nc/journals-in-english/mag/int-journal-of-clinical-pharmacology-and-therapeutics.html>).

CP has gone through a number of development cycles. Concomitantly with a steep growth of the pharmaceutical industry, CP experienced an “age of excitement” [4] in the 1950s and 1960s which led to the foundation of a large number of CP departments worldwide. According to one of the founders of the discipline, Sir Collin Dollery, clinical drug evaluation, which formerly seemed “a matter of gathering testimonials from well known clinicians”, is now a well-designed process and Clinical Pharmacology has become an indispensable part [5]. Although clinical pharmacologists now occupy influential positions in government and regulatory agencies such as EMEA or NICE [4, 6], a widespread feeling emerged in the late 1990s, that CP may not have lived up to its high expectations [7–9] and, in the UK, the number of clinical pharmacologists has been in decline [7], a situation, however, that is contrasted by the sustained growth of CP in other European countries [10]. There is no doubt that the lack of “an organ” and a billable procedure [8] makes a clinical specialty more vulnerable to oblivion in a world where “added value” is frequently reduced to economic concepts and values of a specific brand. The beauty and at the same time “Achilles heel” of CP has always been its enormous breadth, which has expanded further in recent years [11]. Nobody can reasonably claim to be an expert of drug therapy in all therapeutic areas. Likewise it is not credible to claim mastery of clinical therapeutics if one does not participate in up-to-date care of patients. On the other hand, a substantial portion of today’s specialists, who care for patients on a daily basis, have had insufficient training in the principles of pharmacodynamics, pharmacokinetics, pharmacovigilance, epidemiology, drug utilization and drug development.

The added value of clinical pharmacologists, jointly trained in CP and an organ-based specialty [9], is that they can bring together these scientific principles and specialty practice, and ideally can influence the colleagues around them. This kind of

training model seems to offer the best chance for Clinical Pharmacology to make an impact in healthcare. Indeed, since most prescribing of medicines occurs in the community, CP should also look towards primary care as a future development opportunity.

We live at an eventful time in clinical science when the powerful new forces of genomics, information technology, imaging technology or economics, to name a few, are rapidly changing the science and art of medicine. In practice, this will require even more specialization than before. However, there is also an increasing demand for a more integrated and holistic [9] approach, which can pull all the different strands together [6] to create “added value” in patient care, drug research and drug regulation. In this regard, CP has already provided numerous contributions to medicine [12] and will surely remain successful. Clinical pharmacologists have a vital contribution to make in the new era of molecular [13] and “translational” [14] medicine, continuously expanding numbers of drugs and clinical trials, and desire for “personalized medicine”. Future therapeutic agents, e.g. vaccines or cell- and siRNA-based therapies will be more complex from a PK-PD point of view, and they will also be more costly.

These new challenges will demand well-trained students and physicians, each with a firm grounding in the principles of CP. This kind of training will be necessary to ensure that patients get personalized therapy that maximizes their chances of cure and minimizes the risk of adverse effects. More widely it will be necessary to make sure that hospitals, academia and industry can depend on a supply of individuals who understand the new era of therapeutics.

With this vision in mind, it is arguably more important now than it has ever been that medical students are exposed to CP in their curriculum and that the relevant knowledge and competencies are unequivocally demonstrated before a career in medicine even begins [15–17].

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References

1. Roden MD (2008) Principles of Clinical Pharmacology. In: Fauci AS, Braunwald E, et al. Harrison's Textbook of Internal Medicine, 17th edn. McGraw Hill, New York, pp. 27–39
2. WHO Technical Report Series No. 446 (1970) Clinical pharmacology, scope, organisation and training: a report of a WHO study group.

3. Sjöqvist F, Orme M (1999) Preface to the European guide. In: Brosen K (ed.) *A Guide to Training in Clinical Pharmacology in Europe*. Odense University Press, Odense, pp. 7–10
4. Dollery CT (2006) Clinical pharmacology – the first 75 years and a view of the future. *Br J Clin Pharmacol* 61: 650–665
5. Dollery CT (1987) The future of clinical pharmacology and therapeutics. *Clin Pharmacol Ther* 41(1): 1–2
6. Aronson JK (2006) Clinical pharmacology: past, present and future. *Br J Clin Pharmacol* 61: 647–649
7. Maxwell SRJ, Webb DJ (2006) Clinical pharmacology – too young too die? *Lancet* 367: 799–800
8. Fitzgerald GA (2007) Clinical pharmacology or translational medicine and therapeutics: reinvent or rebrand and expand? *Clin Pharmacol Ther* 81: 19–20
9. Honig P (2007) The value and future of clinical pharmacology. *Clin Pharmacol Ther* 81: 17–18
10. Schellens JH, Grouls R, Guchelaar HJ, Touw DJ, Rongen GA, de Boer A, Van Bortel LM (2009) The Dutch vision of clinical pharmacology. *Clin Pharmacol Ther* 85(4): 366–368
11. Waldmann SA, Christensen NB, Moore JE, Terzic A (2007) Clinical pharmacology: the science of therapeutics. *Clin Pharmacol Ther* 81: 3–6
12. Dollery CT (2008) The scientific contribution of clinical pharmacology. *Europ J Clin Pharmacol* 64: 99–106
13. Dollery CT (2008) Clinical pharmacology in the molecular era. *Clin Pharmacol Ther* 83: 220–225
14. Aronson JK, Cohen A, Lewis LD (2008) Clinical pharmacology – providing tools and expertise for translational medicine. *Br J Clin Pharmacol* 61: 647–649
15. Flockhart DA, Usdin Yasuda S, Pezzullo JC, Knollmann BC (2002) Teaching rational prescribing: a new clinical pharmacology curriculum for medical schools. *Naunyn Schmiedeberg Arch Pharmacol* 366: 33–43
16. Aronson JK, Barnett DB, Breckenridge AM, Ferner RE, Jackson P, Maxwell SR, McInnes GT, Rawlins MD, Ritter JM, Routledge P, Walley TJ, Webb DJ, Williams D, Woods KL (2009) The UK's NHS and pharma: need for more clinical pharmacologists. *Lancet* 373(9671): 1251–1252
17. European Science Foundation (2009) Investigator driven clinical trials. A European Syllabus for Training Clinical Investigators. (<http://www.esf.org/research-areas/medical-sciences/publications.html>)

CHAPTER 2

Current issues in drug development

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1 Historical success

Historically, pharmaceutical therapy has been extraordinarily successful in combating and alleviating various diseases. Common life threatening diseases, most notably infections, have extremely satisfying therapeutic success rates and many serious diseases like diabetes mellitus or some forms of cancer have become chronic, stable diseases and do not lead to extreme shortages of life years any longer. Prime examples for success stories in drug development are (1) the massive prolongation in the life span of patients infected with HIV due to various combinations of highly active antiretroviral therapies (HAART), (2) the reduction of gastric ulcer and gastric cancer due to therapies aiming at eradication of *Helicobacter pylori* and (3) the targeted therapeutic approach for chronic myeloic leukaemia (CML) by means of imatinib mesylate (Gleevec). Whereas success rates in (1) are determined by a combination of a large number of powerful drugs which were rapidly developed by industry in response to the challenge posed by the HIV pandemic, success rates in (2) are determined by the discovery of an entirely new and/or previously overlooked concept for the pathogenesis of gastric diseases, i.e. a *Helicobacter* infection and in (3) by an intense effort to address the single molecular aberration responsible for CML, i.e. a mutation in the fusion protein-kinase bcr-abl.

These 3 success stories underline the fact, that success in drug development is driven by different variables and reflects more an art than a process, which can be reduced to robotic tools like high throughput screening or combinatorial chemistry. There is no doubt that historically overall drug development has been extremely productive. A recent analysis identified a total number of >20,000 drug products available today, 2/3 of which target 10 gene families (see Table 1) [1]. Interestingly, however, there are only ~1300 unique drugs of which ~1200 are “small molecule” drugs, 2/3 of which can be administered orally, and ~170 are “biologic” drugs [1].

Keywords: Druggable genome, drug targets, innovation, IMI, critical path, contract research organization (CRO), drug approval process, academic medicine, confidence crisis, public opinion

Table 1 Gene-family distribution of current drugs per drug substance

| Gene family | Percentage of FDA-approved drugs |
|--|-------------------------------------|
| Rhodopsin like GPCRs | 26.8 |
| Nuclear receptors | 13 |
| Ligand gated ion channels | 7.9 |
| Voltage gated ion channels | 5.5 |
| Penicillin binding protein | 4.1 |
| Myeloperoxidase like | 3 |
| Sodium neurotransmitter symporter family | 2.7 |
| Type II DNA Topoisomerase | 2.3 |
| Fibronectin type III | 2.1 |
| Cytochrome P450 | 1.9 |
| Rest | 30.7 |

The family share as a percentage of all FDA-approved drugs is displayed for the top ten families. Beyond the ten most commonly drugged families, there are a further 120 domain families or singletons for which only a few drugs have been successfully launched. Data based on 1357 dosed components from >20,000 approved products, FDA, December 2005. GPCR, G-protein-coupled receptor (modified from Ref. [1])

Also owing to the demographical trend of an ageing population, there is ample room for improvements and yet completely unmet medical needs as we have only modestly successful therapies for many neurological conditions like Alzheimer’s disease (see Case Study Chapter 20) and also success rates for many common cancers are still far from satisfying.

2 The dawn of a molecular era, the “druggable genome” and market fragmentation

The hope for a renaissance in drug development was fuelled by the publication of the human gene sequence by the Human Genome Project (HUGO) in 2000. HUGO revealed that humans harbour approx. 30,000 genes, which give rise to more than 150,000 transcripts. Besides its implications about our insights in human biology (Table 2) this data also led to an important stimulus in drug research.

Recent studies indicate that today’s pharmaceuticals exert their action on approx. 500 drug targets [1, 2] and based on HUGO data and assessment of ligand binding domains concluded that the number of potential therapeutic targets might be around 10,000 [3]. However, a closer look at potentially druggable targets and disease modifying genes reveals a probably more realistic and conservative number of a maximum of approx. 600–1000 novel drug targets [1].

Table 2 Comparison of the druggable genomes of selected eukaryotes

| | <i>Homo sapiens</i> | <i>Drosophila melanogaster</i> | <i>Caenorhabditis elegans</i> | <i>Saccharomyces cerevisiae</i> |
|---|---------------------|--------------------------------|-------------------------------|---------------------------------|
| Total number of predicted genes | ~30,000 | 13,601 | 18,424 | 6241 |
| Number of proteins in proteome | 21,688 | 13,849 | 17,946 | 6127 |
| Number of estimated druggable targets | 3051 | 1714 | 2267 | 508 |
| Percentage that are predicted druggable targets (%) | ~10–14 | 12 | 12 | 8 |

Reproduced and adapted from Ref. [3]

The use of genome-wide association studies (GWAs), in particular, has enabled to associate genetic variants with particular diseases and it is hoped that they may provide new footholds on the long and difficult path to better treatment [4]. However, to date, hopes that genomic high throughput tools would provide a large number of additional druggable targets were not fulfilled as a number of WGAs in large populations showed that for common conditions like coronary heart disease or diabetes only few novel markers could be identified, which also show only modest risk associations. A publication on a WGAs in large sample of patients with seven common diseases [5] revealed associations at many previously identified loci and a large number of further signals likely to yield additional susceptibility loci. However, the novel variants were characterized by a modest effect size (that is, per-allele ORs between 1.2 and 1.5) and even these estimates are believed to be inflated. The data suggest that, for any given trait, there will be few (if any) large effects, a handful of modest effects and a substantial number of genes generating small or very small increases in disease risk [5]. Thus, to date, genomic medicine has not provided a “quick-fix” for a declining productivity in drug development. There is evidence that productivity of pharmaceutical industry – after a period of robust growth – is in decline and as of today no clear trend upwards can be noted (Fig. 1). Ten years ago the Biotechnology industry, which has started the biotechnological era of pharmaceutical development mostly existed in parallel to “Big Pharma” and was perceived as a panacea for the productivity problem of the pharmaceutical industry. Nowadays the barriers between those two concepts of drug development have become increasingly blurred due to large number of mergers and acquisitions and there is increasing scepticism, that biotechnology *per se* will constitute a strong enough force for pharmaceutical growth.

Another trend, which started a decade ago was the “end of the blockbuster” (see Chapter 22). Industry could no longer rely on chemical products which may be prescribed to millions of patients but moved its attention to fragmented and high cost niche- or specialist-biotechnology markets, e.g. oncology or rheumatology. This trend is reflected by a substantial increase in the number of pipeline specialist drugs and a superior economic growth of companies which have adopted this trend

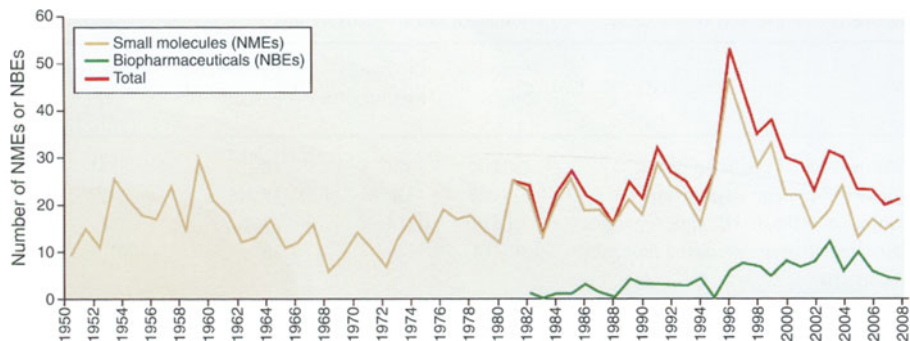


Fig. 1 Timeline of approvals of new molecular entities (nMEs) and new biological entities (nBEs) by the US Food and Drug Administration (FDA) between 1950 and 2008 (Adapted from Ref. [6])

early on (personal communication from IMS health). The perceived “end of the blockbuster” and the adoption of genomic medicine, in its extreme form called “individualization” has also posed a conceptual problem for clinical trial methodology (see Chapters 8 and 22). Randomized controlled trials (RCTs), which comprise large numbers of trial subjects, are focused on the statistical type 1 error (i.e. aiming to safeguard the risk of false positive results), often at the cost of reproducibility. Increasing individualization of therapeutics, however, due to much smaller sample sizes, will move the type 2 error (i.e. the risk of overlooking an effect) into the limelight again.

However, it is evident that the coincidence of current breakthroughs in genomics and information technology will shape a different concept of medicine and therapeutics. Although the consequences are not entirely clear and “pharmacogenomics” has yet failed to fulfil its promise, e-health and genomic health will have a substantial impact on the routine of medicine, not unlike the coincidence of breakthroughs in chemistry and experimental pharmacology at the beginning of the 20th century, which has influenced the last century.

3 Innovation and stagnation

The last decades have faced an increasing focus on codification of every minor facet in the industry – a situation which has produced a false sense of control over drug development [6]. In contrast to this perceived situation of total control, there is little doubt today that drug development faces a crisis in productivity. Over the last decade development costs have risen steadily but the number of new chemical entities (NCEs), which have been developed are in decline.

An outlier seems to be the vaccine area, which seems to prosper (see also Chapter 20). An immediate reaction was the steep increase in mergers and acquisitions and an increased activity in non-core activities like nutrition/“nutraceuticals” or integrated healthcare. One reason for the innovation gap might have been related to a focus on the promotion, patent extension and amendment of existing drugs, including “mee toos”, rather than development of new ones. Interestingly, “first to market” might not be an appropriate goal as there is sufficient evidence that follow-on innovations, even relatively late ones, can and do succeed economically [7]. A recent analysis came to the conclusion that investments in promoting existing products come at a long term cost – i.e. an increase in annual profits but a decrease in long-term value [8].

Therefore, at the beginning of 2000 it became clear that, at the current level of R&D, the traditional concepts were no longer a guarantee for robust growth. FDAs 2004 paper about innovation and stagnation in the pharmaceutical industry has led to a number of worldwide initiatives to salvage drug R&D like the US critical path initiative, a US strategy intended to transform the way FDA-regulated products are developed and used. (<http://www.fda.gov/ScienceResearch/SpecialTopics/CriticalPathInitiative/>). Another reaction was the implementation of the European Innovative Medicines Initiative “IMI”, a partnership between the European Community and the European Federation of Pharmaceutical Industries and Associations (EFPIA) with the aim to support the faster discovery and development of better medicines (<http://www.imi-europe.org/>). These concepts aim for radical changes in drug development and approval processes. Besides various “molecular” and “-omics” approaches (see Chapters 15 and 17) there is now an increasing focus on efficacy and toxicology biomarkers (see Chapter 16) and imaging technology (see Chapter 14) to foster drug research. At the level of drug regulation there is also an increasing awareness that traditional ways of judging drugs and granting market authorization may be outdated and may be one reason for the lack of productivity (see Chapters 3 and 23). Although no leading approach has emerged so far the need for an entirely novel conceptual framework is undisputed and also reflected by regulatory documents and the foundation of novel committees like the committee of advanced therapeutics at the EMEA (see Chapter 21).

Despite all discussions about declining research productivity and potential solutions, it is interesting to note that the rate of production of new drugs has been constant in the past 60 years [6]. A recent review states that “nothing that companies have done has affected their rates of new drug production” [6]. The decline following 1996 was sometimes interpreted as a return to historical normality following an atypical increase in the early 90s, rather than an absolute decline (Fig. 2). On the other hand it is clearly a matter of concern, that no company is able to produce the average of 2–3 NMEs per year, needed to match their growth expectations.

4 The development of EBM methodology

An important force that has come into play during the last 20 years and has influenced the way we perceive drug development and therapeutic interventions is our conceptual framework of clinical trial theory (see Chapters 8, 9, 12 and 13), i.e. the tool of the RCT which had ultimately led to the development of evidence based medicine (EBM) and health technology assessment (HTA). Whereas success has traditionally been measured on the basis of individual observations or testimonials by experts (see Chapter 1), EBM has rightfully raised the bar for successful drug therapy and was wholeheartedly embraced by regulators and reimbursement agencies (see Chapters 2 and 3).

5 Issues in preclinical and clinical drug development

5.1 Target and drug candidate identification

Current drug development strategies are focussed on validated single drug targets and Gleevec represents the ultimate example for the success of this targeted “clean drug” approach. Still, many successful drugs, which were not developed by a rational approach but rather empirically exert their action on more than a single target and thus represent a more “dirty” form of drugs. In the last two decades, successful drug discovery and development has been shaped by robotic technologies like combinatorial chemistry and high-throughput screening (HTS), a revolution in the development of fluorescent – and transporter probes and quantitative structure activity relationship (QSAR) approaches, which are now well-established platforms for discovery of lead compounds. HTS comprises the screening of large chemical libraries for activity against biological targets automatized assays and large-scale data [9]. To date, most emphasis has been put on quantitative screening capacity, whereas for the future many experts in the field propose a greater focus on physiological relevance, content and quality [9]. It is likely that future finding strategies will be much more project-related, tailor-made, and better integrated into the broader drug discovery efforts [9].

5.2 Preclinical drug development

A topic that has caused substantial concern in recent years was the large number of drugs that showed toxicity in late drug development or even after drug approval and the concomitant lack of predictivity of preclinical data [10]. Likewise current tools to assess carcinogenicity are under discussion and there is agreement that genotoxicity tests *in vitro* are not very specific and produce a high and unacceptable occurrence of irrelevant positive results [11]. One notable example where preclinical safety signals did not necessarily indicate toxicity was a phase I trial where six volunteers had to be

admitted to an ICU after administration of an activating CD28 T-cell super-antibody that was considered “safe” in animals (see Chapter 7). Thus, lack of severe toxicity in animal models should never be viewed as a guarantee of safety in man. The generation of meaningful preclinical data is therefore crucially dependent on the selection of a relevant biological model and an appropriate species and may not be viewed as a standard battery of tests similar to conventional chemicals. On the other hand approval of the novel and promising drug candidates may be burdened by preclinical data showing signs of toxicity. One example is the antifungal micafungin, where administration in animals was associated with the development of focal preneoplastic foci of altered hepatocytes (FAH). FAH were shown to precede hepatocellular carcinomas [12] and, thus, the EU product label indicates that “The decision to use micafungin should take into account a potential risk for the development of liver tumours (see Section 4.4). Micafungin should therefore only be used if other antifungals are not appropriate.” (<http://www.ema.europa.eu/humandocs>). Micafungin was non-genotoxic in *in vitro* assays and may be classified as a non-genotoxic (and potentially carcinogenic) chemical [13]. This view, however, stands against clinical experience and the lack of a toxicity signal with several 100,000 patients in Japan where micafungin was approved 4 years earlier (see also Case Study in Chapter 18).

5.3 Clinical drug development

The structure of clinical drug development has changed significantly in the last years, both conceptually [14] and structurally [15, 16]. Whereas in previous decades the clinical study environment has been dominated by big pharmaceutical companies and academic medical centres (AMCs), the field has been taken over by contract research organizations (CROs) and site management organizations (SMOs) over the past decade. Annual CRO-industry revenues have increased from about \$7 billion in 2001 to an estimated \$17.8 billion today; of more than 1000 CROs in operation, the four largest are now billion dollar companies [15]. Simultaneously, a lack of funding for independent clinical research and a lack of well-educated young clinical researchers have become obvious and several programmes have been established to build capacity and human capital in clinical research. The establishment of independent clinical research has, for many reasons, including topic selection and reimbursement questions, become a main goal in many countries. In a recent European Medical Research Council (EMRC) position paper [17] the top five recommendations to strengthen independent clinical trials in Europe were: (1) to improve the education, training and career structure and opportunities for scientists involved in patient-oriented clinical research; (2) to increase levels of funding for IDCT; (3) to adopt a “risk-based” approach to the regulation of IDCT; (4) to streamline procedures for obtaining authorization for IDCT; (5) to ensure that IDCT are carried out with an appropriate number of patients to produce statistically reliable results so that the trials are “correctly powered”.

A key problem of declining pharmaceutical industry productivity is the increasing cost of conducting clinical trials. The way clinical trials are conducted nowadays is determined by large trials with clinical endpoints which are rigid from a design perspective, costly and most importantly take a lot of time. Some experts therefore argue that moving from the traditional clinical development approach based on sequential, distinct phases towards a more integrated view that uses adaptive design tools and Bayesian methodologies to increase flexibility and maximize the use of accumulated knowledge could have an important role in achieving these goals [14]. In Europe a recent public consultation paper on the functioning of the European “Clinical Trial Directive” (CTD) (http://ec.europa.eu/enterprise/sectors/pharmaceuticals/files/clinicaltrials/docs/2009_10_09_public-consultation-paper.pdf) stated that there is widespread criticism that the CTD has led to a significant decline of the attractiveness of patient-oriented research and also had a negative impact in terms of administrative costs. In particular for academic sponsors of clinical trials costs can reach prohibitive levels. Besides (1) clinical trial and EBM methodology, (2) an increasingly complex

Table 3 Improving the Drug-Approval Process through “Economic Darwinism”

| Problem | Proposed solution | Comments |
|--|---|---|
| No long-term safety data No direct head-to-head comparative studies | Granting of extended period of exclusivity for drugs with data that demonstrate long-term safety | Study design requires preapproval by the FDA Will usually involve comparative studies |
| Phase 4 commitments not fulfilled | Granting of extended period of exclusivity only when phase 4 commitments are met | Present completion rate very low Currently no credible sanction |
| Inability to ensure timely conversion of surrogate and biologic marker end points to clinically meaningful end points | Approval based on biologic marker or surrogate marker – granting of limited period of exclusivity Granting of extended exclusivity only when converted to clinically meaningful end point | Some biologic markers and surrogate markers will not correlate to meaningful clinical benefit, and drugs approved on the basis of such end points will lose extended exclusivity |
| No incentives for drug development with high commercial risk No encouragement to make a paradigm shift rather than replicative strategies | Granting of additional (beyond current) extension of exclusivity for predefined high-need, high-risk areas Use of biologic markers and surrogate markers possible, but with limits described above | Achieving consensus independent of commercial and other pressures is key Use of an independent body such as NAS or IOM* to define high-need, high-risk areas Number of designated high-need, high-risk areas restricted to 5–10 |

*NAS denotes the National Academy of Sciences, and IOM the Institute of Medicine
Reproduced and adapted from Ref. [16]

legislative framework for patient centred clinical research and (3) declining willingness of the public to pay for costly pharmaceuticals in light of available (bio)generics, there has also been [4] a steep increase in regulatory demands on drug development (see Chapter 2) and there is ongoing discussion on reshaping the drug approval process radically [16], e.g. by granting limited period of exclusivity and an emphasis on post-marketing commitments on drug safety and efficacy (Table 3).

6 The role of academic medicine

To date, academia has played only a modest direct role in pharmaceutical development. However, there is certainly a huge indirect impact of academic training and intellectual transfer and it has frequently been pointed out that drug development flourishes in clusters of universities. In the future this impact may even increase, mostly in clinical development as industry-independent clinical research may offer a promising alternative to today's landscape of clinical trials. A major hurdle is the lack of public funding, a situation which is in contrast to the public outcry about industry interests in clinical research (see EMRC paper). Unfortunately, also academia undergoes substantial changes as described in a BMJ publication on the possible future scenarios of academic medicine [18, 19] and scenarios where academic medicine only flourishes in a private sector as a commercial business activity or succeeds by entertaining the public and media are certainly not desirable.

7 Confidence crisis and public opinion

Fuelled by a number of high profile failures (e.g. “Vioxx” or “Lipobay”) and inappropriate behaviour of stakeholders the pharmaceutical industry came under scrutiny and sometimes also became victim of public campaigns. In a widely discussed book Marcia Angell, former editor-in-chief of the *New England Journal of Medicine* claimed that the pharmaceutical industry suffers from corruption and makes the case that a substantial portion of industries revenues is spent for marketing rather than R&D [20]. It is frequently claimed that the average development cost of a new pharmaceutical is about 1000 Mio dollars. According to Angell, however, this number is inflated by marketing costs as well as opportunity costs and interest. Angell argues that valuable R&D work is performed by the public sector e.g. at the NIH and at universities. Likewise Jerome Kassirer, also former editor-in-chief of the *New England Journal of Medicine*, argues in his book “On the take” [21] that big business corrupts physicians who accept fees for promoting special products. Kassirer puts several conflicts of interest between companies and doctors into focus and advocates for a ban of industry gifts to medical personnel and full disclosure of financial incentives.

A 2009 survey in Austria (www.pharmig.at) revealed an astonishing image of the pharmaceutical industry in the general public. A substantial portion of the public (50%) believes that the industry is rather devoted to the shareholder value and profits than to healthcare (40%) and only 39% believe that drug products on the market have been tested adequately. On the other hand, more than 60% of the population and 42% of physicians believe that average costs for a successful drug development programme are less than € 50 Mio – which is in stark contrast to an estimated average total pre-approval cost estimate of \$ 802 Mio [22].

8 Conclusion

We currently witness a transition phase from a situation of a well established, highly esteemed process of drug development to a fragmented system without a dominant paradigm. There is a widespread feeling that traditional concepts in drug development are outdated. However, it is likely that international initiatives like IMI, adoption of novel tools by regulators and increased cooperation will shape the new landscape of drug development.

Case Study: Pfizer

Pfizer (www.pfizer.com) is currently the largest biopharmaceutical company worldwide with revenues of ~\$ 70 billion. Pfizer was founded by two cousins from Germany, Charles Pfizer and Charles Erhart in 1849 as a fine-chemicals business in Brooklyn, New York. During WWII Pfizer started with mass-production of penicillin and became the world's largest producer. The first pharmaceutical resulting from the discovery programme was oxytetracycline. In 1967 the antibiotic doxycycline was introduced and in 1976 prazosin, a blood pressure lowering drug. In the 1980s prioxicam and nifedipine were launched. Other important drugs developed by Pfizer were fluconazole, an antifungal, sertraline, an antidepressant, amlodipine for control of hypertension and the antibiotic azithromycin.

A major breakthrough with enormous publicity was the launch of Viagra[®] (sildenafil citrate) for erectile dysfunction in 1998. In 2000 Pfizer and Warner-Lambert merged and atorvastatin, a cholesterol lowering drug, first synthesized in 1985 at Parke-Davis-Warner-Lambert, became the top-selling drug for Pfizer and the number 1 branded pharmaceutical in the world with a patent protection expiring in 2011. After 2000 Pfizer launched a number of novel drugs, including

ziprasidone, a new antipsychotic, voriconazole and antitubercular, two antifungals, pregabalin, for treatment of neuropathic pain, sunitinib, an oral multi-kinase inhibitor and maraviroc, a new HIV drug.

In 2006 Pfizer announced the stop of its trial programme for torcetrapib, an LDL-lowering and HDL-inducing compound after a \$1 billion investment. In September 2008 Pfizer, according to a Wall Street Journal report, intended to drop efforts to develop medicines for heart disease, obesity and bone health to focus on more lucrative areas such as cancer and Alzheimer's disease. On October 15, 2009, Pfizer acquired Wyeth to form the world's largest pharmaceutical company.

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References

- Overington JP, Al-Lazikani B, Hopkins AL (2006) How many drug targets are there? *Nat Rev Drug Discov* 5(12): 993–996
- Drews J, Ryser S (1997) Classic drug targets. *Nat Biotechnol* 15: 1318–1319
- Hopkins AL, Groom CR (2002) The druggable genome. *Nat Rev Drug Discov* 1: 727–730
- Donnelly P (2008) Progress and challenges in genome-wide association studies in humans. *Nature* 456(7223): 728–731
- The Wellcome Trust Case Control Consortium (2007) Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 447: 661–678
- Munos B (2009) Lessons from 60 years of pharmaceutical innovation. *Nat Rev Drug Discov* 8: 959–968
- Cohen FJ (2006) Entry order as a consideration for innovation strategies. *Nat Rev Drug Discov* (AOP, published online 10 March 2006; doi:10.1038/nrd2009)
- Weiss D, Naik P, Weiss R (2009) The ‘big pharma’ dilemma: develop new drugs or promote existing ones? *Nat Rev Drug Discov* 8: 533–534
- Mayr LM, Bojanic D (2009) Novel trends in high-throughput screening. *Curr Opin Pharmacol* 9(5): 580–588
- Greaves P, Williams A, Eve M (2004) First dose of potential new medicines to humans: how animals help. *Nat Rev Drug Discov* 3(3): 226–236
- Kirkland D, Pfuhler S, Tweats D, Aardema M, Corvi R, Darroudi F, Elhajouji A, Glatt H, Hastwell P, Hayashi M, Kasper P, Kirchner S, Lynch A, Marzin D, Maurici D, Meunier JR, Müller L, Nohynek G, Parry J, Parry E, Thybaud V, Tice R, van Benthem J, Vanparys P, White P (2007) How to reduce false positive results when undertaking in vitro genotoxicity testing and thus avoid unnecessary follow-up animal tests: report of an ECVAM workshop. *Mutat Res* 30, 628(1): 31–55

12. Bannasch P, Haertel T, Su Q (2003) Significance of hepatic preneoplasia in risk identification and early detection of neoplasia. *Toxicol Pathol* 31(1): 134–139
13. Bolt HM, Foth H, Hengstler JG, Degen GH (2004) Carcinogenicity categorization of chemicals – new aspects to be considered in a European perspective. *Toxicol Lett* 151(1): 29–41
14. Orloff J, Douglas F, Pinheiro J, Levinson S, Branson M, Chaturvedi P, Ette E, Gallo P, Hirsch G, Mehta C, Patel N, Sabir S, Springs S, Stanski D, Evers MR, Fleming E, Singh N, Tramontin T, Golub H (2009) The future of drug development: advancing clinical trial design. *Nat Rev Drug Discov* 8(12): 949–957
15. Shuchman M (2007) Commercializing clinical trials – risks and benefits of the CRO boom. *N Engl J Med* 357(14): 1365–1368
16. Wood AJ (2006) A proposal for radical changes in the drug-approval process. *N Engl J Med* 355(6): 618–623
17. European Science Foundation: Investigator driven clinical trials (2009) http://www.esf.org/fileadmin/links/EMRC/FL_IDCT.pdf
18. ICRAM (the International Campaign to Revitalise Academic Medicine) (2004) Agenda setting. *BMJ* 329: 787–789
19. Clark J (2005) Five futures for academic medicine: the ICRAM scenarios. *BMJ* 331: 101–104
20. Angell M (2005) The truth about drug companies: how they deceive us and what to do about it. Random House Trade Paperbacks (August 9, 2005) ISBN-10: 0375760946
21. Jerome PK (2004) On the Take: How Medicine's Complicity with Big Business can Endanger Your Health. Oxford University Press, USA (October 18, 2004) ISBN-10: 0195176847
22. DiMasi JA, Hansen RW, Grabowski HG (2003) The price of innovation: new estimates of drug development costs. *J Health Econ* 22(2): 151–185

Further reading

- Drews J (2006) Case histories, magic bullets and the state of drug discovery. *Nat Rev Drug Discov* 5(8): 635–640
- Drews J (2003) Strategic trends in the drug industry. *Drug Discov Today* 8(9): 411–420
- Drews J (1995) Intent and coincidence in pharmaceutical discovery. The impact of biotechnology. *Arzneimittelforschung* 45(8): 934–939

CHAPTER 3

Current issues in drug regulation

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The role of drug regulatory agencies is to protect and promote public health. In everyday practice, this broad mandate translates into two distinct objectives: first, into an obligation to protect patients against ineffective or harmful drugs, and second, to protect patients against the consequences of untreated disease. The first objective results in a gatekeeper function and obliges regulators to apply stringent standards of assessment and to deny marketing authorization where deemed necessary. By contrast, the second objective requires regulators to support and enable drug development – with a view to ensuring that patients have access as early as possible to safe and effective drugs.

This chapter summarizes the processes put in place in the European Union (EU) to ensure that regulators can meet these objectives, and briefly describes some of the challenges surrounding drug approval. The technical term in the EU for drugs is “medicinal product” and we will use that term throughout the text.

1 The drug regulators’ decision-making

When approving new medicinal products, regulatory authorities need to be convinced that the (pharmaceutical) *quality* of the product fulfils predefined standards and that *safety* and *efficacy* are in a favourable balance; this is sometimes referred to as “Q–S–E”, or the first three hurdles a new drug has to pass on its route to market. While the issues around adequate product quality appear manageable in most instances, this is often not the case when it comes to large and complex molecules, such as biologicals [1, 2].

Keywords: European Union, market authorization, human medicinal products, regulatory affairs, centralized procedure, Mutual Recognition Procedure, Decentralized Procedure, risk–benefit assessment, relative effectiveness, pharmacovigilance, signal detection

Table 1 The regulators dilemma: “Regulators are confronted with a growing number of external needs, stakeholders, and their interests and concerns. All of these factors influence, or seek to influence, the timing of marketing authorization, which determines the time at which patients gain access to new drugs. The conundrum results from the fact that some of these external forces, although often legitimate in their own right, are pointed in different directions and become irreconcilable. HTA health technology assessment.” [3]

| | |
|--|---|
| Request for shorter timelines with higher level of uncertainty | Need for more or larger studies with delayed market access |
| <i>Industry</i> Require favourable conditions for innovation <i>Patients and carers</i> Demand early access to potentially lifesaving drugs | <i>Payers, prescribers and HTA assessors</i> Request comparative efficacy and effectiveness data <i>Media and the scientific community</i> Demand more thorough safety assessment after repeated market withdrawals |
| <i>Unmet medical needs (examples):</i> Ageing popultions, epidemiology of obesity, diabetes | <i>Excess medicalization</i> Obesity, metabolic syndrome, mood disorders |

Assessment of safety and efficacy is even more challenging [3]. Considering that no drug is devoid of potential safety issues, the benefits expected from drug treatment have to be weighed against potential harm; this is often referred to as the “benefit-risk balance”. The definition of an acceptable trade-off between safety and efficacy is not straightforward and invariably requires value judgements. Moreover, the balance is a dynamic process and benefit-risk may change as more information about a new medicinal product emerges when it is used in a large population and under everyday conditions (as opposed to clinical trial conditions).

Regulators are therefore finding themselves in a mounting dilemma: the need to balance early market access with the need for comprehensive benefit-risk data (Table 1). Setting the regulatory evidence requirements very high might not only stifle innovation but could also delay or inhibit patients’ access to effective treatment. Pharmaceutical industry and some patient advocacy groups strongly emphasize the point that these are undesirable consequences, particularly in therapeutic areas characterized by a high degree of unmet medical need. On the other hand, lowering the regulatory entry barrier might lead to insufficient knowledge about the benefits and risks of newly authorized medicinal products and thus harm patients. Detrimental consequences could result from unidentified risks or lack of efficacy in real life settings. It is widely assumed that the benefits from a range of medicinal products authorized in developed countries are debatable. It is difficult to predict how the regulators’ dilemma will play itself out in the years ahead.

2 Authorizing a medicinal product in the EU

We have described that quality, safety and efficacy are the main pillars for assessing a medicinal product. Depending on the type of product, each pillar may carry different

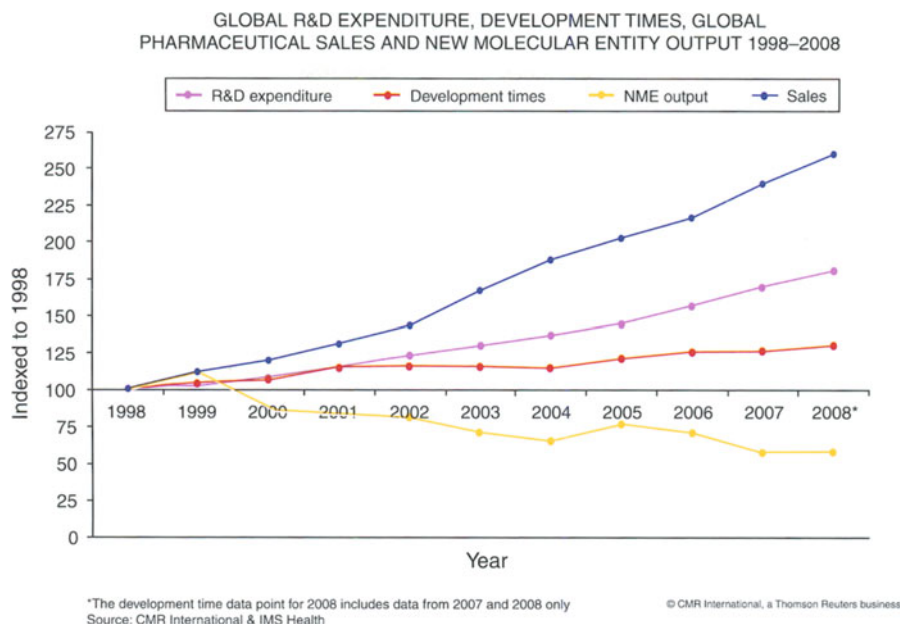


Fig. 1 *Development time*: Time taken from compound code assigned to first world launch. *Global pharmaceutical sales*: The revenue from global sales of ethical pharmaceuticals. This includes finished products, bulk sales and royalties from licensed-out ethical pharmaceuticals. *New Molecular Entity (NME)*: A new chemical entity or biological (including products of biotechnology) that has not been previously available for therapeutic use in man and are destined to be made available as a “prescription only medicine”, to be used for the cure, alleviation, treatment, prevention or *in vivo* diagnosis of diseases in man. Vaccines, new salts, pro drugs, metabolites and esters of existing compounds and certain biological compounds (e.g. antigens) are not classified as NMEs. Combination products are excluded from the list unless one or more of the constituents of the combination product has never been previously available. *R&D expenditure*: This includes salaries and all other personnel-related expenditure, expenditure related to consumable materials and supplies, and an appropriate share of overheads to cover administration, depreciation/amortization, space charges, rent, etc. The expenditure on R&D conducted by means of grants or contracts to other companies or institutions, and proportional expenditure for joint ventures should be included. This definition excludes capital R&D expenditure. **CMR International Performance Metrics Programme/Source**: *R&D expenditure*: Industry R&D Investment Programme; *Development times*, *NME output*: Annual Survey of New Molecular Entity First Launches/New Medicine Launches 2008: A complete guide to NMEs launched worldwide; *Global pharmaceutical sales*: Sales data supplied by IMS Health

weight. Currently, only around 30 medicinal products containing a new active substance (NAS; the definition includes new chemical and new biological entities) are authorized every year in the EU as compared to about 600–700 generics. These figures are broadly similar in all major drug markets, as the innovation pipeline appears to be drying up (Fig. 1). The increasing investment in pharmaceutical research and development over the past decade, coupled with a decrease in output of New Active

Substances reaching the market is often referred to as the current “productivity deficit” of pharmaceutical research.

For medicinal products containing a NAS, evaluation of safety and efficacy are paramount. However, when assessing generics the main issues are quality and bioequivalence [4]. This topic is addressed in more detail in Chapter 23. Another rather hard to delineate area refers to so called biosimilars. Biosimilars are replicas of authorized biologicals. As biologicals are large complex molecules the bioequivalence approach as mentioned above is not sufficient. Apart from quality data the applicant also needs to submit clinical data on efficacy and to a certain extent also on safety and immunogenicity (see Chapter 23).

There are currently four regulatory pathways how a medicinal product can obtain a market authorization in the EU. On one end of the regulatory spectrum is the centralized procedure where a single marketing authorization is followed by a single assessment procedure and – if favourable – results in a single marketing authorization valid in all EU member states. On the other end is a purely national process. In between are the Mutual Recognition Procedure and the Decentralized Procedure.

2.1 The Centralized Authorization

The legal time frame for an authorization procedure in the EU typically takes 210 days, excluding a clock-stop period where the marketing authorization applicant has time to answer a list of questions raised during the assessment procedure. A range of medicinal products are obliged under EU law to undergo the so called Central Authorization Procedure (Table 2) [5]. The advantage of this procedure is that the best available expertise in Europe can be acquired and that an approach fully harmonized across all member states can be established. The advantage for pharmaceutical companies is a

Table 2 Medicinal products requiring a central authorization in the EU

(a) Medicinal products developed by means of biotechnological processes:

- recombinant DNA technology,
- controlled expression of genes coding for biologically active proteins in prokaryotes and eukaryotes
- hybridoma and monoclonal antibody methods.

(b) Medicinal products for human use containing a new active substance and the treatment of:

- acquired immune deficiency syndrome,
- cancer,
- neurodegenerative disorder,
- diabetes,
- auto-immune diseases and other immune dysfunctions,
- viral diseases.

(c) Medicinal products that are designated as orphan medicinal products [Regulation (EC) No 141/2000]

single point of entry and that finally authorization is issued by the European Commission which is binding for all member states.

The central authorization procedure is coordinated by the European Medicines Agency (EMA). The working body, which proposes to the European Commission to accept or to reject an application, is the Committee for Medicinal Products for Human Use (CHMP). This committee is comprised of experts nominated by individual Member States, and additional experts. For each application procedure the CHMP selects from among its members one so-called Rapporteur and one Co-Rapporteur who independently, together with their assessment team based at the national agency, assess the marketing application dossier in depth and provide two separate assessment reports. Other CHMP members are free to assess parts or the complete dossier. Further, there is a peer-review process in place for quality-assurance of assessment reports. The results of all assessments are discussed at defined time points at the CHMP's monthly meetings. During the assessment process, the CHMP can avail itself of the expertise represented in several "scientific working parties", including those for quality, safety, efficacy, pharmacovigilance and, since 2009, also in the Committee for Advanced Therapies (CAT) [6].

At predefined time-points the applicant receives a list of questions, which need to be addresses satisfactorily.

An important feature during the early stages of development of a medicinal product is to ensure that its development plan is in line with what regulators will expect to see when assessing quality, efficacy and safety at the time of market authorization. Therefore procedures for provision of scientific advice by regulatory agencies to sponsors of drug development programmes have been established both in the EU and US. The EU scientific advice procedure is carried out by the Scientific Advice Working Party of the CHMP. In many member states national scientific advice is also available. Experts from Member States are coordinated by the EMA; it is a relatively rapid procedure, taking about 70 days [7]. Sponsors of a drug development programme can discuss the suitability of their planned development including details of non-clinical and clinical study designs. The majority of requests for scientific advice refer to phase 3 clinical trials.

2.2 The Mutual Recognition Procedure (MRP) and the Decentralized Procedure (DCP) [8]

If a new medicinal product is not legally required to go through the central authorization procedure, companies can chose to obtain marketing authorization *via* the Mutual Recognition Procedure or a Decentralized Procedure. The Mutual Recognition Procedure is used when a product is already authorized in one member state and the company intends to extend its marketing authorization to other member states. When using this procedure the company selects as the so-called reference member state, which

is where the medicinal product is authorized. The competent authority of that member state performs the primary assessment within 210 days. When a company decides to ask for authorization in other member states, it selects so-called concerned member states. The reference member state's competent authority forwards the assessment report and concerned member states' agencies then may take up to 90 days for their assessment.

If the product is not authorized in any of the member states the company may select the Decentralized Procedure. Here the applicant selects a reference member state and concerned member states and submits the application simultaneously to all of them. The competent authority of the reference member state performs the primary assessment but liaises earlier with the concerned member states. Overall this is a faster procedure than the Mutual Recognition Procedure and allows for earlier harmonization.

In 2007 a total of 441 MRP and 392 DCP were handled within the EU Member States. The figures for 2008 are 411 MRP and 734 DCP [9]. About 80% of these procedures are generic applications, the others being new medicinal products usually from a known class and not necessarily new active substances. Overall it concerns products with a relatively well-known safety profile.

2.3 The national procedure

There is also a national procedure, which is often of interest for small companies and larger pharmacies which serve a local market. For example, the Austrian regulatory agency received 164 and 134 applications in 2007 and 2008, respectively [10]. Almost 100% of these procedures concerned generic applications, herbal medicines or homeopathic products. The risk to public health may be considered to be limited, provided that product quality is satisfactory. National authorizations may serve as a base for a Mutual Recognition Procedure later on.

While the issues around new medicinal products containing NAS attract more interest from a scientific and public health perspective, the daily business of many national regulatory authorities in the EU is mainly defined by generics applications.

3 Regulatory life-cycle management of medicinal products

Once on the market, a medicinal product undergoes, on average, about three regulatory lifecycle changes per year, so called variations. Two of these are usually minor, such as a change of the market authorization holder's address or, say, the printing on the outer package, but, on average, one variation is expected to be major, such as widening or restriction of indications or insertion of warnings in the summary of product characteristics and the patient information leaflet.

In the following we will focus on products containing NAS, which in the EU are mainly authorized through the Centralized Procedure. NAS are necessarily associated with a higher degree of uncertainty about their benefits and risks. This may translate into greater risks to patients for two reasons: First, we do not understand their safety profile very well. Second, we have only information on efficacy but not effectiveness.

The regulatory life-cycle for centrally authorized products is described on the EMA's homepage in the section on European Public Assessment Reports [11].

3.1 From efficacy to post-marketing relative effectiveness assessment

In the EU, efficacy is defined as “the extent to which an intervention does more good than harm under ideal circumstances”, where “ideal circumstances” refers to conditions of (pre-marketing) clinical trials. Efficacy data are typically considered when regulators make their first-time benefit-risk assessment and are the basis of marketing authorization. By contrast, “Effectiveness is the extent to which an intervention does more good than harm when provided under the usual circumstances of healthcare practice” [12]. The distinction is relevant as it addresses the well-described efficacy-effectiveness gap, implying that drug treatment usually yields better results in the controlled environment of clinical trials than under the conditions of usual care [13]. The gap is in large part due to the fact that in clinical trials highly selected patients are treated in a closely monitored environment – to maximize benefits while minimizing risks.

Moreover, there is only scarce information on relative effectiveness at the time of marketing authorization. Relative effectiveness (called comparative effectiveness in the current debate in the US), is defined in the EU “as the extent to which an intervention does more good than harm compared to one or more intervention alternatives for achieving the desired results when provided under the usual circumstances of healthcare practice”. It has been pointed out that “new and approved does not always mean new and improved” [14], and information on post-marketing relative effectiveness is increasingly demanded by patients and healthcare decision-makers [15].

Note that relative effectiveness may mean more than comparing two medicinal products. In some therapeutic situations, there may be drug and non-drug interventions available. Smoking cessation, for example, can be achieved with the support of medicinal products, such as nicotine replacement products (e.g. gums, patches and inhalers), bupropion (an atypical antidepressant acting as a norepinephrine and dopamine reuptake inhibitor, and nicotinic antagonist) and varenicline (a partial nicotinic receptor agonist). There is, however, also behavioural therapy and the provision of financial incentives to induce smoking cessation [16]. From a patient perspective, it will be of interest to assess the relative effectiveness of all of these interventions.

It is anticipated that, in future, post-marketing life cycle management will include some form of effectiveness and relative effectiveness assessment.

3.2 Pharmacovigilance and signal detection

Even when a medicinal product containing a NAS has been studied in several thousand patients before accessing the market, “with every new drug, the safety profile is incomplete, and there is always more to come” [14]. This is illustrated for example by the observation that first-in-class biologicals are four times more likely to be subject to regulatory action than follow-on products. Such actions were observed with a frequency of 12 per 1000 months of observation after marketing authorization [17].

According to the International Conference on Harmonization (ICH), a safety database containing about 1500 patients is recommended at the time of market authorization of a NAS [18]. For NAS's intended for long-term treatment, this includes a minimum of 300–600 patients treated for 6 months and 100 patients treated for a year and ensures that acute and delayed adverse drug events can be detected if they occur as frequent as 0.5–5%. While the ICH recommendation is fairly general, regulatory practice is influenced by the size of the beneficial effect, safety observations made during the earlier development, the degree of unmet medical need, and whether a large clinical safety database can practically be achieved. In rare diseases this might pose a challenge. In an analysis of data submitted for market authorization to the EMA, the size of pivotal trials ranged from 12 to over 34,000 patients [19].

For statistical reasons less frequent adverse drug reactions can only be detected after market authorization, when large numbers of patients are being treated. This is where pharmacovigilance comes into play. For the past decades, the main pillars of pharmacovigilance have been spontaneous reporting of putative adverse drug reactions observed by healthcare professionals, signal detection, and safety communication.

There are several limitations to this approach, the main being an under-reporting rate higher than 90% [20]. Some adverse events may remain unreported if left to healthcare providers only. Therefore some countries have extended the concept to including consumer/patient reporting. This increased reporting rates but reduced the quality of reports. Apart from underreporting, selective reporting together with the difficulty to assess causality also poses problems with this method. Finally, a very large database is necessary to be able to perform meaningful signal detection. There is now in the EU a single large database, Eudravigilance, where all member states upload their pharmacovigilance case-reports. Eudravigilance enables the use of new methods, such as the proportional reporting ratio [21],

Table 3 Regulatory actions by the Austrian agency related to and triggered by pharmacovigilance activities

| | 2007 | 2008 | 2009 |
|--|------|------|------|
| Case reports originating from Austria | 2089 | 2885 | 3325 |
| Change of SPC and PIL | 998 | 1527 | 825 |
| Quality defects | 181 | 167 | 200 |
| Recalls of medicinal products/batches | 25 | 23 | 26 |
| Public letters to healthcare providers | 30 | 14 | 5 |

to mine data for safety signals. Preliminary research results are encouraging and indicate that improved methodology along with a large database may allow detection of signals earlier than was the case over the past years. Nonetheless, we need to bear in mind that signals are just that – there is no way around a thorough assessment of the signal and other supporting data by experts.

In most cases, concerns over drug-safety affect several EU member states, sometimes the whole EU, and signal detection and verification activities are now coordinated at the EMA level not only for centrally authorized products: the Pharmacovigilance Working Party advises the CHMP on safety issues which in turn agrees on EU-wide action plans, where necessary supported by decisions from the European Commission. Such action plans may include the suspension of a medicinal product (see Case study below), recalls of batches, the restriction of an indication, insertion of warnings in the summary of product characteristics (SPC) and patient information leaflets (PIL), and information to healthcare providers and the public.

Once an action plan has been formulated, its further steps are executed at the national level. In Table 3 we describe, as an example, the work and regulatory actions by the Austrian agency related to and triggered by pharmacovigilance issues [10].

3.3 Risk management plans (RMPs)

Recognizing the largely reactive and spontaneous nature of conventional pharmacovigilance, new EU regulation introduced the concept of Risk Management Strategy [22]. This has resulted in a requirement for industry to submit, under defined conditions, and at the time of application for a marketing authorization: “A detailed description of the pharmacovigilance and where appropriate of the risk management system which the applicant will introduce”. This requirement translates in practice into submission of a RMP, “a set of pharmacovigilance activities and interventions designed to identify, characterize, prevent or minimize risks

relating to medicinal products, including the assessment of the effectiveness of those interventions”.

The RMP has three components: (i) the “safety specification”, i.e. what is known about a medicinal product, (ii) the pharmacovigilance plan, the aim of which is to add to knowledge on suspected risks and to fill in gaps where knowledge is insufficient and (iii) an evaluation of the need for risk minimization activities, and, where applicable, a risk minimization plan.

Under the RMP concept, pharmacovigilance plans are more proactive in nature than routine pharmacovigilance, and may encompass a broad spectrum of study methodologies including randomized controlled studies, pragmatic clinical trials, registries, and various types of observational studies [23]. Risk minimization activities may range from educational materials for patients and/or healthcare providers to limiting pack size to informed consent or controlled distribution.

The adoption of the proactive risk management approach may be considered a paradigm shift in medicines regulation. The future challenge will be to communicate to all stakeholders the knowledge gained from the RMP activities to the benefit of public health.

3.4 When should a medicinal product be authorized?

The case story below – on a monoclonal antibody – is presented to illustrate the difficulties a regulatory body faces when assessing the benefit-risk balance of new medicinal products. This product’s efficacy was moderate but it was intended for patients with a disabling, though non-fatal, condition who had failed previous therapy or were intolerant to alternative therapies. When put on the EU market the product was under close scrutiny by the CHMP, particularly from a pharmacovigilance perspective: there were nine pharmacovigilance-triggered regulatory actions post authorization. These ranged from listing additional adverse effects in the summary of the product characteristics to issuing special warnings and, finally, suspension of the marketing authorization in February 2009: the modest effect in a usually non-fatal disease was not deemed important enough to outweigh the small but real risk of an often fatal condition, progressive multifocal leukoencephalopathy (PML). At the time of authorization this risk potential was not known. To detect the risk of PML before marketing authorization would have required the exposure of a substantially larger number of patients over longer periods of time than is realistic, considering the constraints of modern drug development.

The case story also illustrates the trade-off between accepting risk and supporting development of new treatment options that regulators – and society at large – need to make. Considering the broad range within society of moral values, risk aversion or acceptance, and willingness to support innovation, most will agree that this is no small feat.

Case Study: Efalizumab (Raptiva)

Efalizumab (Raptiva) was authorized in the EU in 2004 for the treatment of adult patients with moderate to severe chronic plaque psoriasis who have failed to respond to, or who have a contraindication to, or are intolerant to other systemic therapies including cyclosporine, methotrexate and PUVA [24].

Psoriasis vulgaris is a chronic, inflammatory skin disorder that affects 0.5% up to 3% of world's population. It is a T-cell mediated immune disorder in which CD4+ and CD8+ memory T cells stimulate the hyperproliferation of keratinocytes. Although rarely life threatening, psoriasis is frequently disabling and often compromises quality of life.

Efalizumab, the active ingredient of Raptiva, is a recombinant humanized monoclonal immunoglobulin G1 (IgG1) antibody with immunomodulatory properties. It binds specifically to the CD11a subunit of LFA-1 (lymphocyte function-associated antigen-1, a leukocyte cell surface protein) and inhibits the binding of LFA-1 to ICAM-1, -2 and -3 (intercellular adhesion molecules 1, 2 and 3) which interferes with lymphocyte adhesion to other cell types. LFA-1 is present on activated T-lymphocytes, and ICAM-1 is up-regulated on endothelial cells and keratinocytes in psoriasis plaques. By preventing LFA-1/ICAM binding, efalizumab may alleviate signs and symptoms of psoriasis by inhibiting several stages in the immunologic cascade: primary T-lymphocyte activation in lymph nodes, T-lymphocyte trafficking into psoriatic lesions, T-lymphocyte interaction with keratinocytes, secondary activation of T-lymphocytes in plaques, and release of pro-inflammatory cytokines.

At the time of market authorization safety-data was based on an overall exposure of about 2500 patient years and the medicinal product was considered to "appear safe and well tolerated". On March 17, 2009, the marketing authorization was suspended in the EU and the company withdrew voluntarily raptiva's market authorization from the USA market on April 8, 2009. The Committee for the Human Medicinal Products, the EMA's decision-making body decided that the risk-benefit ratio was no longer suitable for the following reasons: The beneficial effect was considered "modest", the disease, although negatively impacting a patients life, usually not being life-threatening and there were increasing safety issues. Being a selective immunosuppressant the risk of opportunistic infections is increased [17] and there were finally four cases of progressive multifocal leukoencephalopathy (PML). PML is a rare and usually fatal disease presumed to be caused by a reactivation of the ubiquitous Jakob-Creutzfeldt virus in patients with a depressed cell-mediated immunity. Since the introduction of antibodies for the treatment of various diseases PML has been observed under efalizumab (Raptiva), but also natalizumab (Tysabri) and rituximab (Mabthera). Natalizumab is author-

ized for the treatment of relapsing remitting multiple sclerosis in defined patients [25] and rituximab (Mabthera) is authorized for the treatment of patients with chronic lymphocytic leukaemia, Non-Hodgkin's lymphoma and rheumatoid arthritis [26].

Disclaimer

The views expressed in this article are the personal views of the authors and may not be understood or quoted as being made on behalf of or reflecting the position of AGES or the European Medicines Agency, or one of its committees or working parties.

References

1. Schneider CK, Schäffner-Dallmann G (2008) Typical pitfalls in applications for marketing authorisation of biotechnological products in Europe. *Nat Rev Drug Discov* 7: 893–899
2. EMEA/CHMP-think-tank group on innovative drug development (2007) Innovative drug development approaches. www.ema.europa.eu/pdfs/human/itf/12731807en.pdf (accessed 13.01.2010)
3. Eichler HG, Pignatti F, Flamion B, Leufkens H, Breckenridge A (2008) Balancing early market access to new drugs with the need for benefit/risk data: a mounting dilemma. *Nat Rev Drug Discov* 7: 818–826
4. Tschabitscher D, Platzer P, Baumgärtel C, Müllner M (2008) Generic drugs: quality, efficacy, safety and interchangeability. *Wien Klin Wochenschr* 120: 63–69
5. Regulation (EC) No. 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency. ec.europa.eu/enterprise/sectors/pharmaceuticals/files/eudralex/vol-1/reg_2004_726_cons/reg_2004_726_cons_en.pdf (accessed 13.01.2010)
6. Committee for Advanced Therapies (CAT); CAT Scientific Secretariat; Schneider CK, Salmikangas P, Jilma B, Flamion B, Todorova LR, Paphitou A, Haunerova I, Maimets T, Trouvin JH, Flory E, Tsiftoglou A, Sarkadi B, Gudmundsson K, O'Donovan M, Migliaccio G, Ancāns J, Maciulaitis R, Robert JL, Samuel A, Ovelgönne JH, Hystad M, Fal AM, Lima BS, Moraru AS, Turcáni P, Zorec R, Ruiz S, Akerblom L, Narayanan G, Kent A, Bignami F, Dickson JG, Niederwieser D, Figuerola-Santos MA, Reischl IG, Beuneu C, Georgiev R, Vassiliou M, Pychova A, Clausen M, Methuen T, Lucas S, Schüssler-Lenz M, Kokkas V, Buzás Z, MacAleenan N, Galli MC, Linē A, Gulbinovic J, Berchem G, Fraczek M, Menezes-Ferreira M, Vilceanu N, Hrubisko M, Marinko P, Timón M, Cheng W, Crosbie GA, Meade N, di Paola ML, Vandendriessche T, Ljungman P, D'Apote L, Oliver-Diaz O, Büttel I, Celis P (2010) Challenges with advanced therapy medicinal products and how to meet them. *Nat Rev Drug Discov* 9: 195–201
7. Annual Report of the European Medicines Agency (2008) www.ema.europa.eu/pdfs/general/direct/emeaar/AnnualReport2008.pdf (accessed 08.02.2010)
8. Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use (Consolidated version: 30/12/

- 2008). ec.europa.eu/enterprise/sectors/pharmaceuticals/files/eudralex/vol-1/dir_2001_83_cons/dir2001_83_cons_20081230_en.pdf (accessed 13.01.2010)
9. CMD Statistics (2008) www.hma.eu/fileadmin/dateien/Human_Medicines/CMD_h_/Statistics/2008_MR_DC_Art29_Stats.pdf (accessed 28.08.2009)
10. Austrian Federal Office for Safety in Healthcare. National figures for Austria. www.basg.at/news-center/statistiken/ages-pharmmed (accessed 28.08.2009)
11. EMA. The European Public Assessment Report. <http://www.ema.europa.eu/htms/human/epar/a.htm> (accessed 08.02.2010)
12. High Level Pharmaceutical Forum. Core principles on relative effectiveness. http://ec.europa.eu/pharmaforum/docs/rea_principles_en.pdf (accessed 18.09.2009)
13. Institute of Medicine. To err is human: Building a safer health system. www.iom.edu/Object.File/Master/4/117/ToErr-8pager.pdf (accessed 18.09.2009)
14. Anderson GM, Juurlink D, Detsky AS (2008) Newly approved does not always mean new and improved. *JAMA* 299: 1598–1600
15. Eichler HG, Bloechl-Daum B, Abadie E, Barnett D, König F, Pearson S (2010) Relative efficacy of drugs: an emerging issue between regulatory agencies and third-party payers. *Nat Rev Drug Discov* 9(4): 277–291 (Epub 2010 Feb. 26)
16. Volpp KG, Das A (2009) Comparative effectiveness – thinking beyond medication A versus medication B. *N Engl J Med* 361(4): 331–333
17. Giezen TJ, Mantel-Teeuwisse AK, Straus SM, Schellekens H, Leufkens HG, Egberts AC (2008) Safety-related regulatory actions for biologicals approved in the United States and the European Union. *JAMA* 300: 1887–1896
18. International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use (1994) The extend of population exposure to assess clinical safety for drugs intended for long-term treatment of non-life-threatening conditions. E1 www.ich.org/LOB/media/MEDIA435.pdf (accessed 19.03.2010)
19. Müllner M, Vamvakas S, Rietschel M, van Zwieten-Boot BJ (2007) Are women appropriately represented and assessed in clinical trials submitted for marketing authorisation? A review of the database of the European Medicines Agency. *Int J Clin Pharmacol Ther* 45: 477–484
20. Pirmohamed M, Breckenridge AM, Kitteringham NR, Park BK (1998) Adverse drug reactions. *BMJ* 316: 1295–1298
21. Bate A, Evans SJ (2009) Quantitative signal detection using spontaneous ADR reporting. *Pharmacoepidemiol Drug Saf* 8: 427–436
22. Committee for Medicinal Products for Human Use (CHMP) (2005) Guideline on Risk Management Systems for Medicinal Products for Human Use. www.ema.europa.eu/pdfs/human/euleg/9626805en.pdf (accessed 05.02.2010)
23. Wise L, Parkinson J, Raine J, Breckenridge A (2009) New approaches to drug safety: a pharmacovigilance tool kit. *Nat Rev Drug Discov* 8: 779–782
24. EMA. EPAR Raptiva. www.emea.europa.eu/humandocs/Humans/EPAR/raptiva/raptiva.htm (accessed 08.02.2010)
25. EMA. EPAR Tysabri. www.emea.europa.eu/humandocs/Humans/EPAR/tysabri/tysabri.htm (accessed 08.02.2010)
26. EMA. EPAR Mabthera. www.emea.europa.eu/humandocs/Humans/EPAR/mabthera/mabthera.htm (accessed 08.02.2010)

CHAPTER 4

Current issues in drug reimbursement

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1 Introduction

Reimbursing pharmaceuticals is considered, in most developed countries, an important part of delivering healthcare, be it by the state – or so-called “Beveridge Systems”, by private health insurance providers, or by independent, non-profit statutory institutions – the so-called “Bismarck” system [1, 2]. Notably, this has also been the case for Medicare in the USA due to the Medicare Prescription Drug Improvement and Modernization Act, which was passed in 2003 [3].

The reimbursement of pharmaceuticals deals with a fascinating array of ethical, social, economic and scientific questions, such as whether to reimburse contraceptives, which is the most equitable type of copayment or how to take the economic contribution of the local pharmaceutical industry into account with regard to reimbursement decisions. A comprehensive overview of the reimbursement situation in Europe is provided by the Pharmaceutical Pricing and Reimbursement Information Report, which was commissioned by the European Commission, Directorate-General Health and consumer Protection and the Austrian Federal Ministry of Health, Family and Youth [4]. These reports are by their nature ephemeral, because reimbursement systems change frequently, reflecting political, demographic and economic as well as scientific changes. This is why PPRI maintains Country Reports, which are supposed to be updated by the individual participating countries [5]. This chapter will focus on a part of the scientific evaluation process which is the domain of clinical pharmacology.

Representatives of pharmaceutical companies are fond of saying that, after a drug has received marketing authorization, it is certified to be efficacious, and therefore has to be reimbursed without further ado. For reasons that are beyond the scope of this article, few healthcare systems can afford to pay for all pharmaceuticals without further

Keywords: Reimbursement, relative effectiveness, health technology assessment, Medicines Evaluation Committee, Transparency Directive

Table 1a Comparison of the questions asked by regulators and reimbursers when assessing pharmaceuticals

| Marketing authorization | Evaluation for reimbursement |
|-------------------------|--------------------------------------|
| Quality | What are the available alternatives? |
| Efficacy | Is the new drug better? |
| Safety | Is the price worth the difference? |

scrutiny of their effectiveness and/or price. This scrutiny is, however, different from the marketing authorization process (see Table 1a). The “fourth hurdle” is here to stay, and clinical pharmacologists can do a great deal to help make it equitable to patients and fair to providers.

Evaluation for reimbursement is usually conducted soon after marketing authorization, so assessment and appraisal for reimbursement is often based on a subset of the data generated for marketing authorization. However, the questions which are asked are quite different. The fact that the data were not generated to answer these questions (right side of Table 1a) is a source of frustration.

2 Reimbursement principles in general

Basically, most institutions want to be able to provide pharmaceuticals, even if they are very expensive, to those patients who truly need them. Conversely, they usually discourage the use of ineffective drugs, even if they are cheap.

Generally, marketing authorization is considered a necessary, albeit not sufficient precondition for reimbursement, because paying for (i.e. buying) drugs which are not authorized at all or only authorized for certain indications would make the granting of a marketing authorization somewhat pointless. This is an ideal point of view, because in practice exceptions are made. Some countries make exceptions in the case of “compassionate use” (however, a good case can be made for pharmaceutical companies not profiting from compassionate use), another could be the case where “off-label” use cannot be monitored. A third, more controversial case is where the pharmaceutical company which “owns” the drug is not willing to apply for marketing authorizations in a certain indication, even though there are data to show that it could be efficacious. The “poster child” example for this is the anti-angiogenic agent bevacizumab for the treatment of age-related macular degeneration [6]. Bevacizumab is not licensed for this indication, nevertheless it is widely used and reimbursed, e.g. by Medicare [7]. Bevacizumab is far less costly than ranimizumab, which is licensed, but far more expensive [8]. The fact that commercial reasons can prevent a drug being licensed in an indication which could be useful and important from a public health point of view is

Table 1b Points to consider for added therapeutic benefit

-
- Efficacy
 - Effectiveness
 - Adverse effects
 - Applicability/appropriateness
 - Ease of use
 - Experience
 - Quality of life
-

arguably a shortcoming of the current system of licensing drugs for marketing authorization.

However there are usually several drugs available for a given indication – this is even the case for some orphan diseases (see the drugs licensed by the EMEA for pulmonary hypertension, for example¹). So, it is more common to compare new drugs to already licensed alternatives. Drug committees are a common instrument to make sense of the market. They may be in-house, such as hospital drug committees. More elaborate systems involve an external assessment, such as provided by NICE [9] or IQWiG [10] or HAS [11]. Other examples of such committees would be Canadian Agency for Drugs and Technologies in Health [12] or the Pharmaceutical Benefits Advisory Committee of Australia [13].

The task before the persons working with or in such committees is, ultimately, the same: to find out whether a given drug is as good as other options, or if is better, how much better. This will lead to a decision on whether to reimburse the drug, in some cases preceded by negotiations on price and/or limitations of some kind on reimbursement.

Such deliberations are in marked contrast to the way regulatory agencies consider data [14]. Core items which are considered during the reimbursement evaluation process have been identified by MEDEV (see below). How to value benefits in these dimensions – whether by way of pharmacoeconomic models which translate such benefits into quality-adjusted life-years, as proposed by NICE (among others) or by way of cost-effectiveness analyses, as proposed by IQWiG – is another fascinating topic which cannot be discussed here.

3 Relative effectiveness – background

How to get answers to all these questions? Again, many countries employ a standardized process for assessment. This may involve an application by a pharma-

¹ Drugs from three classes: PDE inhibitors (Sildenafil), prostanoids (iloprost) and endothelin antagonists. In the latter, three substances received marketing authorization, namely bosentan, ambisentan and sitaxentan – which is surely proof that the European Orphan Drug Regulation (25) succeeded in encouraging the marketing authorization of orphan drugs.

ceutical company as is the case in Austria [15] or the Netherlands [16]. Other institutions, such as NICE or IQWiG, are requested to assess certain drugs on the basis of need for guidance.

Regardless of how the drug to be assessed is chosen, institutions are fond of bemoaning the inadequacy of the data, which they have at their disposal for forming a sound basis for decision-making. One complaint is that the clinical trials submitted at the time of an application for reimbursement do not reflect how the drug will perform under “real-life” conditions, because the clinical trial setting does not reflect “real life” [17]. Another shortcoming is the absence of clinical trials with an active comparator. This controversy has been the subject of a recent publication which investigated the clinical trials submitted to the EMA for marketing authorization [14].

Here is a brief survey of data submitted in Austria for reimbursement.

4 Relative effectiveness case study – data submitted for reimbursement in Austria

Anna Bucsics and Valerie Nell-Duxneuner

4.1 Introduction

Applications for inclusion in the Austrian “Code of Reimbursement” (positive list drugs of reimbursed for ambulatory care) must be made online (www.sozialversicherung.at). Applicants must specify the “degree of innovation” for their product on a scale of 1 (no innovation, e.g. for generics) to 8 (first-ever treatment of a disease). The claimed patient benefit must also be specified on a scale of 1 (no additional benefit, e.g. for generics) to 6 (major benefit for all patients who can be treated with the drug in question). No more than three clinical trials can be submitted as “key” studies with the application to support these claims. Other studies are considered supplementary. A checklist must be submitted with the “key” trials; all other trials will be considered supplementary. We wanted investigate to which extent applications were based on studies with active comparators.

4.2 Methods

The document management system (Doxis®) was queried for all applications for inclusion of a new product between October 2005 and January 2010. Applications involving active substances referring to substances with the same ATC Code Level 5 (biosimilars, new formulations, applications based on bridging studies) and applications with insufficient data for full assessment. Subsequently applications

for highly innovative drugs (first-ever drug treatment, first ever treatment of a disease) were excluded because these drugs were, by definition, not eligible for studies with active comparators. The “key studies” submitted with these applications were then hand-reviewed by two reviewers to see whether they included an active comparator. Trials which specified switching from one drug to another without a control group or with a “before-after” design were not considered as having an active comparator. Classification conflicts were resolved by discussion.

4.3 Results

The original query yielded 109 distinct active substances specified as new active substance by the applicant. After the elimination process described above, there were applications for 87 active substances (see Tables 2 and 3).

Approximately half of all applications (49%) included “key” trials with a comparator which was considered appropriate for making reimbursement decisions, e.g. with a comparator from the most similar pharmacological or chemical class. A slightly higher proportion of the applications (55%) included a trial with the appropriate, i.e. similarly efficacious dose of the comparator (regardless of whether the comparator was considered suitable or not).

Table 2 Classification by applicant

| | |
|--|----|
| Number of substances in applications | 87 |
| “Z5” Classification – new active substance with known active principle (e.g. similar substances available at ATC Level 4) – “me-too” | 44 |
| “Z6” Classification – novel mechanism of action for a disease for which drug treatment already available | 43 |
| Applications with “key” trials (max = 3 key trials/application) | |
| One key trial | 13 |
| Two key trials | 31 |
| Three key trials | 43 |

Table 3 Applications with active comparators in key trials

| | |
|---|----|
| Number of applications for Z5 and Z6 | |
| No key trial with active comparator | 28 |
| One key trial with active comparator | 30 |
| Two key trials with active comparator | 19 |
| All three key trials with active comparator | 10 |

4.4 Interpretation

It is important to keep in mind that the “key” trials submitted here are those which the applicants consider important to buttress their reimbursement claims *versus* the payers. Thus, these trials are not necessarily identical to the “pivotal” trials submitted for marketing authorization. This means that they may well be of lower quality – the survey did not consider the quality of the submitted trials (trial size, patient selection, duration, endpoints, blinding and dropouts, etc.). Moreover, the appraisal regarding whether a comparator or a dose was suitable or not is subjective. Nevertheless, it is worth noting that even for substances which the applicant classified as an alternative (albeit possibly a better one) to already existing pharmacotherapeutic options, less than half of the submitted “key” trials (95/204) involved an active comparator. So, there is an indication that the evidence base for appraisal of pharmaceuticals for reimbursement is far from ideal and reflects the situation described by Eichler et al. [14], even though our survey only deals with drugs used in an ambulatory setting and excludes some innovative cancer drugs, such as biologicals used mostly in hospitals in Austria. This underlines the issue that the knowledge base for reimbursement is hardly more evolved than at the time of marketing authorization.

5 MEDEV – local payers vs. global payers

The Medicines Evaluation Committee was established in 1998. It is an informal group of specialists in the field of evaluating pharmaceuticals for reimbursement. It is hosted by the European Social Insurance Platform in Brussels [18]. Since January 2009, ESIP exists as a legal entity under Belgian law and represents a strategic alliance of over 40 national social security organizations across Europe. ESIP may ask the specialists in the MEDEV Committee for advice on specific topics concerning pharmaceuticals. “The principal purpose of MEDEV is to provide the national health insurance organizations and other competent bodies with timely analyses about drug related trends and innovations at both national and European level.

In addition, with the overall objective of providing a necessary counterweight to the pharmaceutical industry, especially at EU level, MEDEV aims to support the EU’s activities in formulating drug policies by giving input from the point of view of the statutory health insurers’ and other competent authorities. MEDEV can offer expert advice to all EU bodies from the earliest stage of the pharmaceutical decision-making process and help them analyze the possible impact of drug-related policies on national health schemes” [18].

Over the years, representatives from more than 20 countries have participated in MEDEV. Since most important new drugs are developed for distribution throughout the European Union (as well as the USA), most countries deal with the same drugs and the same “pivotal” clinical trials in which they were tested. Exchange of information about and experience with new (and not-so-new) therapeutic approaches has proven to be invaluable. We developed some core items which are covered by the evaluation process in most countries (see Table 1b).

MEDEV also offers the opportunity for discussion of “case studies” and to seek the best practice in dealing with specific problems of reimbursement, such as optimal provision of orphan drugs. The informal nature of MEDEV is an asset because it involves a minimum of organizational overhead but it means that MEDEV cannot be a full-fledged player in the European scene. Members of MEDEV have, however, represented ESIP at the Pharmaceutical Forum, which was a full-scale European endeavour.

6 The Pharmaceutical Forum

The European Union began as an economic union and has traditionally regarded the pharmaceutical industry as an important economic sector. This is reflected, *inter alia*, in the strong interest the European Commission has taken in the pricing and reimbursement of pharmaceuticals, despite the fact that providing healthcare is subject to the principle of subsidiarity. The European Medicines Agency was originally attached to the Directorate General for Enterprise. Only recently was the EMA moved to the Directorate General responsible for health [19], thus reflecting the situation of comparable agencies, e.g. the Food and Drug Administration, which is under the auspices of the Department of Health and Human Services [20]. The most important piece of legislation for reimbursers in the EU is the Transparency Directive (Directive 89/105/EEC), which regulates the decision-making around the pricing and reimbursement of pharmaceuticals. It came into effect in 1989, and established the “Consultative Committee for the implementation of Directive 89/105/EEC relating to the transparency of measures regulating the pricing of medicinal products for human use and their inclusion in the scope of national health insurance systems” [21].

The European Commission has undertaken several initiatives in pursuing a single market for pharmaceuticals. These initiatives have evolved to become more transparent and inclusive since the time of the Bangemann Round Table [22]. The subsequent initiative, the so-called “G-10” included input from several stakeholders, notably also payers [23].

G-10 recommendation on relative effectiveness (RE) by the High Level Group on innovation and provision of medicines recommendations for action (G-10) [23]:

Recommendation 7:

The Commission should organize a European reflection to explore how Member States can improve ways of sharing information and data requirements to achieve greater certainty and reliability for all stakeholders, even if the decisions they take may differ.

The objective is to foster the development of health technology assessment (HTA), including clinical and cost effectiveness, in the Member States and the EU; to improve the value of HTA, to share national experiences and data while recognizing that relative evaluation should remain a responsibility of Member States.

The Pharmaceutical Forum was created by the Commission in 2003 to deal with three of the 11 topics identified by the G-10, namely “patient information”, “relative effectiveness” and “pricing and reimbursement”. The topics were entrusted to three Working Groups, each with representation of all relevant stakeholders in addition to relevant Member States. The stakeholders are listed in Box 2. In addition, representatives of the European Parliament and EMEA, as the European Medicines Agency was then called, participated.

Stakeholders of the Pharmaceutical Forum [24]

- European Federation of Pharmaceutical Industries and Associations
- European Generic medicines Association
- European Self-Medication Industry
- European Association for Bioindustries (EuropaBio)
- European Association of Full-Line Wholesalers
- European Patients Forum
- Standing Committee of European Doctors
- Pharmaceutical Group of the European Union (community pharmacists)
- Association Internationale de la Mutualité
- European Social Insurance Platform

Getting all these divergent interest groups to agree on anything at all about something as vague but with such profound economic implications as RE was no easy task. The recommendations coming from the working group therefore can only be seen as the least common European denominator for this topic. However innocuous the output may be, it is not without merit, because it shows the feasibility of European cooperation on the subject of RE. That such a cooperation is useful should be obvious, the obstacles perhaps less so.

The mandate of the Working Group on Relative Effectiveness [24] was as follows:

To help Member States apply relative effectiveness systems in order to allow containment of pharmaceutical costs as well as a fair reward for innovation. Relative effectiveness systems are relatively new for many Member States and rather complex. Nevertheless the outcome of relative effectiveness is promising as they will help allow identify the most valuable medicines, both in terms of clinical efficiency as of cost-effectiveness, and will help set a fair price for these medicines. The Working Group will bring experiences of different Member States and of industry together in order to further develop this promising field.

Work began with discussions on the definition of RE. The following working definitions, based on those proposed by Brian Haynes [26] were adopted:

- *Efficacy: is the extent to which an intervention does more good than harm under ideal circumstances.*
- *Relative efficacy: can be defined as the extent to which an intervention does more good than harm, under ideal circumstances, compared to one or more alternative interventions.*
- *Effectiveness is the extent to which an intervention does more good than harm when provided under the usual circumstances of health care practice.*
- *Relative effectiveness can be defined as the extent to which an intervention does more good than harm compared to one or more intervention alternatives for achieving the desired results when provided under the usual circumstances of health care practice.*

These definitions are somewhat different from the definition of comparative effectiveness for the purpose of comparative effectiveness research in the US [27], because the Pharmaceutical Forum was focused on pharmaceuticals.

7 Results

7.1 Good principles

The first deliverable of the working Group on Relative Effectiveness was a set of good principles governing relative effectiveness assessments (see Box 3). These principles were accompanied by a checklist for use of the principles of relative effectiveness assessment.

Good practice principles for relative effectiveness assessment [28]

The aim of relative effectiveness (here-after RE) assessment is to compare healthcare interventions in practice in order to classify them according to their practical therapeutic

value². Differences between the objectives and priorities of different national healthcare systems may create differences in the way in which healthcare interventions will be valued relative to one another. In the EU context, this means that a relative effectiveness assessment is most likely to be meaningful at the national (local healthcare) level. However there is considerable³ value of stimulating exchange of information, methodologies and experiences between the relevant national authorities. The first step in assessing relative effectiveness is an assessment of relative efficacy. Working definitions of these terms have been developed in the course of the full Working Group's deliberations (see here-after). While the rules and processes within a given healthcare system should be established by discussion among the local stakeholders concerned, some classical principles of public administration are likely to be generally relevant. The working group suggests the following principles for the Pharmaceutical Forum to endorse, for non-binding use as appropriate in Member States.

1. Individual Member States may use RE assessments for different purposes. Decisions on the detailed operation of RE assessments, including methods and relevant stakeholders⁴, are most appropriately made at a national level.
2. RE assessment processes, selection of products to be assessed, working methodologies and quality assurance processes should be transparent to all parties and evidence-based.
3. Relevant stakeholders should be able to contribute to the development of assessment methodologies. The purpose of RE assessment and the organization(s) responsible for its conduct should be clearly identified.
4. RE assessment processes should remain separate from product market authorization procedures (though this does not mean that they are necessarily performed by different organizations).
5. RE assessment processes should be time-framed, and should minimize or avoid causing unnecessary procedural delays consistent with any associated Transparency Directive requirements where applicable.
6. RE assessments should be capable of addressing transparently uncertainty in the evidence base, and the methodological challenge of translating evidence on relative efficacy and other appropriate available data into conclusions on relative effectiveness.
7. The sources of evidence which are to form the relevant RE input should be specifically discussed among the identified key stakeholders, who should each be able to submit evidence or argumentation for appraisal.
8. RE assessment should include comparison with the most appropriate healthcare interventions. Such comparison should build on the results of active controlled clinical trials, where available.

² EFPIA quotes: "The aim of RE assessment is to compare healthcare interventions in practice in order to determine their practical therapeutic value".

³ EFPIA suggests deleting the adjective "considerable".

⁴ Relevant stakeholders include patients and health professional organizations, the pharmaceutical industry and social insurers.

9. When concluded, outcomes should be communicated in a clear and timely manner to all interested parties. Communication by means of publishing the supporting evaluation on a publicly accessible website is strongly encouraged.
10. RE assessments should be capable of subsequent revision and updating as the evidence base develops.
11. RE assessments should aim to identify areas in which the evidence base on an intervention could most usefully be developed in the future.

7.2 Data for relative effectiveness assessment

The WG on RE also undertook a survey of data availability on relative effectiveness assessments [29]. This undertaking demonstrated the complexity and current intractability of the topic of RE. For instance, there is no clear-cut boundary between the concepts of efficacy and effectiveness – indeed, the fact that not all languages of the European Union differentiate among the concepts of efficacy, effectiveness and efficiency is an obstacle in itself! While it may be fairly uncontroversial to assign clinical trials which assess surrogate parameters (e.g. blood pressure, blood glucose or LDL) to the category of trials assessing (relative) efficacy, one might debate whether to assign well-designed clinical trials assessing endpoints such as morbidity or hospitalization to the category of efficacy trials (because they are conducted in a well-controlled environment) or to the category of effectiveness (because they assess real-life endpoints). A major problem is that at present there seems to be an inverse relationship between the robustness of the database with regard to internal validity *vs.* robustness in regard to external validity. To wit, randomized controlled trials have been the state of the art with regard to internal validity. However, due to the rigorous methods used to ensure quality control, such as inclusion and exclusion criteria, monitoring etc., these trials cause concern about the transferability of the results into everyday practice, i.e. external validity. On the other hand, so-called pragmatic trials which reflect everyday care, are much more susceptible to bias issues and channeling, which compromises their internal validity [14]. This is not really new, but it is encouraging to see that these problems are recognized as such in the process of high-level decision making.

To further muddle the issue, despite the definitions adopted by the WG on RE, some Member States consider RE assessment to include the assessment of cost-effectiveness – i.e. that which Haynes describes as efficiency. This is not a purely semantic issue, because it involves procedural differences, which may lead to discrepancies with regard to the qualifications and competencies of the teams involved at specific stages of the assessment process. For example, it might well make a difference whether “effectiveness” is assessed by a clinical pharmacologist on the basis of clinical data (be it surrogate or

“hard” clinical “endpoints”) or whether “effectiveness” is assessed on the basis of cost-effectiveness data. In the latter scenario, it is not unusual to present summary data based on pharmacoeconomic models. It is tempting, but dangerous, to accept such models without rigorous scrutiny of the clinical data which are input into these models. Separating effectiveness from cost-effectiveness seems to be a good way to avoid such pitfalls. It also helps in coordinating people from several disciplines who need to review such models.

7.3 Networking

As shown by the MEDEV experience, it was obvious, even before the Pharmaceutical Forum process, that networking and sharing information would provide benefits for all parties involved in providing advice about RE assessment for decision-making. Pharmaceutical companies which develop new drugs have a limited dataset which is submitted for marketing authorization. In many cases, a distinct subset of the whole dataset for MA is submitted for reimbursement. The fact that at present, many reimbursement agencies assess the same or a similar subset of the MA data for reimbursement is an obvious reason to share the results of the first assessment with other entities which are subsequently confronted with the same dataset. The obstacles to solving this problem are procedural, legal, and political. Whether it is legitimate to for one agency to share its experience with a specific drug with another agency confronted with the same decision options is not an issue of clinical pharmacology. A common assessment of pharmaceuticals, which is, from the scientific point of view, an obvious solution to the problem of a score of countries doing the same thing, faces obstacles of a more political nature. It may be scientifically feasible to perform an *assessment* of relative effectiveness which is acceptable all to the countries involved for many new pharmaceuticals, but there is concern that this will not always be the case, for example because the locally prevailing treatment in one country may define a comparator which is not realistic in other countries.

A more important concern involves the *appraisal* process which follows the assessment, and which ultimately involves value judgments based on local values. Of course, currently in the European Union, local values vary widely, depending on the cultural, demographic, infrastructural and of course economic givens of a country, just to name a few. There is much concern that a common assessment will prejudice the decision-making process in such a way that the local values cannot be sufficiently taken into account – for example that systems in economically less developed regions may be confronted with the problem of paying for drugs at prices which they cannot afford, because of positive assessments made by richer countries. Once these issues are solved, clinical pharmacologists will enthusiastically share their experiences – since there is no scientific reason for not doing so at present.

The WG on RE recommended to the Forum to make use of existing networks to foster cooperation instead of installing a new network [30]. Ultimately, EUnetHTA was entrusted with the task of taking the work of the Pharmaceutical Forum on RE forward.

8 Looking forward

8.1 EUnetHTA

EUnetHTA stands for the European Network for Health Technology Assessment. The EUnetHTA Project 2006–2008 was supported by a grant from the European Commission (DG SANCO). The follow on project will be the EUnetHTA Joint Action (European Network for HTA Joint Action), funded by a grant agreement which has been signed by the EU Executive Agency for Health and Consumers and the Co-ordinator (National Board of Health of Denmark) on behalf of 33 partners in 23 EU Member States and Norway. The activities of the Joint Action will be a continuation of the EUnetHTA Project (2006–2008) and the activities of the Pharmaceutical Forum on relative effectiveness. One of the Work Packages of this Joint Action will deal with the issue of relative effectiveness, as defined by the Pharmaceutical Forum, others will explore the techniques for collaboration among national bodies [31].

One of the first steps in this endeavour was the start of a collaboration between the EMA (the European Medicines Agency) and EUnetHTA on improving and expanding the contribution of the European Public Assessment Report (EPAR) to relative effectiveness assessments [32].

EUnetHTA has developed a Core Model™ for Health Technology Assessment. This model is somewhat different from the procedure used to assess pharmaceuticals for reimbursement within the legal framework of the Transparency Directive, the Core Model™ being more comprehensive in nature and less subject to time constraints. It will be interesting to see where the journey along these two quite different paths will lead.

8.2 USA – comparative effectiveness research

Another promising development is the Obama Administration's plan to fund Comparative Effectiveness Research to the tune of around one billion dollars [33, 34]. Although the project is not primarily devoted to comparison of pharmaceuticals, but to comparisons on a much broader basis [27], drug comparisons will at some point also be included. More importantly, we can expect the development of generic methodologies which should prove useful for comparing pharmaceuticals too.

9 In the future – building a european system of assessing relative effectiveness

Based on work of MEDEV, the Pharmaceutical Forum and EUnetHTA, here is an outline of an incremental model of dealing with the issues of relative effectiveness, based on a paper submitted to the Pharmaceutical Forum by ESIP. The aim of this system would be to aid in decision-making and to provide guidance for further research and work to improve the quality of the decision-making process by improving the science-base for assessing relative effectiveness. This system would not replace the decisions made by the individual payers in each country, but would aim to inform the decision-making process.

9.1 Developing the methodology

There is already a wealth of data on how individual agencies assess relative effectiveness. This methodology needs to be developed further, to find methods for indirect comparisons with robust management of uncertainty, perhaps analogous to the meta-analysis methodology of the Cochrane Collaboration [35].

9.2 Validating estimates of relative effectiveness

The next step after defining standardized methods would be to conduct such comparisons, possibly by EUnetHTA or by individual institutions for sharing. Taking this idea further means making an attempt to verify the results of such comparisons, because these are, in effect, predictions of how the compared drugs will behave in real life. This can be done initially with a pharmacoepidemiological approach, e.g. with the use of drug utilization data. This would provide post marketing information which could be used in conjunction with the system of phased release of medications, as proposed by Ray and Stein [36]. Such a system allows for providing new medications to those who really need them and will benefit most while obtaining further efficacy and safety data in a controlled setting. These data then can be used to extend or limit reimbursement. The criteria should be agreed upon in advance to limit controversy. Such schemes have already been proposed, so the idea does not seem too far-fetched [14].

9.3 Designing and performing the studies we need

The scientific experience amassed with the above type of studies can further be utilized to conduct genuine post-marketing studies to obtain the data that reimbursers and society need to reliably assess relative effectiveness. At this point, the EU could establish an institution to encompass the recommendations made by Ray and Stein [36], i.e. sponsoring and conduct of independent post-marketing studies, on which further reimbursement may be contingent.

By establishing a “European Institute of Health”, a European counterpart to the NIH, as the EMA is the European counterpart of the FDA, Europe would give a much-needed impetus for clinical research, thereby helping preserve the science base in Europe, provide guidance for reimbursers and feedback for pharmaceutical companies with more exact and unbiased data on the true relative effectiveness of their products. Ideally, this would incentivise companies toward developing more innovative products instead of “me-too” products which consume an inordinate amount of marketing resources to promote. In conjunction with the phased release of medications, this would prevent market withdrawal of products with safety issues due to inappropriate use, keeping them available for patients who really benefit.

9.4 Leading the way

A “European Institute of Health” would contribute to reinvigorating the pharmaceutical industry in Europe. It would provide a much-needed independent research foundation on which pharmaceutical companies can build. The example of the development of taxanes in the U.S. shows that this can be commercially extremely beneficial for the companies involved [37]. A well-defined research programme could also help in incentivising and focussing the pharmaceutical industry to develop products for which there is a real need, as proposed by the Dutch initiative on priority medicines for Europe and the world [38]. This could, of course also form the basis for public-private partnerships.

Convincing countries that such an institution is worth financing is, of course, a challenge. The USA, at least, has realized that the benefits are sure to outweigh the cost and is proposing to go ahead with the Comparative Effectiveness Research initiative. On the one hand, the institute would conduct much-needed independent studies, which the pharmaceutical industry has no reason to sponsor (e.g. specifically studies on relative effectiveness, such as ALLHAT [39]). Doing this at the European level instead of a national level only, as in Italy, for example, would leverage the economies of scale provided by the European Union. On the other hand, it would provide expertise and guidance for the pharmaceutical industry, which would, ultimately, benefit. Europe would be well advised to establish an institute to help achieve the goal of becoming the most innovative economic unit in the world.

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Disclaimer

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References

1. Bismarck versus Beveridge: Social Insurance Systems in Europe CESifo DICE Report 2008 [cited 1 March 2010. 69–71]. Available from: http://www.cesifo-group.de/pls/guestci/results.run_query?v_it_folder_list=2888&v_page_title=CESifo%20DICE%20Report%204/2008%20&v_num_files=25&v_display_results=S&v_order_by=autor
2. van der Zee J, Kroneman M (2007) Bismarck or Beveridge: a beauty contest between dinosaurs. *BMC Health Ser Res* 7(1): 94
3. Frencher SK Jr, Glied S (2006) The Medicare Prescription Drug Improvement and Modernization Act: prescription drugs and academic medicine. *Acad Med* 81(9): 812–816
4. Vogler S, et al. (2008) PPRI Report. [cited 6 March 2010], Available from: <http://ppri.oebig.at/index.aspx?Navigation=r|2|0->
5. PPRI Pharma Profiles. [cited 6 March 2010], Available from: <http://ppri.oebig.at/index.aspx?Navigation=r|2|1->
6. Steinbrook R (2006) The Price of Sight – Ranibizumab, Bevacizumab, and the Treatment of Macular Degeneration. *N Engl J Med* 355(14): 1409–1412
7. CMS LCD for Intravitreal bevacizumab (Avastin®). [cited 15 Jan. 2010], Available from: http://www.cms.hhs.gov/mcd/viewlcd.asp?lcd_id=29961&lcd_version=5&basket=lcd%3A29961%3A5%3AIntravitreal+Bevacizumab+%28Avastin%C2%AE%29%3AMAC++Part+B%3AFirst+Coast+Service+Options||+Inc.+%2809302%29%3A#top
8. Raftery J, et al. (2007) Ranibizumab (Lucentis) versus bevacizumab (Avastin): modelling cost effectiveness. *Br J Ophthalmol* 91(9): 1244–1246
9. National Institute for Health and Clinical Excellence. [cited 1 March 2010], Available from: <http://www.nice.org.uk/>
10. IQWiG Website. [cited 6 March 2010], Available from: <http://www.iqwig.de/institute-for-quality-and-efficiency-in-health.2.en.html>
11. Haute Autorité de santé [cited 1 March 2010], Available from: http://www.has-sante.fr/portail/jcms/c_5443/english?cid=c_5443
12. Canadian Agency for Drugs and Technologies in Health [cited 1 March 2010], Available from: <http://www.cadth.ca/index.php/en/home>
13. Pharmaceutical Benefits Advisory Committee (PBAC) [cited 1 March 2010], Available from: <http://www.health.gov.au/internet/main/publishing.nsf/content/health-pbs-general-listing-committee3.htm>
14. Eichler HG, Bloechl-Daum B, et al. (2010) Relative efficacy of drugs: an emerging issue between regulatory agencies and third-party payers. *Nat Rev Drug Discov* 9: 277–291

15. Legal Basis for the Austrian Reimbursement List [cited 1 March 2010], Available from: http://www.sozialversicherung.at/portal27/portal/esvportal/channel_content/cmsWindow?p_tabid=5&p_menuid=64740&action=2
16. College voor zorgverzekeringen (CVZ) [cited 1 March 2010], Available from: <http://www.cvz.nl/>
17. Garattini S, Bertele V (2008) Do we learn the right things from clinical trials? *Eur J Clin Pharmacol* 64(2): 115–125
18. ESIP website [cited 6 March 2010], Available from: www.esip.org
19. President Barroso unveils his new team (2009) [cited 6 March 2010], Available from: <http://europa.eu/rapid/pressReleasesAction.do?reference=IP/09/1837&format=HTML&aged=0&language=EN&guiLanguage=en>
20. The Department of Health and Human Services [cited 6 March 2010], Available from: <http://www.hhs.gov/about/>
21. Council Directive 89/105/EEC of 21 December 1988 relating to the transparency of measures regulating the pricing of medicinal products for human use and their inclusion in the scope of national health insurance systems, In DIRECTIVE 89/105/EEC. 1989, THE COUNCIL OF THE EUROPEAN COMMUNITIES: OJ No L 40 of 11. 2. 1989
22. DG III-E3, P.U. Transparency Committee (1999) (Directive 89/105/EEC), 30th March 1999, Summary Report. [cited 6 March 2010], Available from: ec.europa.eu/enterprise/sectors/pharmaceuticals/files/pharmacos/docs/doc99/tc89105_en.pdf
23. European Commission High Level Group on innovation and provision of medicines recommendations for action (2002) [cited 6 March 2010]; Available from: ec.europa.eu/enterprise/sectors/pharmaceuticals/files/phabiocom/docs/g10-medicines_en.pdf
24. The Pharmaceutical Forum (2008) [cited 6 March 2010], Available from: <http://ec.europa.eu/pharmaforum/>
25. Regulation (EC) No 141/2000 of the European Parliament and of the Council of 16 Dec 1999 on orphan medicinal products
26. Haynes B (1999) Can it work? Does it work? Is it worth it? The testing of healthcare interventions is evolving. *BMJ* 319(7211): 652–653
27. Services, U.D.o.h.a.H. Federal Coordinating Council for Comparative Effectiveness Research Report to the President and the Congress (2009) [cited 6 March 2010], Available from: <http://www.hhs.gov/recovery/programs/cer/cerannualrpt.pdf>
28. Core principles on relative effectiveness (2008) [cited 6 March 2010], Available from: ec.europa.eu/pharmaforum/docs/rea_principles_en.pdf
29. Data availability to conduct on relative effectiveness assessments (2008) [cited 6 March 2010], Available from: ec.europa.eu/pharmaforum/docs/rea_data_en.pdf
30. Development of networking and collaboration (2008) [cited 6 March 2010], Available from: http://ec.europa.eu/pharmaforum/effectiveness_en.htm
31. European network for Health Technology Assessment – EUnetHTA [cited 6 March 2010], Available from: www.eunethta.net
32. European Medicines Agency and EUnetHTA Joint Action start collaboration on European Public Assessment Report (EPAR) contribution to relative effectiveness assessments (2010) [cited 6 March 2010], Available from: http://www.eunethta.net/Public/Communication/Press_Releases/European-Medicines-Agency-and-EUnetHTA-Joint-Action-start-collaboration-on-European-Public-Assessment-Report-EPAR-contribution-to-relative-effectiveness-assessments/
33. Overview of the American Recovery and Reinvestment Act of 2009 (Recovery Act) (2010) [cited 6 March 2010], Available from: <http://www.ahrq.gov/fund/cefarr.htm>
34. Steinbrook R (2009) Health Care and the American Recovery and Reinvestment Act. *N Engl J Med* 360(11): 1057–1060
35. The Cochrane Collaboration [cited 6 March 2010], Available from: <http://www.cochrane.org/>

36. Ray WA, Stein CM (2006) Reform of Drug Regulation – Beyond an Independent Drug-Safety Board. *N Engl J Med* 354(2): 194–201
37. NIH-Private Sector Partnership in the Development of Taxol. [cited 6 March 2010], Available from: <http://www.gao.gov/cgi-bin/getrpt?GAO-03-829>
38. Kaplan W, Laing R, and Priority Medicines for Europe and the World (2004) [cited 6 March 2010], Available from: whqlibdoc.who.int/HQ/2004/WHO_EDM_PAR_2004.7.pdf
39. The ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group (2002) Major outcomes in high-risk hypertensive patients randomized to angiotensin-converting enzyme inhibitor or calcium channel blocker vs diuretic: The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). *JAMA* 288(23): 2981–2997

SECTION 2

Clinical Trials

CHAPTER 5

Ethics in clinical research

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1 Development of world-wide standards in clinical research ethics

Physicians engaged in clinical research must address the challenge to determine whether a potential new intervention represents an advance over current methods, whether the new intervention would avoid harms currently incurred, whether it would save lives currently lost. They face the dilemma between the rigorous demands of science necessary to accept the challenge and find the answer and the obligation to deliver individualized and best possible medical care to their patients. The combination of medical research and medical care is a challenging ethical issue and its difficult implications have not been understood for a long time. The dilemma was not addressed, because it was thought that the physician's moral obligation would legitimize his scientific work.

It was a little over 100 years ago when the case of Albert Neisser unmasked the misconception. Graduated from medical school in 1877, Neisser found a job under the well-known physician Oskar Simon at a dermatological clinic in Breslau. Being an outstanding doctor from the start he made at the age of 21 the discovery for which he would become famous – the bacterium responsible for gonorrhea, named after him *Neisseria gonorrhoeae*. In the following two decades Neisser was engaged in research on leprosy, Lupus and in particular syphilis, the public-health-enemy number one in 19th-century Europe. By the turn of the century, Neisser had established himself as a supporter of public-health initiatives. He opposed jailing prostitutes and promoted educating them and the public about sexually transmitted diseases. As a scientist he was impressed and inspired by the successful attempts to develop vaccines against infectious diseases such as rabies (Roux 1885) or diphtheria (Behring 1890). Neisser theorized that the process should work equally well with syphilis. Thus he began inoculating

Keywords: Ethics Committee, ethics, Albert Neisser, Nuremberg Code, declaration of Helsinki, ICH E6 ICH-GCP, Clinical Trial Directive 2001/20/EC

prostitutes, some of whom were minors, by injecting them with an infected serum without their knowledge. The experiment didn't work and many of his subjects came down with the disease. Some of the victims went to trial and caused quite a scandal, though Neisser's colleagues mostly agreed with his practices. He was – incomprehensible from today's point of view – only sentenced to a fine (not because of the damage done to the health of the victims but for not informing them). Politically however the scandal led to the *29 December 1900 Regulation of the Prussian Ministry of Education*. It prohibited all medical interventions for experimental purposes, if the human subject was a minor or not competent. All other interventions required the consent of the human subject in the light of relevant information provided in advance. Thus the Prussian Regulation was among the first such directives to be implemented by the European medical community.

A second important pre-war document addressing ethical issues in clinical research was the *28 February 1931 Reich Circular "Regulations on New Therapy and Human Experimentation"*. It was issued after a scandal involving inoculation of newborns with tuberculosis vaccine at the general hospital of Lübeck [1]. Because of a contamination of the vaccine and lacking experience of the principal investigators 77 children died, and over 100 became ill. The Reich Circular – visionary in its content that is still relevant today – consisted of 14 paragraphs regulating innovative therapy and scientific experimentation. It demanded complete responsibility of the medical profession for carrying out human experiments and explicitly stated that it is the individual physician and the chief physician who are responsible for the well-being of the patient or subject. It also clarified for the first time that, in order to undertake innovative therapy, exploitation of social hardship was incompatible with the principles of medical ethics.

Although the Prussian Act and the Reich Circular addressed the problems of clinical research adequately, they were only national documents. There was no world-wide agreement on how to deal with the issue of clinical research ethics. The development of more generally accepted guidelines dealing with the protection of persons involved in clinical research started not until after World War II as a result of the inhuman Nazi experiments. After the "Doctor's Trial" against Karl Brandt¹ and several others the *Nuremberg Code* was formulated in the year 1947. The Code consisted of 10 points that addressed important principles such as the absolute essentiality of voluntary participation of subjects, informed consent, the right to withdraw, but also issues such as the qualification of the physician, the scientific validity of the project and the risk-benefit assessment. (It is of note that the above mentioned Reich Circular of 1931 contained almost all of the principles cited in the Nuremberg Code).

¹ Karl Brandt (January 8, 1904–June 2, 1948) headed the administration of the Nazi euthanasia programme from 1939 and was selected the personal physician of Hitler in August 1944.

In the same year the Nuremberg Code was written the World Medical Association (WMA) was founded. WMA today has a membership of over 80 national medical associations and represents about 9 million physicians. In 1964 it issued the *Declaration of Helsinki*, one of the most important documents in the history of research ethics as the first significant effort of the medical community to regulate research itself. Although it is not a legally binding instrument, it is widely regarded as the cornerstone document of human research ethics, and physicians engaged in clinical research observe it around the globe. The document has undergone six revisions, one of paramount importance in 1975 when the concept of oversight by an “independent committee” was introduced, thus giving birth to Ethics Committees worldwide. The Ethics Committee of the Medical University of Vienna (then Medical Faculty of the University of Vienna) was founded 3 years later, in 1978.

Another important development regarding clinical research ethics took place in the United States in the wake of the probably most famous unethical post-war clinical study, the “Tuskegee Syphilis Study”. In 1932, prior to the start of World War II, 400 African American males with syphilis had been entered into a study at Tuskegee, Alabama with the intended purpose of documenting the natural history of their disease. However, although by the 1950s penicillin was available and known to be highly effective against syphilis, it was withheld. By the end of the experiment, 28 of the men had died directly of syphilis, 100 were dead of related complications, 40 of their wives had been infected, and 19 of their children had been born with congenital syphilis. The surviving participants were only given treatment in 1972, after the nature of the Public Health Service (PHS)-funded study became publicly known. This was 23 years after publication of the Nuremberg Code.

As a reaction to the scandal the National Commission for the Protection of Human Subjects was created in 1974. This Commission was tasked with studying the ethical principles underlying biomedical and behavioral research on human subjects and to make recommendations to the Congress for the protection of human subjects. The commission produced a number of Reports, the most important issued in the late seventies “Ethical Principles and Guidelines for the Protection of Human Subjects of Research”. It was named the *Belmont Report* [3], for the Belmont Conference Center, where the National Commission met when first drafting the report. It formulates the three fundamental ethical principles for using any human subjects for research:

- respect for persons: protecting the autonomy of all people and treating them with courtesy and respect and allowing for informed consent;
- beneficence: maximizing benefits for the research project while minimizing risks to the research subjects and
- justice: ensuring reasonable, non-exploitative, and well-considered procedures are administered fairly (the fair distribution of costs and benefits to potential research participants).

Today, the Belmont Report continues as an essential reference for Ethics Committees that review research proposals involving human subjects, in order to ensure that the research meets the ethical foundations of the regulations.

The final step of developing world-wide standards in quality of clinical research and research ethics was done with the birth of the International Conference of Harmonization (ICH) at a meeting in April 1990 in Brussels. Representatives of the regulatory agencies and industry associations of Europe, Japan and the USA met to plan an International Conference with the aim to harmonize the requirements and conditions of developing new medicinal products. Topics selected for harmonization were divided into Safety, Quality and Efficacy to reflect the three criteria which are the basis for approving and authorizing new medicinal products.

Guideline ICH E6 (“E” for “Efficacy”), better known als ICH-GCP or *Good Clinical Practice* Guideline represents the global standard for performing clinical research today. It describes the responsibilities and expectations of all participants in the conduct of clinical trials, including investigators, monitors, sponsors and research Ethics Committees. It clearly states in its section “The Principles of ICH GCP” that “Clinical trials should be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki . . .”. In Europe the Clinical Trials Directive (Directive 2001/20/EC) [4], which became effective in 2004, relates to implementation of Good Clinical Practice into European law.

2 Research Ethics Committees today – function and composition

The function of Ethics Committees today is multi-faceted. Primarily established to prevent misconduct in clinical research and to protect patients and healthy volunteers, Ethic Committees also fulfill other roles, two very important objectives being the support of the investigator and his investigational plan, and, secondly, to give public assurance that clinical research is conducted in a transparent and ethical way.

A comprehensive list of tasks assigned to Ethics Committees is given in Directive 2001/20/EC [4]. It lists 11 topics which Ethics Committees have to evaluate in a clinical trial:

- the relevance of the trial,
- its benefits and risks,
- the protocol,
- the suitability of the investigator,
- the quality of the facilities,
- the adequacy of the written information for the patient,
- the provisions of indemnity or compensation in the event of injury,

- insurance to cover the liability of the investigator and sponsor,
- the arrangements for rewarding the investigator and trial subjects,
- relevant aspects of any agreement between sponsor and the site,
- the arrangements for the recruitment of trial subjects,

Given this multitude of tasks it is not surprising that Ethics Committees have to be composed of a number of specialists from various areas, as well as, lay members. Research Ethics Committees in Europe are typically composed of physicians, members from the nursing profession, members with legal expertise, a pharmacist, somebody with ethical expertise, or a philosophical or theological background, a statistician, somebody from a representative patient organization and others. Their main obligation is to review research protocols for clinical trials within a certain timeframe. In many European Member States the review of a research protocol by the Ethics Committee is an integral part of the review of this protocol by the Competent Authority. So Ethics Committees play an even greater role in the evaluation of clinical research.

3 Research Ethics Committees – issues of debate

3.1 Increasing workload

Thirty years ago, when the submission of clinical research projects was scarce, the time of the Ethics Committee for protocol review was limited. However, this has changed drastically over time. In the following the development at Vienna Medical University is given as an example. The graph below (Fig. 1) shows the number of applications at the Ethics Committee over the course of the last 10 years.

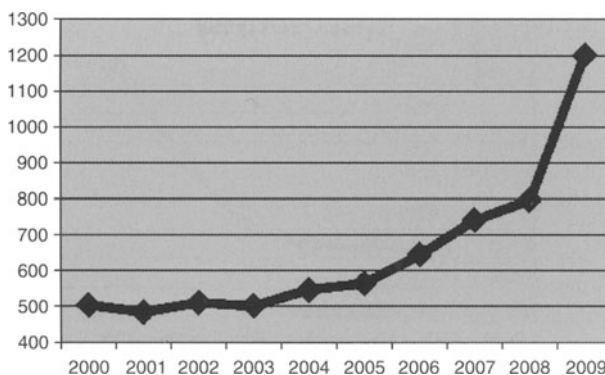


Fig. 1 Number of clinical studies at the Medical University of Vienna in the years 2000–2009

The ethics committee handled 1201 projects in the year 2009, 738 of which were non-interventional projects or projects with minimal risk and minimal burden to the patient. Four hundred and sixty-three projects were more than minimal risk/minimal burden. These latter projects are mostly not only reviewed by the ethics committee members but are subject to additional review by independent experts outside the hospital.

To better handle the large number of study protocols regarded as minimal risk an expedited review process was introduced in March 2004 [5]. The expedited review board is a selected group of IEC members who meet monthly for discussion. A lawyer, a biostatistician and a clinician are permanent members of this board and other specialists are invited as required by the spectrum of trial applications. The appointed reviewers may not reject research applications. If a reviewer would have disapproved the project, it is automatically referred to the standard full review.

It is however not only the number of projects that constantly increases the workload of Ethics Committees. Once a study is approved and has started there is an accompanying flow of reports and notifications comprising a number of issues regarding safety, protocol amendments, administrative changes, updates of various study documents. Of particular impact on the increase in the number of reports was the implementation of the Clinical Trial Directive 2001/20/EC in 2004. In an effort to harmonize pharmacovigilance reports from clinical trials, the Directive has introduced a distinction between suspected unexpected serious adverse reactions (SUSAR), suspected serious adverse reactions and other serious adverse events. Although the intent was to streamline reporting of adverse events, the opposite result was obtained. The graph below (Fig. 2) shows an example. It is obvious that the number of reports and notifications received by the Ethics Committee of the Medical University of Vienna increased by a factor of 3–4 after the introduction of the CT-Directive in 2004.

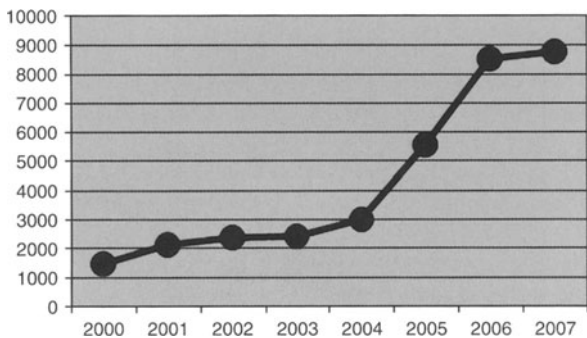


Fig. 2 Number of reports and notifications received by the Ethics Committee of the Medical University of Vienna from 2000 to 2007

Against this background it is conceivable that the role of the ethics committee in safeguarding the well-being of the study participants has become increasingly difficult.

In fact, the European Commission financed in its 7th Framework Programme a one-year project to measure and analyse the direct and indirect impact of the Clinical Trials Directive 2001/20/EC with the aim to determine the most relevant pathways for improvement. All stakeholders in clinical research participated, including academic research organizations and Ethics Committees [6].

4 Compensation for committee members

Except for some Ethics Committees where the members are compensated with a small attendance fee, members are generally working on an honorary basis. The usual argument put forward is that members should not be compensated financially “to avoid any conflicts of interest”. However, looking at the responsibilities and workload involved with work in an ethics committee this argument becomes questionable. For example, the ethics committee of the Medical University of Vienna holds 12 regular meetings per year and another 12 “Expedited Review Meetings” (see above). The estimated total time for a member attending the meetings amounts to about 150 h per year [5]. This does not include time for preparation. Thus, it seems not acceptable to expect unremunerated work in this field. Ethics committees today play an integral role in clinical research, are implemented under various laws and have to perform a highly specialized task. Thus, the hitherto existing attitude towards a remuneration of the persons performing the task may be reconsidered.

In conclusion, Ethics Committees have undergone a 35-year long development. They have grown from small groups of peers voluntarily reviewing protocols of their hospital to institutions implemented under various laws, performing specialized tasks requiring a high level of professionalism.

Case Study: “Roaring sixties” in clinical research – The Beecher Article

The above-mentioned Tuskegee-study of untreated syphilis was not the only example of research to conflict with ethical principles. Nineteen years after the publication of the Nuremberg Code Henry K. Beecher ([7]; Fig. 3) reported 22 examples of research inconsistent with ethics and the Nuremberg Code, but published in the current medical literature. The year of Beecher’s publication, the Public Health Service (PHS) issued a new policy requiring institutional review for

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SPECIAL ARTICLE ETHICS AND CLINICAL RESEARCH*

HENRY K. BEECHER, M.D.†

BOSTON

Fig. 3 Headline of the Beecher Article in 1966

PHS-funded research involving human subjects and laying the procedural foundation for the process of informed consent.

In the following three examples of research described in Beecher's article are given

Effective treatment withheld (example 1 in Beecher's article)

The sulfonamides were for many years the only antibacterial drugs effective in shortening the duration of streptococcal pharyngitis and in reducing its suppurative complications. The investigators in the study took to determine if the occurrence of the serious nonsuppurative complications, rheumatic fever and glomerulonephritis, would be reduced by this treatment. The study was undertaken in spite of the fact that antibiotics, in particular penicillin (available at the time), will prevent these complications. About 500 patients with Group A streptococcus infection were included in the study and treated with a sulfonamide (experimental group) or nonspecific measures ("control group") to see whether rheumatic fever would develop. About 5% of the patients, that is 25 individuals, in both groups (5.4% vs. 4.2%, respectively) developed rheumatic fever. The subjects were not informed, did not consent and were not aware that they had been involved in an experiment.

Willful exposure to toxic doses of drug (example 3 in Beecher's article)

Chloramphenicol is well known to cause aplastic anemia, and it is also known that this toxic effect is related to dose. Nonetheless a study was undertaken to further define the toxicology of the drug. In a double-blind trial on 41 patients doses of 2g versus 6g were tested. Toxic bone marrow depression occurred in 2 of 20 in the 2g-group and in 18 of 21 in the 6g-group. The lower dose was recommended for routine use.

Technical study with unknown risk (example 19 in Beecher's article)

During bronchoscopy a special needle was inserted through a bronchus into the left atrium of the heart. This was done in an unspecified number of subjects, both with cardiac disease and with normal hearts. The technique was a new approach whose hazards were at the beginning quite unknown. The subjects with normal hearts were used, not for their possible benefit but for the benefit of patients in general.

References

1. The Lübeck Trial (1932) *BMJ* 1(3712): 392–393
2. Lemaire F (2006) The Nuremberg doctors' trial: the 60th anniversary. *Intensive Care Med* 32(12): 2049–2052
3. <http://ohsr.od.nih.gov/guidelines/belmont.html>
4. Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001, Article 6; <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2001:121:0034:0044:EN:PDF>
5. Wolzt M, Druml CH, Leitner D, Singer EA (2009) Protocols in expedited review: tackling the workload of ethics committees. *Intensive Care Med* 35(9): 1636–1640 (Epub 2009 June 19)
6. Impact on Clinical Research of European Legislation – ICREL; http://www.efgcp.be/Downloads/confDocuments/Programme_ICREL_2_Dec_2008_final.pdf
7. Beecher HK (1966) Ethics and clinical research. *N Engl J Med* 274: 1354–1360

CHAPTER 6

Good Clinical Practice (GCP) and scientific misconduct

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Good Clinical Practice

1 Introduction

Good Clinical Practice (GCP) is an international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve human subjects.

The ICH–GCP guidelines [1] were developed in order to provide clinical trials with a unified standard across the European Union, Japan and the United States to facilitate the mutual acceptance of clinical data by the regulatory authorities in these jurisdictions. They were adopted at the International Conference on Harmonization (ICH) in 1996. As these are the most generally used, they are the main focus of this chapter.

Compliance with this standard provides public assurance that the rights, safety and well-being of trial subjects are protected consistent with the principles that have their origin in the Declaration of Helsinki, and that the data generated in the trial are valid.

2 Historic background

Knowing about the historic development of clinical research means better understanding of the context of today's clinical research regulatory environment. Many current laws and regulations governing clinical research resulted from a few key events in the history of the drug industry and human subject experimentation, usually associated with very serious consequences. The present day guideline on GCP has evolved through a series of regulation and policy formulations. These are some of the major milestones [2] in the evolution of GCP.

Keywords: Ethics committee, Nuremberg Code, Declaration of Helsinki, ICH–GCP, SOP, SUSAR, quality control

2.1 The Prussian directive and the case of Neisser

The first detailed regulations about clinical research in Western medicine came from the Prussian minister for religious, educational, and medical affairs in 1900. They were issued after critical public discussion and political debate on the Neisser case in the Prussian parliament and set forth the legal basis of disclosure and unmistakable consent.

2.1.1 The Neisser case

In 1898 Albert Neisser, professor of dermatology and venereology at the University of Breslau and discoverer of the gonococcus, published clinical trials on serum therapy in patients with syphilis. In order to find a method of syphilis prevention he injected cell free serum from patients with syphilis into patients who were admitted for other medical conditions. Most of these patients were prostitutes, who were neither informed about the experiment nor asked for their consent. When some of them contracted syphilis, Neisser concluded that the “vaccination” did not work. However, he argued that the women did not contract syphilis as a result of his serum injections but contracted the disease because they worked as prostitutes [3].

2.2 Federal Food and Drugs Act of 1906

By the late 1800s, drug companies were well established and selling thousands of products worldwide, for example Merck (Germany) offered 800 different products in its 1860 catalogue, including quinine, morphine, strychnine and codeine.

In 1898, the Bayer Company sold heroin as “a superior cough suppressant”. By 1899, Bayer was producing about a ton of heroin a year, and exporting the drug to 23 countries.

Patent medicines generated \$75 million in annual sales, Patent medicines were advertised as “miracle cures” and there was more alcohol consumed in patent medicines than sold in liquor stores. The formula for “Peruna”, a popular remedy, was published in early 1900, (1/2 pint of 90% proof spirits, 1.5 pints of water, a flavour cube, a little burned sugar for colour), this spurred the US Congress to pass the Pure Food and Drug Act of 1906 [4]. This Food and Drugs Act of 1906 was the first of more than 200 laws that constitute one of the world’s most comprehensive and effective networks of public health and consumer protections. It required manufacturers to list ingredients contained in their product, and meet the standards of strength and purity established in the United States Pharmacopeia (USP), however, it did not restrict the nature or amount of ingredients.

2.3 The sulfanilamide disaster and the “Food, Drug and Cosmetic Act”, 1938

In 1937, S. E. Massengill, a manufacturer of sulfanilamide tablets, a drug used to treat streptococcal infections, produced liquid version with a sweet raspberry taste [5].

In September, 1937, 240 gallons were shipped across the U.S. By mid-October, the American Medical Association (AMA) had received numerous reports of patients with severe abdominal pain, nausea and vomiting, renal failure and death. One hundred and seven people in 15 states died, including many children. Through the persistence of federal, state, and local health agencies and the effects of the AMA and the news media, most of the elixir was recovered. Of 240 gallons manufactured and distributed, 234 gallons and 1 pint was retrieved; the remainder was consumed and caused the deaths of the victims. It turned out that the compound used to dissolve the tablets into solution was diethylene glycol [6], a deadly poison related to antifreeze. However, the manufacturer had done nothing legally wrong. Therefore the Federal Food, Drug, and Cosmetic Act of 1938 was passed. The FD&C Act completely reformed the public health system. Among other provisions, the law authorized the US Food and Drug Administration (FDA) to demand evidence of safety for new drugs [7].

2.4 Second World War crimes

2.4.1 Unit 731

Unit 731 was a biological and chemical warfare research and development unit of the Imperial Japanese Army that undertook lethal human experimentation during the Second Sino-Japanese War (1937–1945) and World War II. It was responsible for some of the most notorious war crimes carried out by the Japanese.

2.4.2 Nazi experiments

Nazi human experimentation was medical experimentation on large numbers of people by the German Nazi regime in its concentration camps during World War II. Prisoners were coerced into participating; they did not willingly volunteer and there was never informed consent. Quite often the study endpoint was death of the study subject, or the experiments resulted in disfigurement or permanent disability (e.g. Dr. Josef Mengele “Dr. Auschwitz” (1911–1979) twin experiments, Dr. Herta Oberheuser (1911–1978) Sulfonamide-experiments . . .).

2.5 Nuremberg trial and Nuremberg Code

In 1946, the military trial (Nazi Doctors’ Trial) in Nuremberg, Germany, were performed with guilty verdicts for 15 out of 23 defendants, seven received death sentences. In their final judgment, the justices presiding at the trial concluded that human experimentation was necessary for the advancement of medical knowledge, but only if done consistent with the principles they articulated in what has come to be known as the Nuremberg Code.

The Nuremberg Code includes ten principles to guide physician investigators in experiments involving human subjects. These principles, particularly the first principles on voluntary consent, were primarily based on legal concepts because medical codes of ethics existent at the time of the Nazi atrocities did not address consent and other safeguards for human subjects [2].

The pivotal principles are:

- Voluntary consent of the subject must be obtained
- Prior animal experimentation to determine risk must be performed
- Investigators must be qualified medical personnel

The Nuremberg Code was adopted by the United Nations in 1948 and was recognized internationally as a guide to medical research. Although it did not carry the force of law, the Nuremberg Code was the first international document which advocated voluntary participation and informed consent.

2.6 Thalidomide (contergan) tragedy

Beginning in 1959, West German physicians began to prescribe thalidomide to relieve morning sickness and insomnia and 5000 Babies in Germany were born with birth defects.

Dr. Frances Kelsey at the FDA delayed approval of thalidomide by asking the sponsor for more information about neuritis as a possible side effect, but still 2.5 million tablets were distributed as samples to 1270 US physicians. 200,000 American patients received thalidomide, and by the mid-1960s, 10,000 birth defects had occurred worldwide.

In the USA the Kefauver-Harris Amendments of 1962 were inspired by the world wide thalidomide tragedy and the rules for drug safety were strengthened. It was required for drugs to be proven effective as well as safe prior to marketing [7]. The thalidomide tragedy also triggered the development of the Declaration of Helsinki [8].

2.7 Declaration of Helsinki [6]

In 1964, the World Medical Association established recommendations guiding medical doctors in biomedical research involving human subjects. The declaration governs international research ethics and defines rules for “research combined with clinical care” and “non-therapeutic research” [9]. The Declaration of Helsinki is the basis for Good Clinical Practices used today.

3 Development of Good Clinical Practice Guidelines

The term “Good Clinical Practice” Guidelines was first introduced in the late 1980s, the CPMP (Committee for Proprietary Medicinal Products), now CHMP (Committee

for Medicinal Products for Human Use), is responsible for preparing the opinions on all questions concerning medicinal products for human use for the European Medicines Agency (EMA) adopted in 1990 the note for guidance: GCP for Trials on Medicinal Products in the EC [10]. In July 1991 Directive 91/507/EEC modifying 75/318/EEC was issued. It requires the proof of quality, safety and efficacy to the latest state of the art [11]. Further developments led to the Council for International Organizations of Medical Sciences (CIOMS) and World Health Organization (WHO) Guidelines on GCP in 1993 [12], which were followed by the GCP–International Conference of Harmonization (ICH) guideline in 1996 [13]. These ICH–GCP guidelines were developed in order to provide clinical trials with a unified standard across the European Union, Japan and the United States.

In 2001 the European Parliament approved the EU directive 2001/20/EEC 2001 [14] on clinical trials, which was intended to harmonize the principles on conducting clinical trials by making the standards of GCP law in the EU. All member states had to comply with this legislation till May 2004 [15]. So the adherence to ICH–GCP is now a legal requirement within the EU.

4 International Conference of Harmonization (ICH [16])

ICH is a joint initiative (involving both regulators and industry as equal partners) in the scientific and technical discussions of the testing procedures which are required to ensure and assess the safety, quality and efficacy of medicines and consists of six parties that are directly involved, as well as three Observers and the International Federation of Pharmaceutical Manufacturers & Associations (IFPMA). The six parties are the founder members of ICH which represent the regulatory bodies and the research-based industry in the European Union, Japan and the USA. The Observers are WHO, EFTA, and Canada (represented by Health Canada). This group of non-voting members acts as a link between the ICH and non-ICH countries and regions.

4.1 ICH parties

4.1.1 European Commission – European Union (EU)

The European Commission represents all members of the EU. The Commission works through harmonization of legislation and technical requirements and procedures, to achieve a single market in pharmaceuticals to allow free movement of products throughout the EU.

The European Medicines Agency (EMA) has been established by the Commission and is situated in London. Scientific support for ICH activities is provided by the EMA and its Committee for Medicinal Products for Human Use (CHMP) of the EMA.

4.1.2 European Federation of Pharmaceutical Industries and Associations (EFPIA)

EFPIA is situated in Brussels and has, as its members, 29 national pharmaceutical industry associations and 45 leading pharmaceutical companies involved in the research, development and manufacturing of medicinal products in Europe for human use.

4.1.3 US Food and Drug Administration (FDA)

The US Food and Drug Administration has a wide range of responsibilities for drugs, biologicals, medical devices, cosmetics and radiological products. As the largest of the world's drug regulatory agencies FDA is responsible for the approval of all drug products used in the USA.

4.1.4 Pharmaceutical Research and Manufacturers of America (PhRMA)

The Pharmaceutical Research and Manufacturers of America – PhRMA – represents the research-based industry in the USA.

4.1.5 Ministry of Health, Labour and Welfare, Japan (MHLW)

The Ministry of Health, Labour and Welfare has responsibilities for approval and administration of drugs, medical devices and cosmetics in Japan.

4.1.6 Japan Pharmaceutical Manufacturers Association (JPMA)

JPMA represents 75 members (including 20 foreign affiliates) and 14 committees. Membership includes all the major research-based pharmaceutical manufacturers in Japan.

4.2 ICH observers

The observers act as a link with non-ICH countries and regions. The ICH observers are:

- The World Health Organization (WHO)
- The European Free Trade Association (EFTA), currently represented at ICH by Swissmedic Switzerland
- Canada, represented at ICH by Health Canada
- The International Federation of Pharmaceutical Manufacturers & Associations (IFPMA) is a non-profit, Non-Governmental Organization (NGO) representing national industry associations and companies from both developed and developing countries.

4.3 ICH Steering Committee

ICH is administered by the ICH Steering Committee which is supported by the ICH Secretariat. Since the beginning, each of the six co-sponsors has had two seats on the ICH Steering Committee (SC) which oversees the harmonization activities. IFPMA provides the Secretariat and participates as a non-voting member of the Steering Committee. The ICH Observers, WHO, Health Canada, and the European Free Trade Association (EFTA) nominate non-voting participants to attend the ICH Steering Committee Meetings.

5 ICH TOPIC E6, Note of Guidance for Good Clinical Practice (CPMP/ICH/135/95) – the Principles of ICH GCP [1]

ICH-GCP follows two main goals:

- To protect the rights, safety and welfare of humans participating in research
- To assure the quality, reliability and integrity of data collected

The original ICH-GCP-document states the following principles:

1. Clinical trials should be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with GCP and the applicable regulatory requirement(s).
2. Before a trial is initiated, foreseeable risks and inconveniences should be weighed against the anticipated benefit for the individual trial subject and society. A trial should be initiated and continued only if the anticipated benefits justify the risks.
3. The rights, safety and well-being of the trial subjects are the most important considerations and should prevail over interests of science and society.
4. The available nonclinical and clinical information on an investigational product should be adequate to support the proposed clinical trial.
5. Clinical trials should be scientifically sound, and described in a clear, detailed protocol.
6. A trial should be conducted in compliance with the protocol that has received prior institutional review board (IRB)/independent ethics committee (IEC) approval/favourable opinion.
7. The medical care given to, and medical decisions made on behalf of, subjects should always be the responsibility of a qualified physician or, when appropriate, of a qualified dentist.

8. Each individual involved in conducting a trial should be qualified by education, training, and experience to perform his or her respective task(s).
9. Freely given informed consent should be obtained from every subject prior to clinical trial participation.
10. All clinical trial information should be recorded, handled, and stored in a way that allows its accurate reporting, interpretation and verification.
11. The confidentiality of records that could identify subjects should be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s).
12. Investigational products should be manufactured, handled, and stored in accordance with applicable good manufacturing practice (GMP). They should be used in accordance with the approved protocol.
13. Systems with procedures that assure the quality of every aspect of the trial should be implemented.

6 The three “main players” of ICH–GCP – Ethics Committee, Investigator and Sponsor

6.1 Institutional review board/independent Ethics Committee (IRB/IEC)

The main responsibilities of any Ethics Committee are to safeguard the rights, safety, and well-being of all trial subjects and to pay special attention to trials that may include vulnerable subjects. The role of the Ethics Committee is described in detail in Chapter 5.

6.2 Investigator

ICH–GCP defines an investigator as “a person responsible for the conduct of the clinical trial at a trial site. If a trial is conducted by a team of individuals at a trial site, the investigator is the responsible leader of the team and may be called the principal investigator” [1].

In the Declaration of Helsinki, the description of the duties are “to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects” [17].

6.2.1 Investigator’s qualifications and agreements

The investigator should be qualified by education, training, and experience to assume responsibility for the proper conduct of the trial. He should meet all regulatory requirements (e.g. board certified physician) and has to provide evidence of his qualifications.

The investigator should be thoroughly familiar with the use of the investigational product as described in the protocol, and in the Investigational Brochure (IB), which in practice means that he actually has to read the protocol and the IB. Of course he has to be aware of and comply with GCP, which also includes the permission to allow monitoring and auditing by the sponsor and inspecting by the appropriate regulatory authorities.

As the investigator usually cannot perform all of his duties alone, he has to delegate some of his responsibilities. This is acceptable in view of the GCP requirement, but he has to maintain a list of appropriately qualified persons to whom he has delegated significant duties. He has to clearly define and document the responsibilities of all study staff and has to ensure, that all persons involved are adequately informed.

6.2.2 Adequate resources

The investigator has to have adequate resources to perform the clinical trial. That means not only adequate numbers of well trained staff, but also adequate study equipment, which has to be suitable, available, maintained and calibrated.

This requirement also includes that the Investigator should be able to demonstrate a potential for recruiting the required number of suitable subjects within the agreed recruitment period.

One aspect, that quite often creates a problem between investigators and sponsor is that investigators tend to overestimate the number of their patients that can be enrolled in a trial. It is advisable, to look, for example, through the outpatients' records and check each individual patient against the in- and exclusion criteria, to see who would really be eligible for the trial.

6.2.3 Medical care of trial subjects

The investigator's main responsibility is the medical care of the trial subjects; this obligation can lead to the "investigator's dilemma" – "As a clinician, the investigator has duties to provide the patient with optimal care and undivided loyalty. As a scientist, the investigator has duties to follow the rules, procedures and methods described in the protocol" [18]. However the well-being of the individual research subjects takes precedence over any research questions [17].

The investigator should

- ... ensure that adequate care is provided in case of adverse events and intercurrent illnesses (potential conflict with sponsor!)
- ... inform the subject's primary physician
- ... make reasonable effort to ascertain reasons if subject withdraws prematurely

6.2.4 Communication with IRB/IEC

Before start of the trial, a written and dated positive opinion of the ethics committee regarding the study protocol, the written informed consent form and the recruitment procedure is absolutely mandatory.

6.2.5 Compliance with protocol

The signature of the investigator on the protocol is the formal agreement to adhere to the protocol, so the investigator should only sign, when he is in full agreement with the protocol.

There should be no deviation of the protocol without agreement by the sponsor and approval of the Ethics Committee,

- ... except to eliminate immediate hazards to trial subjects
- ... or only logistic or administrative aspects (Change of phone number ...).

6.2.6 Investigational product(s)

The responsibility for investigational products at the trial site rests with the investigator, he has to

- ... maintain records of the product's delivery to the trial site, the inventory at the site, the use by each subject, and the return to the sponsor or alternative disposition of unused product(s).
- ... assure proper storage store.
- ... assure use only in accordance with approved protocol.

6.2.7 Randomization procedures and unblinding

Correct handling according to the protocol of the above mentioned procedures are also the sole responsibility of the investigator, unblinding should only be performed to avoid immediate danger for the study subjects. Premature unblinding of ongoing trials for example for commercial purposes could compromise the integrity of these studies [19].

6.2.8 Informed consent of trial subjects

Informed Consent is “a process by which a subject voluntarily confirms his or her willingness to participate in a particular trial, after having been informed of all

aspects of the trial that are relevant to the subject's decision to participate. Informed consent is documented by means of a written, signed and dated informed consent form" [1].

The investigator is primarily responsible for the ethics and practice of informing persons about their participation in research. No study related procedures can be performed without a signed Informed Consent form!

The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never interfere with the patient-physician relationship [17].

ICH-GCP states: "Neither the investigator, nor the trial staff, should coerce or unduly influence a subject to participate or to continue to participate in a trial" [1].

The investigator has to be careful not to influence the subject in any way. Even if he personally thinks, that the patient would benefit from participation in the planned trial, he may not express this. Here also the question of payment of study subjects needs to be raised.

6.2.9 Should study participants receive payment?

It used to be common practice to pay subjects for participating in research studies, and this practice remains one of the most controversial methods of recruitment [20], the ethical issues about payment are still in discussion. "The predominant concern expressed is that payment of subjects might represent 'undue inducement', by leading to a decrease in either the voluntariness or the understanding with which subjects agree to participate . . . A second concern is that the payment of subjects may result in economically disadvantaged populations' bearing an unduly large share of the risks and burdens of research participation" [21].

"Whether an inducement is an undue influence depends on the amount of the financial incentive, the risk involved in the study, and the financial need of the subject. Excessive payment may limit the volunteers' ability to assess the risk of the experiment and inhibit their freedom to make a sound decision about participation [22].

This discussion is not new, in 1900, the renowned American military surgeon Walter Reed (1851–1902) paid volunteers \$100 in gold for their participation in studies of yellow fever, with an additional bonus of \$100 for the volunteers' heirs in the event of their death [21]. Even with the widespread payment and use of healthy volunteers, currently no consensus regarding the ethical payment of subjects can be found in the literature, federal regulations, or professional guidelines. Concern has been raised about the lack of guidance and dialogue with respect to payment of volunteers [22].

6.2.10 Should there be a different standard for paying healthy subjects as opposed to patient-subjects?

It is quite often assumed that it is legitimate to pay healthy subjects but not patient-subjects for their participation in research [23]. Healthy subjects are usually only motivated by money to participate in research, as they receive in general no benefit from participation. Paying money to healthy volunteers is widely accepted, although concerns about undue inducement and distributive justice may still persist [24]. The amount of money expected by healthy volunteers is based on each subject's perception of study burden and associated risk [25]. The ethics' committee of the Medical University Vienna generally demands, that healthy volunteers receive payment according to the burden on time and other inconveniences (like number of needle punctures, or examinations like gastroscopy), but not for risk.

In contrast, patients in research studies are often considered more vulnerable than healthy subjects, because of the nature of the relationship with their doctor and because of possible confusion about the difference between participation in clinical research and the receipt of clinical care – the “therapeutic misconception” [26]. Payment may not be necessary for recruiting patients for research, especially if they are motivated by an opportunity for therapeutic benefit. However, it is often accepted to compensate patients for their time, to reduce the financial sacrifice that research subjects have to make [24]. If the goal of payment is to show appreciation for their contribution, “patient-subjects are equally deserving and should be paid comparably to healthy subjects” [24].

In case of a non therapeutic research on patients, when the patient does not even have a chance of a benefit, then the patients should be paid as if they were healthy subjects.

6.2.11 Patient information

Before informed consent may be obtained the investigator should fully inform the subject or subject's legally acceptable representative of all pertinent aspects of the trial. This information must be written and oral, and the formulation and wording should be easily understandable.

The patient information must contain information about:

- The purpose of the trial
- Trial procedure
- Randomization (“flip a coin”)/blinding/placebo
- Experimental methods
- Study (scientific and medical section of protocol): number of subjects, duration and design

- Subjects responsibilities
- Risks and inconveniences
- Expected benefits (If there is no intended benefit, the patient has to be informed!)
- Individual vs. general benefit of participation
- Alternative treatment options (risks and benefits)
- Concomitant medication, life-style modifications (diet, sport, handling of machinery)
- Insurance (Insurance company, policy number, contact address, Insurance exclusions (protocol violations by subjects, e.g. concealed medical information or treatments))
- Compensation and payment of subjects
- Confidentiality: Monitor, Auditor, Ethics committee and regulatory authorities will be granted access to the subjects' medical record for verification of clinical trial data, by signing a written informed consent form, the subject is authorizing such access, but the Subject Identification list will be kept confidential. If results are published, the subjects' identity will remain confidential.
- Contact-information for discussion of further details
- Emergency measures (office and mobile contact no)
- Information of third parties (referring physician)?

The most important information on any informed consent form:

Participation in the clinical trial is

- Voluntary
- Can be discontinued at any time
- No disadvantages from withdrawal of consent

6.2.12 The informed consent process

The informed consent process begins with an interview and continues through the study, at each study visit the study subjects should be asked again, if they are still willing to participate. As soon as new information regarding the study becomes available, the participants need to be informed and the consent confirmed. Participation in research must begin as a voluntary activity and remain voluntary. The informed consent process is only then finished, when the study is closed and final reports are issued!

Persons who are vulnerable may not be able to consent freely, and require special protections in the informed consent process.

Vulnerable Populations protected in the regulation are:

- Children and wards of the state
- Prisoners
- Pregnant women and fetuses

Researchers should obtain informed consent from both the pregnant woman and the father, the consent of the father is not necessary if:

- The purpose of the study is to meet the health needs of the mother.
 - The identity or whereabouts of the father can not be reasonably ascertained.
 - The father is not reasonably available.
 - The pregnancy is the result of rape.
- Cognitively impaired persons

Other accepted vulnerable populations are for example, persons, that only speak a foreign language, illiterate persons, financially impaired persons and terminally ill patients.

6.2.13 Records and reports

Accurate and extensive reporting is a major aspect of ICH-GCP to ensure the trustworthiness of the data. Even minor steps need to be documented for transparency, if it is not documented, it is considered not done.

“The investigator should ensure accuracy, legibility and timeliness of the data reported in the CRF . . .” Case Report Form – is a “printed or electronic document, designed to record all of the protocol required information to be reported to the sponsor on each trial subject”.

All, even minor steps involved in data collection and management must be recorded, documented and confirmed in writing (e.g. temperatures in the refrigerator . . .).

6.2.14 Case record form (CRF)

To standardize the documentation of all trial related procedures and results it is mandatory to use appropriate CRFs

- One per subject
- Contains data and other information about each included subject – according to protocol
- Serves as a means for analysis and standardization of obtained data – Source Data

Design of a CRF (minimal requirements)

- Identification under the rules of data-protection laws
- Date, place, study identification

- demographic, ethnic data
- characteristics (smoker, diet . . .)
- Diagnosis, indication for study drug
- Fits entry criteria
- mode of treatment (single dose, daily dose, duration, . . .)
- observation periods
- concomitant medication
- Registration of time points with signature
- Registration of Adverse Events and serious Adverse events
- Start and end of observation period

Data reported on the CRF should be consistent with the Source Data, Any change or correction should be dated, initialled, and explained and should not obscure original entry.

Example:

BP 130/87 ~~130/78~~ Corr. NN Error dd.mm.yyyy

6.2.15 Archiving

All trial related documents need to be archived, the regulations vary from country to country, a minimum of 2 years is required following market approval in the EU or 2 years following development stop. Occasionally prolongation can be legally required or is required by the sponsor. If no regulatory submission was performed, the documents must be stored for at least 5 years after completion. It is recommended to plan for archiving as soon participation in a study is considered.

6.2.16 Progress reports

At least once a year the investigator has to submit a progress report to the ethics committee.

6.2.17 Safety reporting

Accurate safety reporting is a fundamental component of the investigators responsibility to protect trial subjects and future patients.

6.2.18 Serious adverse event

All serious adverse events (SAEs) should be reported immediately to the sponsor [16] and once a year to the Ethics committee.

A serious adverse event is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment and that

- Results in death
- Is life threatening
- Requires or prolongs hospitalization
- Results in significant disability
- Is a congenital abnormality/birth defect

6.2.19 Suspected unexpected serious adverse event (SUSAR)

What is a SUSAR?

- Suspected – probable cause
- Unexpected – not mentioned in reference document
- Serious – ICH criteria
- Adverse – unintended
- Reaction – suspected response

SUSARs are entered into a clinical trial module of the Eudravigilance database, thus creating a single overall database for European regulatory authorities covering clinical trial safety reporting and post-marketing safety reporting [27]. This database is needed to facilitate the review of the safety of the use of these products in the clinical trials. SUSARs have to be reported immediately to the sponsor and if life threatening within 7 otherwise 15 days to the IRB/IEC and the authorities.

6.2.20 Causality assessment

It is usually requested not only to report SAEs and SUSARs, but also to perform a causality assessment.

- Certain – plausible time relationship, response to withdrawal/dechallenge/rechallenge procedure
- Probably/likely – reasonable time sequence to drug intake, response to withdrawal/dechallenge
- Possible – reasonable time sequence could be explained by the underlying disease or another drug
- Unlikely – improbable time relationship disease/other drugs give plausible explanation

6.2.21 Premature termination or suspension of a trial

If a trial is prematurely terminated or suspended for any reason, the investigator/institution should promptly inform the trial subjects, should assure appropriate therapy and follow-up for the subjects, and, where required by the applicable regulatory requirement(s), should inform the regulatory authority(ies) [16].

6.2.22 Final reports

ICH-GCP only demands, that “upon completion of the trial, the investigator, where applicable, should inform the institution; the investigator/institution should provide the IRB/IEC with a summary of the trial’s outcome, and the regulatory authority(ies) with any reports required” [16].

The 2008 version of the “Declaration of Helsinki” [8] goes further and states that “Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available”.

6.3 Sponsor

Per definition the Sponsor is “an individual, company, institution, or organization which takes responsibility for the initiation, management, and/or financing of a clinical trial” [1].

Especially in academic research it sometimes becomes necessary, that the investigator also takes over the responsibility of the sponsor and is then called the “sponsor-investigator”. The obligations of a sponsor-investigator include both those of a sponsor and those of an investigator.

Prior to initiating a trial, the sponsor should:

- define, establish, and allocate all trial-related duties and functions
- utilize qualified individuals (e.g. biostatisticians, clinical pharmacologists, and physicians) throughout all stages of the trial process
- designate appropriately qualified medical personnel, who will be readily available to advise on trial-related medical questions or problems (including outside consultants if necessary)

6.3.1 Quality assurance and quality control

Implementing and maintaining quality assurance (QA) and quality control (QC) systems for clinical trials is essential for sponsors to assure the integrity and reliability of clinical trials and the data obtained from clinical trials. The sponsor is responsible for preparing standard operating procedures (SOPs) for QA and QC systems or to verify, that the use of appropriate SOPs are established at the trial site. Standard Operating Procedures (SOP) are detailed written instruction to achieve uniformity of the performance of a specific function (e.g. blood draw, RR-measurement, spinning down of blood samples . . .).

The sponsor is also responsible for monitoring by way of source data verification, and auditing. These provisions imply that the sponsor is ultimately responsible for conducting and managing clinical trials, and the sponsor's activities must be supported by detailed SOPs, training and education of the monitors, and consensus on the auditing method must be pursued by auditors.

A sponsor may transfer any or all of the sponsor's trial-related duties and functions to a Contract Research Organization (CRO), but the ultimate responsibility for the quality and integrity of the trial data always resides with the sponsor.

6.3.2 Additional responsibilities of the sponsor

These involve trial design, trial management, data handling, and record keeping; he is responsible for the selection of the investigator and the allocation of responsibilities. All aspects regarding the investigational product, including manufacturing, packaging, labelling and coding, supplying and handling and the ongoing safety evaluation of the Investigational Product(s) are also the sponsor's duty. As all these aspects are self explanatory in the ICH-GCP Guidelines [1], they are not further discussed here.

7 Deficiencies of GCP

7.1 Is ICH-GCP just a “bronze standard”? [28]

Critical voices mention several deficits of the ICH-GCP Guidelines. First of all it might be said that the term “Good Clinical Practice” is a misnomer as it does not relate to clinical practice at all, but, rather, to the conduct of clinical research [28].

Although the guideline's goals of documenting informed-consent, safety of participants, and integrity of data are worthy, its development process is said to be based on the weakest approach of Guideline development, informal consensus [29], instead of evidence based guideline development. Also the document has no identified authors or

contributors and the scientific basis of its recommendations is not known. It is generally agreed that guidelines need to be updated regularly [30], ICH-E6 has not been updated since 1996, and no timetable for revision is specified.

7.2 ICH–GCP and academic research

The regulatory burden of ICH–GCP is said to obstruct high quality science and might have become the biggest single threat to research carried out in academia [31]. The EU Clinical Trials Directive must also take some blame. Set up in 2004 to improve the quality and safety of trials and to harmonize and simplify application processes across Europe, it has been heavily criticized by academics [32].

McMahon et al. [33] raised serious concerns, “that the onerous procedural requirements for data management and documentation stipulated by ICH are deterring academic research where registration of a new pharmaceutical entity is not an objective. The rigid bureaucracy of GCP as defined by ICH has already been recognized as an impediment to clinical research . . .”, especially as the ICH guideline on GCP provides extremely detailed instructions on data management and reporting of trials. Also this system of regulatory bureaucracy in clinical trials has increased costs dramatically, but some “aspects of clinical trials regulatory structure, such as monitoring/auditing review and adverse event reporting may constitute a waste of money and resources. Misdirected data collection and adverse events reporting divert valuable resources and hamper development of large, simple clinical trials powered to definitively answer important research questions. Careful scrutiny of the utility of current or proposed regulatory schemes is required to ensure the integrity of human subjects’ research and to enhance the effectiveness of research dollars” [34].

8 Summary

Despite several misgivings ICH–GCP is another important milestone in the development of clinical research. ICH–GCP helps to ensure the safety of clinical trial subjects and that the data collected from clinical studies can be relied upon and thus protecting future patients.

Research misconduct

“It seems paradoxical that scientific research, in many ways one of the most questioning and sceptical of human activities, should be dependent on personal trust. But the fact is that without trust the research enterprise could not function”, is a famous quote by Arnold Relman, Editor, NEJM, 1983. Research fraud undermines the scientific enterprise and corrodes trust both among scientists and between scientists and the public [35].

1 What is research misconduct?

The Joint Consensus Conference on Misconduct in Biomedical Research [36] was convened in 1999 in order to debate, address and offer guidance on key questions because “every single case [of fraud and misconduct] reduces public confidence, abuses the use of public and charitable funds, and causes insult and frustration to the vast majority of careful, honest workers”.

The following definition was agreed upon [36]:

“Behaviour by a researcher, intentional or not, that falls short of good ethical and scientific standards”.

Effective June 16, 2005, the United States Public Health Service, which administers its integrity programme through the Office of research integrity (ORI) defined research misconduct as [37]:

“Fabrication, falsification, or plagiarism, in proposing, performing or reviewing research, or in reporting research results”.

2 Forms of research misconduct

- Honest mistakes
- Gift authorship, guest authorship, ghost-writing and exclusion of rightful authors
- Plagiarism: appropriation of another person’s ideas, processes, results or words without giving appropriate credit
- Undeclared post hoc sub group analyses
- Withholding of unfavourable data
- Fabrication and falsification of data

- Fabrication: inventing of patients and making up of data or results and recording or reporting them.
- Falsification: Manipulating research materials, equipment or processes, or changing or omitting data or results such that the research is not accurately represented in the research record.
- Unethical treatment of research subjects.

3 How common is research misconduct or fraud?

Usually professionals and the public focus on headline-grabbing cases of scientific misconduct, but researchers should not longer ignore a wider range of questionable behaviour that threatens the integrity of science. In a survey published in nature [38], the authors surveyed several thousand early- and mid-career scientists, who are based in the United States and funded by the National Institutes of Health (NIH), and asked them to report their own behaviours.

| Self reported misbehaviour [38] <i>n</i> = 3247 | |
|---|--|
| 0.3% | Scientists falsifying or “cooking” research data |
| 0.3% | Ignoring major aspects of human-subject requirements |
| 0.3% | Not properly disclosing involvement in firms whose products are based on one’s own research |
| 1.4% | Relationships with students, research subjects or clients that may be interpreted as questionable |
| 1.4% | Using other scientist’s ideas without obtaining permission or giving due credit |
| 1.7% | Unauthorized use of confidential information in connection with one’s own research |
| 6.0% | Failing to present data that contradict one’s own previous research |
| 7.6% | Circumventing certain minor aspects of human-subject requirements |
| 12.5% | Overlooking others’ use of flawed data or questionable interpretation of data |
| 15.5% | Changing the design, methodology or results of a study in response to pressure from a funding source |
| 4.7% | Publishing the same data or results in two or more publications |
| 10.0% | Inappropriately assigning authorship credit |
| 10.8% | Withholding details of methodology or results in papers or proposals |
| 13.5% | Using inadequate or inappropriate research designs |
| 15.3% | Dropping observations or data points from analyses based on a gut feeling that they were inaccurate |
| 27.5% | Inadequate record keeping related to research projects |

4 Conclusion

Every case of misconduct is difficult for those accused, for those making the allegations, for the institutions involved, for the funding agencies, and for the profession. Public esteem for science and scientists can only be harmed when ego and career are valued more highly than the accuracy of the scientific literature and the welfare of the public [39]. Each and every one of us can contribute to fight research misconduct and fraud by being 100% honest ourselves.

Case Study: Medical research in a global world

On the 17th of Dec. 2000, the Washington Post [40] brought the story of a 1996 medical experiment conducted by Pfizer researchers in Kano, Nigeria, during a major meningitis epidemic.

The Story of the girl No. 6587-0069

- She was 10 years old and weight only 41 pounds. She lived in Nigeria, and in April 1996 she suffered from meningitis.
- Somehow the girl found help: “Doctors Without Borders” had erected a treatment centre solely in an effort to save lives.
- Researchers for Pfizer Inc. had set up a second centre.
- They were using Nigeria’s meningitis epidemic to conduct experiments on children with what Pfizer believed was a promising new antibiotic – a drug not yet approved in the United States.
- Doctors working with Pfizer drew spinal fluid from the girl, gauged her symptoms.
- They gave her 56 mg of Trovan.
- A day later, the girl’s strength was evaporating, Pfizer records show, and one of her eyes froze in place.
- On the third day, she died.
- Pfizer records are explicit.
- Action taken: “Dose continued unchanged”.
- Outcome: “Death”.

The full picture during an epidemic of meningokokkal meningitis in Nigeria in 1996, Pfizer sent physicians to the Kano Infectious Diseases Hospital to conduct a study involving 200 sick children, comparing the efficacy of its new oral antibiotic trovafloxacin (Trovan) with the FDA-approved antibiotic ceftriaxone

(Rocephin). Trovan had never been tested in children in its oral form [41]. The open label phase 3 trial, in which half the children were given Trovan and the other half received a low dose of Rocephin, was conducted over a 2-week period, and then allegedly the Pfizer team abruptly left. According to the families, “the tests caused the deaths of eleven children, five of whom had taken Trovan and six of whom had taken the lowered dose of ceftriaxone, and left many others blind, deaf, paralyzed, or brain-damaged” [42]. The story in the Washington Post 40 described the slow death of a 10-year-old girl known only as Subject 6587-0069 [43]. The researchers, who were working for Pfizer, monitored her dying without modifying her treatment, following the protocol designed to test their antibiotic Trovan (trovafloxacin) in children.

After the exposé was published, the families of the Kano subjects brought suit against Pfizer in Nigeria and, later, in the United States, charging the company with conducting medical experiments without informed consent [43].

The central allegation is that Pfizer “failed to secure the informed consent of either the children or their guardians and specifically failed to disclose or explain the experimental nature of the study or the serious risks involved” or “to inform them that alternative treatment proven to be effective was immediately available from Médecins sans Frontières at the same facility” [42].

In Feb. 26, 2009 AFP announced that the US drug giant Pfizer has agreed to settle a multi-billion dollar damages case with 200 alleged victims of a drugs trial in Kano, in northern Nigeria [44].

Conclusion

The globalization of clinical research is a relatively recent phenomenon. Glickman et al. reviewed 300 articles reporting the results of clinical trials in the New England Journal of Medicine (NEJM), the Lancet, and the Journal of the American Medical Association (JAMA) in 1995 and 2005 and found that the number of countries serving as trial sites outside the United States more than doubled in 10 years [45], thus the increasing globalization of clinical research trials calls for more effective ethical and legal rules to protect both research subjects and scientific integrity [45].

Acknowledgement

“Most people say that it is the intellect which makes a great scientist. They are wrong: it is character”. Albert Einstein.

References

1. <http://www.ema.europa.eu/pdfs/human/ich/013595en.pdf>
2. Abraham S, Grace D, Parambi T, Pahuja S (2009) Milestones in development of good clinical practice. *Internet J Health* 9(1)
3. Vollmann J, Winau R (1996) Consent in human experimentation before the Nuremberg Code. *BMJ* 313: 1445–1447
4. http://www.fohbc.com/PDF_Files/Peruna_JSullivan.pdf
5. http://www.gmptrainingsystems.com/files/u1/pdf/Sulfanilamide_article.pdf
6. Medicine: Post-Mortem. *Time* magazine. December 20, 1937. <http://www.time.com/time/magazine/article/0,9171,758704,00.html>
7. <http://www.fda.gov/RegulatoryInformation/Legislation/default.htm>
8. <http://www.wma.net/en/30publications/10policies/b3/index.html>
9. <http://research.unlv.edu/OPRS/history-ethics.htm>
10. CPMP Working Party on Efficacy of Medicinal Products Note for Guidance: Good Clinical Practice for Trials on Medicinal Products in the European Community (1990) CB-55-89-706-EN-C
11. Anhalt E (1993) The development of good clinical practice in the EEC and in Germany. *Methods Find Exp Clin Pharmacol* 15(4): 217–222
12. No-authors, International Ethical Guidelines for Biomedical Research Involving Human Subjects. Prepared by the Council for International Organizations of Medical Sciences (CIOMS) in collaboration with the World Health Organization (WHO). http://www.cioms.ch/frame_guidelines_nov_2002.htm
13. No-authors, ICH–GCP; <http://www.emea.eu.int/pdfs/human/ich/013595en.pdf>. 1997
14. European Parliament. Directive 2001/20/EC of the European Parliament and Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the member states relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use. *Official J European Commun* 2001(121): 34–44
15. Jørgensen A, Bach KF, Friis K (2004) Good Clinical Practice is now Obligatory in Academic Clinical Drug Research in the European Union *Basic & Clinical Pharmacology & Toxicology*, 94: 57–58
16. <http://www.ich.org/cache/comp/276-254-1.html>
17. <http://www.wma.net/en/20activities/10ethics/10helsinki/index.html>
18. Resnik DB (2009) The clinical investigator-subject relationship: a contextual approach. *Philosophy, Ethics, and Humanities in Medicine* 4: 16, 2009; doi: 10.1186/1747-5341-4-16
19. Taylor AJ, Nissen SE (2008) Preliminary observations from preliminary trial results: have we finally had enough? *Circ Cardiovasc Qual Outcomes* 1(1): 54–57,
20. Cohen L (1996) Stuck for money: to screen new drugs for safety, Lilly pays homeless alcoholics. *Wall St J* 14: 1
21. Rothman DJ (1995) Research, human: ethical aspects. In: Reich WT (ed.) *Encyclopedia of Bioethics*. MacMillan, New York, pp. 2248–2258
22. Tishler CL, Bartholomae S (2002) The recruitment of normal healthy volunteers: a review of the literature on the use of financial incentives. *J Clin Pharmacol* 42: 365
23. Lemmens T, Elliott C (2001) Justice for the professional guinea pig. *Am J Bioeth* 1: 51–53
24. Christine Grady (2005) The Payment of clinical research subjects. *J Clin Invest* 115(7): 1681–1687
25. Czarny MJ, et al. (2010) Payment to healthy volunteers in clinical research: the research subject's perspective. *Clin Pharmacol Ther* 87: 286–293
26. Appelbaum P, et al. (1987) False hopes and best data: consent to research and the therapeutic misconception. *Hastings Cent Rep* 17: 20–24

27. http://ec.europa.eu/enterprise/pharmaceuticals/eudralex/vol-10/22_cp_and_guidance_database_susars16_april_2004.pdf
28. Grimes DA (2005) The Good Clinical Practice guideline: a bronze standard for clinical research. *Lancet* 366: 172–174
29. Woolf SH (1992) Practice guidelines, a new reality in medicine. II. Methods of developing guidelines. *Arch Intern Med* 152: 946–952
30. Shekelle PG, Ortiz E, Rhodes S, et al. (2001) Validity of the Agency Healthcare Research and Quality clinical practice guidelines: how quickly do guidelines become outdated? *JAMA* 286: 1461–1467
31. Stewart PM, Stears A, Tomlinson JW, Brown MJ (2008) Regulation – the real threat to clinical research. *BMJ* 337: a1732
32. Godlee F (2008) It's time to change how Europe regulates research. *BMJ* 337: a2986
33. McMahon AD, Conway DI, Macdonald TM, McInnes GT (2009) The unintended consequences of clinical trials regulations. *PLoS Med* 3(11): e1000131. Epub 2009 Nov 17
34. Califf RM (2006) Clinical trials bureaucracy: unintended consequences of well-intentioned policy. *Clin Trials* 3(6): 496–502
35. Professor Sir Peter Lachmann COPE Report 2002: The Research Integrity Initiative: progress report <http://publicationethics.org/static/2002/2002pdf5.pdf>
36. Joint consensus conference on misconduct in biomedical research: 28th and 29th October 1999: consensus statement. The COPE Report 2000. Available at: <http://publicationethics.org/static/2000/2000pdf5.pdf>
37. Department of Health and Human Services. Public Health Service policies on research misconduct; final rule. 42 CFR Parts 50 & 93. Available at: http://www.nacua.org/documents/HHS_ResearchMisconduct.pdf
38. Martinson BC, Anderson MS, de Vries R (2005) Scientists behaving badly. *Nature* 435: 737–738
39. Dingell JD (1993) Misconduct in Medical Research. *NEJM* 328(22): 1610–1615
40. Stephen J (17 Dec 2000) Washington Post: Where Profits and Lives Hang in Balance; Finding an Abundance of Subjects and Lack of Oversight Abroad, Big Drug Companies Test Offshore to Speed Products to Market Series: THE BODY HUNTERS: Exporting Human Experiments
41. Wise J (2001) Pfizer accused of testing new drug without ethical approval. *BMJ* 322(7280): 194
42. Abdullahi v Pfizer (2009) U.S. App. LEXIS 1768 (2d Cir. 2009)
43. Annas GJ (2009) Globalized Clinical Trials and Informed Consent. *NEJM* 360: 20 (nejm.org)
44. <http://www.google.com/hostednews/afp/article/ALeqM5gn2FKY2RU91rq4vfD36UmsCOkwLA>
45. Glickman SW, McHutchison JG, Peterson ED, et al. (2009) Ethical and scientific implications of the globalization of clinical research. *N Engl J Med* 360: 816–823

CHAPTER 7

Phase-I studies and first-in-human trials

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1 Introduction

Clinical drug development is often described as consisting of four temporal phases (phases I–IV) [1]. Phase I starts with the initial administration of an investigational drug into humans, whereas phase-II studies are conducted to explore therapeutic efficacy and phase-III studies to demonstrate or confirm therapeutic benefit of the drug. Phase-IV studies begin after drug approval. However, it is important to note that the phase of development provides an insufficient basis for classification of clinical trials as one type of trial may occur in several phases (e.g. human pharmacology studies are typically conducted during phase I but as well at the other development phases. Nonetheless such studies are sometimes labelled as phase-I studies). In general, drug development is ideally a step-wise procedure in which information gained from early, typically smaller, studies is used to plan and perform larger studies with more detailed objectives.

2 Definition phase I

Phase I starts with the initial administration of an investigational new drug into humans [1]. Studies in this phase of development usually have non-therapeutic objectives and typically involve one or a combination of the following aspects:

- Estimation of initial safety and tolerability

Aim is to determine the tolerability of the dose range expected to be needed for further clinical studies and to determine the nature of adverse reactions that

Keywords: Phase-I studies, First-in-man studies, First-in-human studies, Minimal Anticipated Biological Effect Level (MABEL), No Observed Adverse Effect Level (NOAEL), Single ascending Dose study, Multiple Ascending Dose study, high-risk medicinal products

can be expected. Studies typically include both- single and multiple dose administration:

- In Single Ascending Dose studies (SAD) subjects are given a single dose of the drug. If they do not exhibit any adverse side effects, and the pharmacokinetic data is roughly in line with predicted values, the dose is escalated, and a new group of subjects is then administered a higher dose. This is continued until pre-calculated pharmacokinetic safety levels are reached, or intolerable side effects start showing up, at which point the drug is said to have reached the maximum tolerated dose (MTD).
- Multiple Ascending Dose (MAD) studies are conducted to better understand the pharmacokinetics and pharmacodynamics of multiple doses of the drug. In these studies, multiple low doses of the investigational drug are administered and pharmacokinetic and pharmacodynamic data are obtained. The dose is subsequently escalated for further groups, up to a predetermined level.

- Pharmacokinetics (PK)

Although pharmacokinetic data are usually obtained during all development phases, the initial characterization of pharmacokinetic properties, like drug absorption, distribution, metabolism and excretion, is an important goal of phase I. Pharmacokinetics may be assessed via separate studies or as a part of efficacy, safety or tolerance studies. It is important to characterize drug bioavailability, clearance, possible accumulation of the drug or its metabolites and potential drug-drug interactions. However, some of the more specialized questions, especially drug-drug interactions or pharmacokinetics in certain subgroups, are generally part of later phase studies.

For many orally administered drugs, especially modified release products, it is important to assess the influence of food intake on bioavailability. These studies usually have a cross-over design (see Chapter 8), where identical doses of the investigational drug are given to volunteers, once under fasting condition and once after a meal.

- Pharmacodynamics (PD)

Depending on the drug and the endpoint studied, pharmacodynamic studies may be conducted in healthy volunteers or in patients with the target disease. These data can provide early estimates of activity and potential efficacy and may guide the dosage and/or the dose regimen in later studies.

- Early measurement of drug activity

Preliminary studies of activity or potential therapeutic benefit may be conducted in phase I as secondary objective.

3 General considerations for phase-I studies, trial design and study protocol

As for all trials, an appropriate study design is the basis to gain the desired information. However, phase-I studies have certain aspects that need special considerations.

First of all it is important to differentiate between “real” first-in-man clinical trials and phase I trials, where drugs with an established record of safety in humans are used (e.g. known substances with a new formulation, generic drugs). The former need special caution, especially when the investigational drug belongs to one of the following categories:

- biological molecules with a novel mechanism of action,
- new agents with a high degree of species-specificity and
- new agents with immune system targets.

In more detail this encompasses

- any agent, whose effects might cause severe physiological disturbance to vital body systems,
- has agonistic or stimulatory actions,
- novel agents and novel mechanism of actions, where there is no prior experience,
- species-specific agents, which makes pre-clinical risk-assessment in animal models difficult or impossible,
- agents with high potency (e.g. compared with a natural ligand),
- multifunctional agents (e.g. bivalent antibodies),
- agents with cell-associated targets,
- targets that by-pass normal control mechanisms,
- immune-system targets,
- targets in systems with the potential for large biological amplification *in vivo* [2].

In summary, these agents have either a higher potential of harm to volunteers during first human exposure or the risk may be more difficult to evaluate in pre-clinical development. Investigational drugs that fall into the above described categories do not necessarily pose a high-risk on first-in-human exposure. However, a thorough risk assessment should always be carried out before a first-in-man trial and extensively explained in the study protocol. And in doubt, higher risk should be assumed [2].

4 Preclinical development

The preclinical development of new medicines is addressed by internationally agreed guidelines [e.g. 3, 4]. However, qualitative and quantitative differences may exist in the

biological responses in *in vitro* experiments as compared to *in vivo* or between animals and humans. Thus developers of medicines, research funding bodies and regulatory authorities should expedite the collection of information of unpublished pre-clinical studies relevant to the safety of human exposure, for instance in the form of a confidential database.

5 Choice of subjects, study population

In general there is no anticipated benefit to a patient or volunteer subject in a first-in-man trial for a new medicine. Therefore, the risk to benefit assessment is not usually a major factor in deciding whether such trials should be performed in volunteer patients or in healthy subjects [5]. The most important factors are the safety, rights and well-being of the participants and the value of what can be learned from the trial.

The choice of the study population, i.e. healthy subjects or patients, should be done on a case-to-case basis, considering several factors. The risks inherent in the type of the investigational medicinal product (IMP) – which should be quantified and justified, – its molecular target, immediate and potential long-term toxicity, the lack of a relevant animal model, the relative presence of the target in healthy subjects or in patients, the possibly higher variability in patients, the ability of healthy volunteers to tolerate any possible side effects, the potential pharmacogenomic difference between the targeted patient group and healthy subjects, the patients' ability to benefit from other products or interventions and the predicted therapeutic window of the IMP are some of the factors that have to be taken into consideration [5].

Although there is no anticipated benefit to the subjects in a phase I trial, patients may be more appropriate than healthy volunteers on the basis of a “risk to benefit assessment” in the case of higher risk agents targeted at serious diseases where all therapeutic options for the patient have been exhausted [2]. For example, in the cancer field, there is a history of conducting clinical trials with cytotoxic agents with high potential for toxic effects. The practice has usually been to perform first-in-man trials in patients, which ensures that the intended drug target is present and toxicity arising from both “on-target” and “off-target” effects would be detectable.

However, there may be circumstances where healthy volunteers are more appropriate subjects in a phase I trial, e.g. healthy male volunteers are a relatively homogenic group or where concurrent medication in patients would cause difficulties in the interpretation of results [2].

Both, healthy volunteers and patients, should not be included in a phase I trial, if they are currently in another clinical trial or have participated recently unless justified to prevent concomitant or consecutive exposure to IMPs.

The study protocol has to contain clear in – and exclusion criteria to exactly define study population.

6 Dose finding

The estimation of the first dose in humans is an important element to the safeguard of subjects participating in first-in-human studies. All available information has to be taken into account and dose selection has to be made on a case-by-case basis.

In general, the No Observed Adverse Effect Level (NOAEL; the highest dose level that does not produce a significant increase in adverse effects in comparison to the control group [6]) gives the most important information. It is determined in non-clinical safety studies performed in the most sensitive and relevant animal species, adjusted with allometric factors or on the basis of pharmacokinetics. Finally, the relevant dose is adjusted by appropriate safety factors according to the particular aspects of the IMP and the trial design [5].

For high-risk medicinal products, an additional approach for dose finding should be taken. The novelty of the agent, its biological potency and mechanism of action, the degree of species-specificity, the dose-response curves of biological effects in human and animal cells, dose-response data from *in vivo* animal studies, pharmacokinetic and pharmacodynamic modelling, the calculation of target occupancy versus concentration and the calculated exposure of targets or target cells in humans *in vivo* have to be taken into account [2]. The “Minimal Anticipated Biological Effect Level” (MABEL) approach is one good model for achieving this [2]. The MABEL is defined as the anticipated dose level leading to a minimal biological effect level in humans [5, 7]. The calculation of MABEL should utilize all relevant *in vitro* and *in vivo* data, such as receptor binding and receptor occupancy studies *in vitro* in target cells from human and the relevant animal(s) species and *in vivo* in the relevant animal species; concentration-response curves *in vitro* in target cells from human and the relevant animal species; exposures at pharmacological doses in the relevant species. These data should be integrated in a PK/PD modelling approach for the determination of MABEL [5].

If the different methods lead to different estimates of a safe dose in humans, the lowest value should be taken as the starting point in first-in-man trials with a safety margin. When it is likely that preclinical information may be a poor guide for human response *in vivo*, the starting dose should be calculated to err on the side of caution and further dose increases should proceed with caution since the initial dose may be particularly low and there may be a steep dose-response curve [2].

7 Route and rate of administration

The route and rate of administration should be based on pre-clinical data. Careful monitoring for adverse reactions is a prerequisite. In case of first human exposure to a higher risk agent administered intravenously, a slow infusion is recommended,

which allows monitoring for adverse responses and stopping the infusion if needed [5, 7].

New agents in first-in-man trials should be administered sequentially to human subjects with an appropriate interval between the dosing of subjects to limit the number of people that may be affected by a severe adverse reaction [5, 7].

The intervals should be determined by the kind of adverse reaction that might be anticipated based on the nature of the agent, its target and the intended recipient as well as the potential pharmac- and toxicokinetics and pharmac-toxicodynamics of the agent [2]. Thus, administration of the first dose of the active IMP to a single subject is an appropriate design. A sequential further dose administration within each cohort is strongly recommended and progression to a subsequent cohort should not occur before participants in the previous cohort have been treated and results been reviewed in accordance with the protocol.

The selection of the dose increment between two dose levels follows data gained from non-clinical studies: dose/toxicity or dose/effect relationship. In general, the steeper the increase in the dose/toxicity or dose/effect curves, the lower the dose increment should be selected. Information on exposure, effect and safety from the preceding dose in humans should be taken into account. Since the initial doses may be very low (as outlined above), early cohorts may not show any pharmacological effects. Nevertheless, the precautions for the next cohort should be the same as for the previous [5, 7].

The trial protocol encompasses also processes and responsibilities for decision making about dosing of the subjects, dose escalation and stopping the cohort (stopping rules). It should provide a specific plan for monitoring of adverse events or adverse reactions and in cases with a predictable risk of a certain type of adverse reaction, a treatment strategy should already be described in the protocol [5, 7].

8 Clinical environment

First-in-man studies should be conducted in an appropriate clinical environment supervised by staff with appropriate training and expertise and understanding of the IMP, its target and the mechanism of action. The trial unit should have immediate access to equipment and staff for resuscitation and stabilizing individuals in an acute emergency (e.g. anaphylaxis, cytokine release syndrome, hypotension and cardiac events). Contingency availability of Intensive Care Unit facilities in reasonable proximity should be pre-arranged and standard operating procedures for emergency situations and regular drills are necessary [2]. It is important to inform the trial subjects about what to do if they experience symptoms of an adverse reaction during or after the trial. In this sense the informed consent form (ICF) should be easy to understand for a non-expert reader and provide extensive information about adverse reactions [8].

Communication between clinical investigators and trial subjects before and during a trial, adequate follow-up of trial subjects, insurance cover and the role of Research Ethics Committees are further important points [2].

Generally, first-in-human trials should be conducted as a single protocol at a single site. However, if different sites have to be involved an adequate communication system has to be established.

Case Study: Anti-CD28 antibody first-in-man trial

After written informed consent, eight healthy young male volunteers were enrolled in the first-in-man phase I trial of a novel anti-CD28 monoclonal antibody. This antibody was a recombinantly expressed, humanized superagonist anti-CD28 antibody that stimulates and expands T-cells independently of the ligation of the T-cell receptor [9].

It was hypothesized that this antibody unspecifically stimulates the immune system, which would have helped to overcome the immunodeficiency or immunosuppression occurring in several diseases, like chronic lymphatic leukemia or rheumatoid arthritis. The antibody had been tested *in vitro* and *in vivo* in rabbits and monkeys and the study protocol had been approved by government health authorities and the local ethics committee.

The trial was carried out by a contract research organization that operates an independent clinical trial unit on the premises of a public hospital.

On the trial day, volunteers were randomly assigned to receive the antibody ($n=6$) in a dose of 0.1 mg/kg body weight or placebo ($n=2$). Each subject was then administered an intravenous infusion with either antibody or placebo in a 10 min interval.

After a median of 60 min a series of adverse effects began, initially with headache followed by lumbar myalgia. The subjects were then restless, had varying degree of nausea, vomiting, bowel urgency or diarrhoea. Subsequently, all subjects developed a systemic inflammatory response with erythema and vasodilation. After approximately 4 h hypotension and tachycardia occurred and body temperature rose to 39.5 till 40.0°C. All subjects developed signs of respiratory failure and chest X-rays revealed pulmonary infiltrates one hour later.

All subjects were initially empirically treated in the independent clinical trial unit with hydrocortisone, chlorpheniramine, acetaminophen, odansetron, metaraminol and received lactated Ringer's solution 4 h after the antibody infusion.

After a spurious improvement after 6 h, medical condition of the subjects became worse. Twelve hours after infusion one had to be intubated due to respiratory failure, he had hypotension and lactate acidosis and was transferred to

an intensive care unit (ICU). There was concern that the other subjects would follow a similar course of deterioration, thus all other study subjects were transferred to ICU facilities 16 h after infusion as well.

And indeed, all subjects developed respiratory deterioration, renal impairment, disseminated intravascular coagulation, severe lympho- and monocytopenia and neurologic symptoms in terms of multi organ failure. Four subjects received continuous positive airway pressure, two underwent mechanical ventilation, all six needed renal support by means of continuous venovenous hemodiafiltration and replacement of blood components. All were treated with repeated doses of methylprednisolone, with ranitidine, chlorpheniramine maleate and with an anti-interleukin-2 receptor antagonist antibody.

Four subjects showed a faster recovery and spent average six days in the ICU. A more complex course of disease occurred in two subjects, with one developing peripheral ischemia resulting in patches of necrosis on the fingers and all toes. He was finally discharged from the ICU after 21 days.

Subsequently, all subjects had generalized desquamation over the next month, muscle weakness, as well as neurologic symptoms (varying from headache, difficulties with concentration, short-term difficulties in finding words, delayed hyperalgesia, peripheral numbness) [9].

As described above, this substance clearly is a potential high-risk medicinal product. This implies special requirements.

First, for this type of IMP the ability of non-clinical studies to predict safety issues in humans may be reduced because the nature of the target is more specific to humans – the CD28 T-cell surface receptor shares only 68% of identity of amino acids between mouse and man [10] and the extracellular domain of the human CD28, including a binding loop, differs by four amino acids from the macaque sequence and their T-cells show lower proliferation upon stimulation with anti CD 28 antibodies [11]. However, according to the investigators brochure, 100% homology between the CD28 binding site in humans and monkeys exist and no sequence comparison was included [12]. Information about the effects of this “new” antibody on human T-cells was lacking in the preclinical test phase. Warning bells could have been sounded if the regulators had known that e.g. a superagonistic anti-human CD28 antibody induced rapid depletion of peripheral T-cells in mice with a humanized immune system [11, 13]. Unfortunately, these results were published after the incidence and further studies were invented to elucidate why T-cell activation and subsequent dramatic cytokine storm had not be foreseen by animal experiments in the preclinical test phase [11]. Additionally, some other points of concern about the pre-clinical tests were raised [12]. More transparency in the process of developing new drugs with the possibility of public

review might have prevented the dramatic events. Thus, some experts claim for an open access database for sharing safety information [2].

Secondly, the question has to be asked, why the drug was tested in healthy volunteers rather than in patients. In this trial an agonist drug targeted at compromised immune systems was given to individuals with an intact immune system. And without a doubt this IMP as a high-risk drug should have been administered in a much lower starting dose than selected: The dose was ascertained by a fraction of the NOAEL in cynomolgus monkeys. However, cytokine release was already recorded at a low dose in this species. Therefore a proper starting dose would most probably be much less than a 500th of the concentration causing effects in the monkeys, even assuming the sensitivity of man and monkey being equal [12]. In addition, the IMP was administered to all volunteers at the same time. Most monoclonal antibodies have long plasma half-lives and the animal data from the investigator's brochure show a half-life for this antibody of about eight days. Thus, the full removal from the body would take about a month [12]. In this view, a ten minutes dose interval is clearly too short to observe for drug related adverse events. Simultaneous dosing of eight subjects was a major problem, which stimulated experts to call for tighter regulation of the operational side of first-in-man trials [14]. A longer observation period would have saved the other volunteers from suffering those life-threatening events. Besides, in the investigator's brochure no care was taken to follow-up potential long-term immunosuppressive effects [12].

Another point for discussion is the placebo design of a first-in-man phase I trial. It is important that any decision taken with respect to subsequent dosing at the same dose level and/or dose escalation takes into account the number of subjects that might have received the active drug. The study design including randomization schemes should take this into account [7].

This incidence also raises the question about the qualification of investigators and attending physicians in phase I trials. Experts proposed the development to an accreditation system for principal investigators involved in first-in-man studies, as they are of the opinion that a trained investigator would not have likely accepted the study design for this trial. There was also doubt about adequate qualification of the attending physicians and the time till transfer to the ICU [15, 16]. In the investigator's brochure little guidance is given to doctors on how side-effects can be controlled and treated [12].

Finally, interviews with the victims yielded various motives for participating in this study, from altruism to monetary reward [17]. However, a study revealed that informed consent forms may not have informed participants adequately for consent [18].

Lessons have been learned from this trial and expert groups were formed to improve guidelines for phase I trials [2, 19]. Future needs encompass development of national professional accreditation systems for principal investigators conducting first-in-man trials and certification of adequately trained staff. This could be achieved by developing specialist centres for phase I trial of higher risk and advanced medicinal products. For interests of safety the ultimate goal should be an open access database [2].

Nevertheless, the risk of a serious adverse event in a phase I trial is low when the common safety rules are applied. In the literature only 15 deaths have been published during the last 30 years in Western countries, although 100,000 healthy subjects are dosed every year [20]. In France a recently implemented register revealed a rate of related worrying serious adverse events (SAE) incidence of 0.02% in 15,386 healthy subjects [20]. A Japanese register shows similar data: In 95,780 subjects (included from 1993 to 2003) no deaths occurred and the incidence of related SAEs was 0.02%, all reversible without sequela [21].

However, from specific reactions to anaphylactoid reactions, although rarely, have also been observed at our unit during phase I trials. This is not always an issue of high-risk investigational medicinal products. So awareness and preparedness are indicated and immediate access to resuscitation equipment and regularly trained staff are of utmost importance.

The safety, rights and well-being of subjects, both patients and healthy volunteers, must always be the primary concerns in clinical trials. However, despite of all safety measures the transition from animal to human will remain a critical step, which has been accepted in order to develop innovative new drugs.

References

1. European Medicines Agency (1998) ICH Topic E8 General Considerations for Clinical Trials. Note for guidance on general considerations for clinical trials. EMEA/CPMP/ICH/291/95
2. Duff G (2006) Expert Scientific Group on Phase One Clinical Trials Final Report. Stationery Office, Norwich, UK
3. European Medicines Agency (1998) ICH Guideline S6 Pre-clinical Safety Evaluation of Biotechnology-Derived Products. EMEA/CPMP/ICH/302/95
4. European Medicines Agency (2000) ICH Guideline M3 (R1) Non-clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals. EMEA/CPMP/ICH/286/95
5. European Medicines Agency, Committee for Medicinal Products for Human Use (2007) Guideline on Strategies to Identify and Mitigate Risks for First-in-Human Clinical Trials with Investigational Medicinal Products. EMEA/CHMP/SWP/28367/07

6. Food and Drug Administration (2005) Guidance for industry. Estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers. (www.fda.gov/cder/guidance/index.htm)
7. European Medicines Agency, Committee for Medicinal Products for Human Use (2007) Guideline on Requirements for First-in-Man Clinical Trials for Potential High-risk Medicinal Products. EMEA/CHMP/SWP/28367/2007
8. Paasche-Orlow MK, Taylor HA, Brancati FL (2003) Readability standards for informed-consent forms as compared with actual readability. *N Engl J Med* 348: 721–726
9. Suntharalingham G, Perry MR, Ward S, et al. (2006) Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. *N Engl J Med* 355: 1018–1028
10. Gross JA, St. John T, Allison JP (1990) The murine homologue of the T lymphocyte antigen CD28. Molecular cloning and cell surface expression. *J Immunol* 15: 3201–3210
11. St. Clair EW (2008) The calm after the cytokine storm: lessons learned from the TGN 1412 trial. *J Clin Invest* 118: 1344–1347
12. Kenter MJH, Cohen AF (2006) Establishing risk of human experimentation with drugs: lessons from TGN1412. *Lancet* 368: 1387–1391
13. Legrand N, Cupedo T, van Lent AU, et al. (2006) Transient accumulation of human mature thymocytes and regulatory T-cells with CD28 superagonist in “human immune system” Rag 2^{-/-}γc^{-/-} mice. *Blood* 108: 238–245
14. Jilma B, Shah R (2006) Minimizing disasters during early drug development: a pressing need for an improved guidance. *Nat Rev Drug Discov* 5 www.nature.com.
15. Day M (2006) Agency criticises drug trial. *BMJ* 332: 1290
16. Goodyear M (2006) Further lessons from the TGN1412 tragedy. *BMJ* 333: 270–271
17. Goodyear M (2006) Learning from the TGN 1412 trial. *BMJ* 332: 677–680
18. Knapp P, Raynor DK, Silcock J, Parkinson B (2009) Performance-based readability testing of participant materials for a phase I trial: TGN 1412. *J Med Ethics* 35: 573–578
19. Schneider CK, Kalinke U (2007) Nach dem TGN1412-Zwischenfall. Prinzipien der Bewertung von First-in-Man-Studien mit monoklonalen Antikörpern durch das Paul-Ehrlich-Institut. *Bundesgesundheitsbl-Gesundheitsforsch-Gesundheitsschutz* 50: 1213–1220
20. Sibille M, Donazzolo Y, Lecoz F, Krupka E (2006) After the London tragedy, is it still possible to consider phase I is safe? *Br J Clin Pharmacol* 62: 502–503
21. Kumagai Y, Fukazawa I, Momma T, et al. (2006) A nationwide survey on serious adverse events in healthy volunteers studies in Japan. *Clin Pharmacol Therap* 79: P71

Further reading

The European Medicines Agency and the American Food and Drug administration provide various guidelines for requirements for phase I trials, which can be downloaded from the home pages: <http://www.emea.europa.eu> and <http://www.fda.gov>.

CHAPTER 8

Clinical trials – interventional studies

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1 Introduction

It is considered that the first properly controlled trial in history was performed by James Lind. This Scottish surgeon and ship's doctor was the first who conducted a trial with an appropriately controlled design from a modern point of view. In 1747 when scurvy was a common disease among sailors James Lind administered different acidic substances to 12 sailors affected with scurvy to test who benefits most. Five pairs of the seamen were given vinegar, mustard and garlic purges, and elixir of vitriol. The sixth pair was given two oranges and one lemon per day and recovered within 6 days. Objective and reliable evaluation of appropriate treatments against diseases has become a great need in medical research especially in the last century. Today healthcare professionals are required to base their decisions on the highest level of evidence. Evidence based medicine aims to rationalize this decision process in medical treatment and legitimates a certain treatment – or rejects it. In the process of finding the best treatment available it became obvious that different kinds of clinical trials might not provide the same level of evidence and differences between study designs are more than trivial. Today clinical trials are currently seen to have the highest level of evidence and to be the “Gold Standard” in clinical research.

2 Types of clinical trials

There are two fundamental types of clinical trials: Observational studies and interventional trials, where the effect of a standardized regimen is being tested against a comparator or no treatment. An observational study is a kind of study in which certain outcomes are measured without an additional intervention for study participants. The researcher does not influence the outcome or the study conditions in any way. A typical

Keywords: Randomization, blinding, clinical trial, Framingham, James Lind, endpoints, trial design, interim analysis

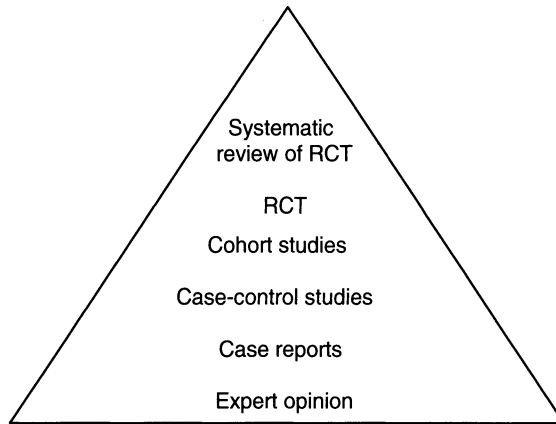


Fig. 1 Randomized clinical trials (RCT)

example of an observational study is the Framingham heart study which started in 1948. In this study data from 5 209 adults of the small town Framingham (MA, USA) were included to identify cardiovascular risk factors. Much of today's knowledge of risk factors and progression of cardiovascular diseases is based on this longitudinal study. Observational studies have clear advantages like lower costs and broader range of patients but they are not seen to be as robust as interventional trials, where a clear cause-effect relationship can be established (Fig. 1). The current chapter focuses on the second type of trials: interventional studies.

In contrast to observational studies controlled trials are regarded as gold standard in evidenced based medicine [1]. In these studies the investigator uses interventional techniques to investigate predefined scientific questions in subjects exposed to a treatment or a control condition.

However, some diseases are particularly complex, and the salutary effect of an intervention might not necessarily be causal for the improvement of signs or symptoms, but rather act as bystander of a natural cause of disease.

2.1 Purpose

The purpose of an interventional clinical trial is to answer predefined scientific questions about the efficacy and reliability of interventions to prevent, diagnose, and treat diseases.

2.2 Definition

An interventional trial is defined as a prospectively planned biomedical or health-related experiment in humans. Every clinical trial has to fulfill certain criteria. It has to

be based on a well defined scientific question and has to follow a pre-defined protocol. Every interventional clinical trial has to use a control group for comparison with the interventional group. The associated features randomization, control group selection and blinding are quality characteristics.

3 Randomization

3.1 The basic idea, like most good things is very simple [2]

Randomization is an essential part and a quality criterion in scientific research. Every clinical trial compares the influence of different treatments. To do this comparable study groups are needed which is difficult to achieve because of the number of known and unknown confounders. In spite of restrictive exclusion and inclusion criteria every study participant still differs in so many factors that equality across groups is impossible to gain. Randomization is a generally accepted means to deal with this problem. It tries to ensure that treatments or subject characteristics which may influence study outcomes are randomly distributed across groups. In randomization every participant has a clear defined probability to get allocated to a certain treatment regimen. Important is that the assignment cannot be predicted which minimizes the chance of bias.

Sir Austin Bradford Hill is pivotal in the context of randomization. He was a pilot in the first civil war but left the army due to an infection with tuberculosis. Afterwards he studied economics and became later a professor for statistics. In “Principles of Medical Statistics” he was the first to instead of notice: emphasize the tremendous importance of randomization in clinical trials, an idea that originally came from agriculture research. Due to his influence the first clinical trial using properly randomized treatment and control groups was conducted in 1948. This study carried out by the Medical Research Council investigated the use of streptomycin in the treatment of tuberculosis. Due to the highly variable course of disease the introduction of the control group enabled comparison and reliability of the data. The tremendous success of streptomycin was demonstrated and became a hallmark of treatment.

Although it sounds paradox because chance decides about the assignation of the participants, randomization is one of the simplest but most powerful tools in scientific research.

The need for randomization has clear reasons. It minimizes the possibility of conscious and unconscious selection bias that might occur if the observer or the participant chooses the assignment to a certain intervention group. Furthermore randomization tends to produce comparable groups. This provides a basis for an assumption-free statistical test of the equality of treatments.

Properly performed randomization consist of two processes: The first step guarantees that the participants are randomly allocated to the different study groups. The

second process called allocation concealment keeps those who are involved in the study unaware of upcoming assignment [3].

Several possibilities exist to randomize participants which can be summarized as three types: simple randomization, permuted block randomization and adaptive randomization.

3.2 Simple randomization

This kind of randomization can be achieved by a toss of coin. If head turns up the participant will be assigned to the intervention group, if tail turns up the participant will be assigned to the control group. This creates two approximately equal study groups. In practice this method is not used because it is not reproducible and not controllable. Randomization with a list of random numbers would be a more feasible method. A simple randomization list can be generated by assigning treatment A for example to the numbers 0, 1, 2, 3, 4 and treatment B to the numbers 5, 6, 7, 8, 9 and numbers then blindly drawn. This will create a randomization list. The advantage of simple randomization is already revealed by the name: it is very simple to generate and implement. The main disadvantage is that there will be an imbalance in the number of participants especially in smaller study groups: With $n = 20$ on two treatments A and B, the chance of a 12:8 split or worse is approximately 0.19. With $n = 100$, the chance of a 60:40 split or worse is approximately 0.025 if numbers are not controlled in advance.

3.3 Permuted block randomization

Block randomization is probably the most common method for the assignment of participants. The randomization occurs in subgroups – so called blocks. The principal advantage of block randomization is that it allows numerically balanced study groups throughout the whole enrolment process. An imbalance due to a possible change in the characteristics of the study population over time (e.g. change in life circumstances) is avoided. Furthermore, in case of premature end of enrollment this process still yields balanced study groups. The implementation of block randomization is simple: For example in a study with two treatments A or B and a block size of four participants we have six possible permutations: AABB, ABAB, BBAA, BABA, ABBA and BAAB. Each block is linked to a numbers 1–6. With the use of a random number list a block randomization can be achieved.

3.4 Stratified randomization

Another subtype of randomization is called stratified randomization. Stratified randomization refers to the situation in which characteristics of participants are

thought to affect the response to a treatment. In such situations it is advantageous to sample each group (stratum) independently. This provides balanced study groups with respect to a various combination of prognostic variables. Such variables are for example age, gender, tumour status, study centre etc. To achieve balanced groups it is advisable to use permuted blocks. Simple randomization would easily produce numerical imbalance in the sub-groups. This is an example of a study population in which age, gender and glucose tolerance are prognostic variables.

| | Age (yr) | Sex | Glucose tolerance |
|----|----------|--------|-------------------|
| 1. | 20–35 | Male | Normal |
| 2. | 36–50 | Female | Pre-diabetes |
| 3. | 51–70 | | Diabetes |

Our example would require $3 \times 2 \times 3 = 18$ strata which elucidates the main disadvantage of the study design. In a study with 144 participants and 2 therapies this randomization will result in 4 participants in each treatment group which has a low power to detect differences between groups (“over-stratification”).

3.5 Pseudo – randomization

Some treatment allocations are often incorrectly regarded as randomization methods. Randomizations according the date of birth, social security number, patient’s initials or just the alternating assignment (e.g. ABABAB) are not acceptable methods of randomization. There is no random component in the assignment and bias can easily occur.

3.6 Allocation concealment

In properly randomized clinical trials allocation concealment is an indispensable part that is often spuriously confused with blinding. Allocation concealment is a process that prevents predictability of treatment during the assignment to secure strict implementation of a random allocation sequence. It shields those who are responsible for admitting participants into the study and consequently prevents selection and confounding biases. Studies in the past underlined the importance of allocation concealment. They have shown that inadequate allocation concealment resulted in up to 40% larger estimates of effect [3]. Sequentially-numbered, opaque sealed envelopes, sequentially numbered containers, pharmacy controlled, and central randomization are standard methods for the implementation of allocation concealment [4].

4 Blinding

4.1 Human behaviour is influenced by what we know or believe

Blinding is a powerful tool in clinical research to minimize bias. During a study there are many situations where the researcher or the participant can influence the study outcome. For example, if the researcher is interested in the success of a new treatment he could take influence in many ways: He could be not so strict in declaring adverse events, he could influence the participant's attitude and encourage or discourage him/her to continue study participation. Further the investigator could be overprotective in study groups believed to receive an inferior treatment. Outcome assessment is a further source of bias. Subjective endpoints such as pain are more susceptible to unwanted influence than hard outcome results such as mortality. Hence, blinding to the treatment prevents bias of outcome assessment and is more important when subjective or soft endpoints are used.

On the other hand the participant's knowledge may also influence the study results. Psychological and physiological effects can arise. The believe of a patient in getting a new promising treatment, or a readily available treatment already changes his attitude towards response to the treatment. When he believes that he was assigned to what he perceives as an inferior intervention he may not comply well and probably will not adhere to procedures and follow up as stringently. Furthermore, placebo is less effective as a time control if participants are informed because the psychological component of taking a treatment would be lost.

Blinding is a way to reduce and prevent these ascertainment-, information-, and observer bias. Blinding means to make treatments undistinguishable from each other and needs more effort than just keeping the name of the treatment hidden. In the best way blinding provides that neither the researcher nor the participant knows the assignment to a certain treatment.

To achieve blinding several components have to be considered. The appearance of the drug like color or form is important. It gives a clue to its identity and possibly changes the response and adherence to treatment as shown in previous studies [5]. Also differences in smell, taste, or mode of delivery already allow conclusions for the drug identity.

In some cases the different daily pattern of administration complicates the ability of blinding. Under such circumstances a “double dummy” trial design can help. Double dummy means that each drug has an identical looking placebo and the participant always has to take both therapies with only one containing verum drug. Characteristic side effects of a certain drug may also unblind a study. In such cases the blinding has to be extended to match the side effect profile.

Basically blinding can be classified in to the following different types: open-label, single blind and double blind. Open label means that the study is performed without any

blinding; both the researcher and the patient are informed about the treatment. Although it is not recommended to perform open label studies in some cases blinding is not feasible. Sham surgery might not be acceptable on ethical grounds. An open-label study certainly has advantages such as lower cost and simplified logistics: In a single blind study only one part (investigator or patient) is informed about the treatment, while the other part (investigator or patient) is blinded. Double blind studies are currently the best approach in reducing the risk of bias. The patients and the investigators will remain unaware of the patient's assigned treatment throughout the whole trial.

5 Different study designs

The aim of a clinical trial is to answer specific scientific questions ("study hypothesis"). This study aim has to be defined in advance in the study protocol. The clinical trial will test and reject the hypothesis by statistical means. Some study designs are suited better than others to address specific scientific questions. In order to identify and select the individual best study design the objective of the present clinical trial should be determined: What is the primary question and what are the subsidiary questions that should be answered [6]? Subsequently, the study design has to be chosen accordingly since it is an important factor for the validity of a clinical trial.

5.1 Parallel group design

One of the most common methods in clinical investigation is the parallel group design (Fig. 2) which is considered to have the highest power and to be most reliable [7]. In a parallel design each patient receives only one treatment throughout the observation period.

In a comparative study a predefined number of subjects are randomized into two or more usually equally sized groups. The simplest model would be the two group parallel

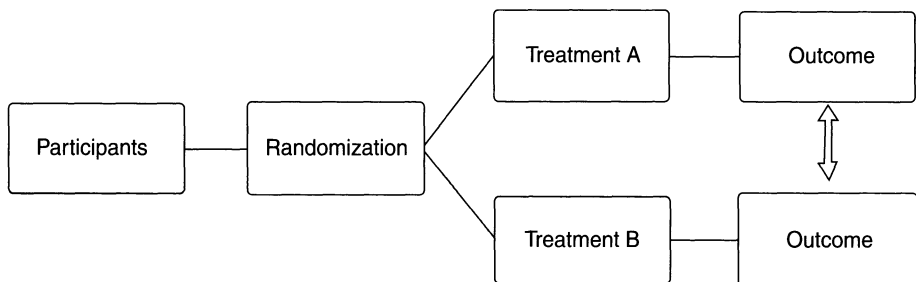


Fig. 2 Parallel group design

design, in which one subject receives either the interventional or the control treatment. At the end the outcome is compared.

5.2 Cross-over design

The cross-over design is another popular design. In contrast to the parallel design each subject receives all treatments being studied and therefore acts as his own control. In the simplest 2×2 cross-over design half of all subjects are randomized to treatment order AB and half of the subjects are randomized to treatment order BA (i.e. in reverse order). The cross-over trial has some advantages. The fact that every subjects serves as own control reduces inter-individual variability, which is a great source of variance. As a consequence a smaller sample size suffices to detect differences between treatments. A main disadvantage in cross-over studies is the carryover effect which means that the effect of the first period still persists in the second period and might influence the outcome. To prevent carry over a long washout period should be established. Another limitation is that cross-over designs need chronic stable disease conditions with little within-subject variability. In psychopharmacology trials, for example, this study design would hardly be applicable due to variable course of psychiatric disorders during therapy [8].

5.3 Factorial design

This study type may be considered when more than just one intervention is being studied. A factorial design allows investigating the individual effects of two or more treatments as well as the effects of their combination in the same trial. The simplest factorial design is the 2×2 factorial design addressing two intervention comparisons: A vs. not-A, and B vs. not B. The participants are first randomized to one of two levels factor 1 (A vs. not A) and afterwards to one of two levels factor 2 (B vs. not B). The physician health study is a typical example using the 2×2 factorial design. This study

| | | |
|--|-------------------------|------------------------------|
| beta carotin vs. not beta carotin (cancer) | | aspirin vs. not aspirin (MI) |
| beta carotin | placebo | |
| aspirin | aspirin + beta catotine | |

Fig. 3 Factorial design

investigated the effect of aspirin on the risk of myocardial infarction and the effect of Beta Carotin on the risk of cancer. The participants were assigned to one of four possible combinations (Fig. 3). The main result of the study showed a positive effect of aspirin in the treatment of myocardial infarction. Due to the study design this important study could be conducted at a fraction of the cost of a parallel group study.

The study type also has limitations. Before choosing the factorial design the possibility of interactions between the interventions should be accounted for. In the case of interactions the power is lower and hence larger study groups are required. Beside the 2×2 design there are higher complex factorial designs feasible like a $2 \times 2 \times 2$ factorial design when there is a third intervention. This is currently applied in the Women Health Initiative study where the effects of postmenopausal hormone replacement therapy, diet modification, and calcium and vitamin D supplements on heart disease, fractures, and breast/colorectal cancer are studied.

6 Study endpoint

A study endpoint is a prospectively defined outcome marker which reflects the main study goal. It should be appropriate to answer the main objectives of the study, should be precisely defined, measurable and should reflect validated aspects of the disease process [9]. Full remissions of all disease symptoms, a disease relapse or mortality are regarded as hard study endpoints whereas pain or quality of life are regarded as soft study endpoints. The fact that studies with soft endpoints are not directly related to the disease process and that they need subjective assessment minimizes their acceptance by some expert's [10]. A clinical study should only contain one or a maximum of two primary endpoints to allow reliable result interpretation. The primary endpoint should be chosen to be sufficient to fully characterize the treatment effect of the intervention. To gain additional information about an intervention a secondary endpoint can be introduced. Over-interpretation of this secondary aim might occur when the primary endpoint has not demonstrated statistical significance [11]. A group of endpoints integrated into one primary endpoint is called composite endpoint. This allows a smaller study group due to the higher endpoint event rate and a broader view on the benefit of a treatment [12]. However, an effect on a composite does not necessarily mean that all individual endpoints are affected or influenced in a consistent way.

7 Interim analyses

Interim analyses are important to estimate a treatment effect during an ongoing study. They may be implemented to detect early differences between treatments. Interim analyses require an unblinded data monitoring committee and enable an assessment of

safety, efficacy and futility on the basis of predefined statistical cutoffs. Beside lower costs earlier study termination can be beneficial for the participants because either exposure to an inferior treatment can be abbreviated or earlier excess to superior treatments can be achieved.

Case Study: The cardiac arrhythmia suppression trial (CAST)

The cardiac arrhythmia suppression trial (CAST), a double-blind, randomized interventional multicenter trial investigated the effects of three class I anti-arrhythmic drugs in patients with myocardial infarction and ventricular ectopy/non-sustained ventricular tachycardia. Responders to anti-arrhythmic treatment with reduction of ventricular ectopies were identified in a test phase. The CAST study consisted of two parts: CAST I and II. CAST I tested the effects of flecainide or ecainide *versus* placebo on morbidity and mortality in 1455 patients following a parallel group design. After 10 months of follow up 63 of 755 subjects died in the anti-arrhythmic drug treatment arm and 26 deaths occurred in the placebo group ($n = 743$). Due to this excess mortality in the interventional arm the study was stopped prematurely. CAST II compared the effects of moricizine *vs.* placebo on deaths due to ventricular arrhythmias and overall survival in a parallel group design. The CAST II study was also stopped prematurely because of increased mortality in subjects randomized to receive moricizine. A meta-analysis of 51 randomized clinical trials with a total of 11,712 patients has confirmed the potential harmful effect of class I anti-arrhythmic agents in this selected group of patients. The results of the CAST study led to a fundamental change in the treatment of patients with ventricular arrhythmias after myocardial infarction.

References

1. Berwick DM (2008) The science of improvement. JAMA 299: 1182–1184
2. Cochrane AL (1989) Archie Cochrane in his own words. Selections arranged from his 1972 introduction to “Effectiveness and Efficiency: Random Reflections on the Health Services” 1972. Control Clin Trials 10: 428–433
3. Schulz KF (2001) Assessing allocation concealment and blinding in randomised controlled trials: why bother? Evid Based Nurs 4: 4–6
4. Schulz KF, Grimes DA (2002) Allocation concealment in randomised trials: defending against deciphering. Lancet 359: 614–618

5. de Craen AJ, Roos PJ, Leonard de Vries A, et al. (1996) Effect of colour of drugs: systematic review of perceived effect of drugs and of their effectiveness. *BMJ* 313: 1624–1626
6. Chow SC, Liu J (1998) *Design and Analysis of Clinical Trials: Concepts and Methodologies*. John Wiley & Sons, New York
7. ICH Expert working group (1998) Statistical principles for clinical trials. In ICH Harmonized Tripartite Guideline. http://www.pmda.go.jp/ich/e/e9_98_11_30e.pdf
8. Kenneth LD, Charney D, Coyle JT, et al. (2002) *Neuropsychopharmacology. The Fifth Generation of Progress*. Lippincott Williams & Wilkins, Philadelphia
9. Bacchieri A, Cioppa G (2007) *Fundamentals of Clinical Research*. Springer Verlag
10. Asmar R, Hosseini H (2009) Endpoints in clinical trials: does evidence only originate from ‘hard’ or mortality endpoints? *J Hypertens* 27 (Suppl 2): S45–S50
11. O’Neill RT (1997) Secondary endpoints cannot be validly analyzed if the primary endpoint does not demonstrate clear statistical significance. *Control Clin Trials* 18: 550–556; discussion 561–557
12. Cannon CP (1997) Clinical perspectives on the use of composite endpoints. *Control Clin Trials* 18: 517–529; discussion 546–519

CHAPTER 9

Observational studies

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1 Introduction

Epidemiologists have used observational studies for a long time to explore effects of infectious and non-infectious exposures on health outcomes. Outstanding people who performed milestone epidemiological research include Ignaz Semmelweis (1818–65), William Farr (1807–83), John Snow (1813–58), or later Sir Richard Doll (1912–2005). They all gave examples of the classical epidemiological approach where harmful exposures were examined. After Sir Austin Bradford Hill had published his legendary randomized trial on the benefits of streptomycin for pulmonary tuberculosis in 1948 [1], interventional studies seemed to gradually displace observational research in the field of clinical pharmacology, because they permitted fair comparisons at much higher levels of internal validity. An important breakthrough of this concept was seen after Archie Cochrane had published his very influential book on effectiveness and efficiency in 1972 [2].

On the other hand, little attention was paid to adverse drug reactions for a long time, even though there is a text by Louis Lewin from 1881 about the untoward effects of drugs [3]. The increasing interest in adverse drug reactions from the early 1950s is expressed in a successful textbook on side effects of drugs by Leopold Meyler [4]. Driven by the thalidomide disaster, notification systems for adverse drug reactions were established [5]. Nonetheless investigations on adverse drug reactions were still conceived as descriptive research. An illustration of properly using analytical observational studies to investigate adverse drug reactions was published only in 1978 [6], and increasingly established epidemiological methods were utilized in pharmacology. With the availability of large databases the methods of observational research are still being advanced [7].

Keywords: Pharmacoepidemiology, pharmacovigilance, adverse drug reaction, effectiveness, drug utilization, bias, confounding, cohort study, case-control study, meta-analysis

Table 1 Applications of observational studies in drug research

Adverse drug reactions

- unexpected short term
- long-term adverse effects
- medication errors

Effectiveness in

- daily life practice
- long-term use
- vulnerable populations
- new populations

Drug interactions

Effects of drugs with well-established use (constraints to randomize)

Patterns of drug utilization

Pharmacoeconomics

There is no doubt that randomized controlled trials are the gold standard method to study the effectiveness and efficacy of pharmaceuticals. There are, however, also some limitations of the randomized trials that may be outweighed by observational studies. Randomized trials are usually powered to detect short-term efficacy and are often not designed to detect rare, but still important side effects [9]. Results from suitable trial cohorts cannot be necessarily generalized to the actual daily life practice in more vulnerable co-morbid populations. In particular effectiveness of interventions in elderly people, children or pregnant women are often not established by premarketing trials, as well as drug interactions. Further, we live in a world that has a number of remedies available. Some are marketed for a long time and for some there is no sufficient contemporary evidence of their effects – benefit or even more importantly the harms. Is there sufficient equipoise for starting a randomized trial, implying that we withhold a drug that is marketed for decades to a study population, despite many people believe in its benefits? Recently also applications in pharmacoeconomics are reported [10]. Contemporary applications of observational studies in drug research are presented in Table 1.

Accordingly observational research is an important complementary tool to randomized research in clinical pharmacology. As outlined below the major challenge in observational research is the methodological complexity and flexibility. Good research skills and methodological knowledge [11], and sufficient training in critical appraisal for those who apply the results are therefore required.

As a consequence of using epidemiologic methods in clinical pharmacology the term *pharmacoepidemiology* was coined, resulting in prominent textbooks (e.g. Strom's textbook of Pharmacoepidemiology) and the formation of associations like the International Society of Pharmacoepidemiology. On their website [12] they give a good definition:

“Pharmacoepidemiology may be defined as the study of the utilization and effects of drugs in large numbers of people. To accomplish this study, pharmacoepidemiology borrows from both pharmacology and epidemiology. Thus, pharmacoepidemiology can be called a bridge science spanning both pharmacology and epidemiology. Pharmacology is the study of the effect of drugs and clinical pharmacology is the study of effect of drugs in humans. Part of the task of clinical pharmacology is to provide a risk benefit assessment for the effect of drugs in patients. Doing the studies needed to provide an estimate of the probability of beneficial effects in populations, or the probability of adverse effects in populations and other parameters relating to drug use may benefit from using epidemiological methodology. Pharmacoepidemiology then can also be defined as the application of epidemiological methods to pharmacological issues.”

We provide a short methodological introduction to special issues in observational studies, and exemplify the two most important study design types with a cohort study and a case control study example.

2 Methodological principles

Key elements of any epidemiological (analytical) study are the *study population* (P), the *exposure* (E) or risk factor, *controls* (C), and the *outcomes* (O) or endpoints of interest (which may be remembered by the acronym PECO), and the *type of study design*. These elements compose the study objective and need to be precisely defined at planning stage to choose the appropriate study methods in order to minimize errors (bias, confounding) during conduct and misinterpretations when analyzing the study. The elements also need to be described when reporting a study to allow judgement of the validity of study results and their interpretation [14].

In pharmacoepidemiologic studies, exposure is the use of a drug (or several drugs), controls are those not exposed to the drug, and outcomes are beneficial and adverse effects of the drug(s) of interest. A special situation is the case-control design that has the same elements, however, here controls are defined as those who have not experienced the outcome (see below).

2.1 Study population

Epidemiological studies are hardly ever performed in the general population. First, a population of interest is defined, i.e. the population to whom the study question is relevant. For example, a study on prostate cancer would only consider male subjects. Of this population, a study sample is drawn and study findings in this

sample are inferred to be true for the overall population of interest. Thus, the study sample must be as representative as possible of the population of interest [13].

The process of selecting the study population determines the *generalisability* of study results, also termed *external validity* of the study. This process involves active selection, sampling strategies, and inherent selection mechanisms.

The population of interest is defined by eligibility criteria that describe person, place, and time of study participation. Depending on the scope of the study, narrow or wide eligibility criteria may be chosen for explanatory (proof-of-concept) studies or pragmatic studies, respectively [15]. Sometimes, random samples are drawn from the population of interest for feasibility reasons. The basic principle aims at assuring equal probabilities of being sampled (EPSEM, equal probability selection methods) with some extensions like stratified or cluster sampling [16]. In practice, however, inherent selection problems may result from restricted access to eligible subjects (catchment area of hospitals, referral mechanisms, limited resources, etc.). Another important selection mechanism is subjects' consent to study participation which varies by setting and depending on the study design type.

Generally, experimental studies have more selected study populations, because of more restrictive eligibility criteria and because the study intervention and complex follow-up measures result in lower consent rates. Observational studies usually have less selected study populations, thus have better external validity. For descriptive studies, a representative study population is most important, as any reported frequencies are related to the study population as the only frame of reference. Thus, external validity is the main methodological criteria for descriptive studies.

The definition and process of selecting the study population needs to be reported in detail to allow readers to judge the generalisability of results [14].

Pharmacoepidemiologic studies generally comprise large study populations of more than 10,000 persons that allow detecting rare outcomes in order to supplement information from pre-marketing trials that usually include a few hundred to few thousand patients. Because of their scope, pharmacoepidemiologic studies should ideally be close to population-based in order to be representative of drug utilization and drug benefit and safety in all subsets of the population (Strom 2006).

2.2 Data sources

Pharmacoepidemiologic studies need to collect data on drug exposure and outcome, underlying disease and demographic data to delimit the study population, and further clinical and life-style data to control for confounding.

Data sources used for pharmacoepidemiologic studies are automated databases, pharmacy records, or physician records [7, 8]. Automated databases are the most valuable data source because they are large, usually complete, and their use is cost-efficient. There are two general types of databases, administrative databases

and medical records databases. *Administrative databases* are most commonly claims databases for reimbursement for prescriptions or other services from health insurances. Such databases provide the most valid data on drug exposure. However, their data on disease and outcome data are frequently less reliable. Data sets may be restricted to variable extent to population subsets, for example in a health insurance claim databases to those with coverage by that insurance system (e.g. Medicaid in the US). *Medical records databases* are specifically generated for research purposes and make use of the electronic documentation of diagnostic and therapeutic records both on in-hospital and out-patient care. Prominent examples are the *General Practice Research Database, GPRD*, in the UK [18] and the *PHARMO system of record linkage*, in the Netherlands [19]. Medical records databases usually have more valid disease and outcome data. However, their completeness may be an issue. One general limitation of automated databases is lack of information on potential confounders such as clinical factors and, particularly, life-style factors. Different databases are frequently linked to combine complementary information and also to validate some of the information. Another approach for completion and *external validation* of data is by review of the original medical charts or by interviewing patients [20, 21]. The latter approaches are very resource intensive, and data obtained from patients is the least reliable [22].

2.2.1 Exposure

In the context of PE studies, exposure is the use of a drug (or several drugs). Information on drug exposure is obtained from prescription records, either health insurance claims databases, pharmacy or physicians records, post-marketing monitoring of specific drugs, or from drug sales records (e.g. IMS Health). The latter are usually not based on individual person data, and can therefore only be used for descriptive studies. Certain exposure data can only be obtained from medical records or directly from patients such as compliance with medication, intermittent drug use for symptom relief, use of over-the-counter drugs, or information on life-style factors [7].

Drug exposure is challenging to measure as it is subject to much variation that may be more or less accurately documented in the data source used. First, drug exposure is time-dependent, as patients take drugs for different durations and sometimes intermittently. In most cohort studies, the time of drug initiation for each individual is fixed as the starting point of observation, and sometimes the observation is censored when the drug is stopped. The extent to which time-dependent information is important and needs to be dealt with in the analysis depends on whether the drug effect is immediate or delayed, reversible after stopping the drug or permanent, idiosyncratic or cumulative. Second, drugs may be

taken at different dose levels and it is usually relevant to determine whether an effect is dose-dependent. The straightforward approach is to compare different dose strata. However, doses levels in individuals may also change over time. Such complex situations can only be dealt with by treating exposure as time-dependent and dose-dependent variable in mixed linear regression models.

Multiple factors (demographic, socio-economic, life-style factors and health status) determine the use of a drug. These factors which will likely differ between users and non-users of the drug and might therefore act as confounders. Consequently, the basic prerequisite is to collect as much information as possible on these factors. How to control for confounding will be discussed later.

2.2.2 Outcome

Outcomes in PE studies are beneficial and adverse effects, and data on the economic impact of the drug(s) of interest. Data sources for outcome data are national disease and mortality statistics, health surveys, reportable disease registries, primary care and ambulatory care records, hospital admission and discharge records, disease-specific registries, post-marketing monitoring of specific drugs, and spontaneous reporting to adverse drug reaction surveillance programmes [7].

Important properties of outcome parameters are that they should be sensitive to the exposure effect, clinically relevant, objective, and feasible to determine. There is some trade-off in fulfilment of each of these requirements.

In general, we differentiate clinical outcomes and surrogate outcomes. *Clinical outcomes* may be *outcome events* such as mortality or morbidity, i.e. occurrence or disappearance of disease, which can be ascertained with objectivity. These are complemented by *patient-reported outcomes*, subjective parameters such as perception of pain, physical status and quality of life, which are more difficult to assess but are most relevant to the patient.

Biometric parameters such as physical measures, laboratory parameters, radiographic results, are used to objectify the diagnosis of disease. Used as isolated parameters, they may serve as *surrogate outcome* only if their association with the clinical outcome in question has been well established by previous studies.

2.3 Measurement issues

We can distinguish three principal ways to acquire information in quantitative observational clinical research. We can (1) *observe* events or conditions *as assessors* (2) *ask* study participants either by written questionnaires, structured face-to-face interviews, or telephone interview or (3) *measure* physical (e.g. anthropometrics) and chemical quantities or use biomarkers.

Either method has benefits and disadvantages and it depends on the type of information that we are interested in, the feasibility of the method, ethics, the number of necessary measurements, the available budget, and so on. Therefore decisions have to be made in every single study, and it always needs critical appraisal to assess whether the decisions were reasonable and adequate.

Every method should have sufficient validity (close to the anticipated true values), should be reliable (have a good reproducibility between different observers and within individual observers over repeated measurements) and should be responsive to the effect of interest (measure at the right scale).

Measurements of exposure may be more distant (like long-term drug intake, probably measured best by questionnaires, though hampered by a potential social desirability bias, or recall problems), or more proximate by measuring drug concentrations (with the problem of measuring at the wrong site if the target tissue is not easily accessible, the wrong metabolite or at the wrong time). If markers of susceptibility are measured we sometimes have to decide between phenotypic and genotypic tests. If biomarkers are used as early outcomes to predict later clinical disease this usually saves observation time, sample size (many assays give numeric results which are statistically more efficient) and accordingly cost, but must be an essential step in the development of a disease. If they are not a necessary cause or are only intermittently produced they will underestimate clinical outcome, if they are not a sufficient cause or are non-specific they will overestimate the clinical outcome.

Most pharmacoepidemiologic research measures health-related events. Person, place, time, and social context are minimal required information to set research findings into context. No matter whether information is measured qualitatively or quantitatively the definition of a case is critical for the conduct and reporting of research: (1) Which method was used to measure exposures, confounders and outcomes, (2) Which boundaries were used when data were categorized (e.g. in diseased/case or healthy/control, in exposed *versus* unexposed) and (3) What was the unit of analysis (a person, a transient health event, an organ, a cluster of people from a district, etc.)

2.4 Measures of association and impact

As a matter of culture we tend to think chance in terms of probability or *risk*. If we want to compare the risk between exposed and non-exposed groups, the ratio of the risks of outcome in the two groups is an obvious solution and well known as the *risk ratio* ($RR = \text{risk}_{\text{exposed}} / \text{risk}_{\text{controls}}$). If the RR equals one, the exposure has no effect on the outcome. If the $RR > 1$ the exposed group has a higher risk for the outcome, and given the outcome is adverse a $RR < 1$ indicates a protective exposure, as this is usually reported in clinical trials with beneficial effects. This measure of effect is therefore preferred wherever possible. The same concept may be used if we incorporate observation time into our frequency measure and get event *rates* (events/person-time).

The corresponding effect measure is the *rate-ratio*. *Hazard ratios* are a comparable measure, taking into account time-to-event information. An alternative way to describe effects is the *risk difference* between the comparison groups ($RD = \text{risk}_{\text{exposed}} - \text{risk}_{\text{controls}}$). If this risk difference represents a causal effect, it may also be called the *attributable risk* (AR), and can be easily used to calculate the number needed to treat or harm ($NNT = 100/AR$). If mortality is the outcome, the number needed to treat represents the number of people that have to be treated (exposed to the intervention) to avoid one death. This number must be seen in the context of disease frequency and severity.

Measures of effect provide us with valuable information about the relative risk in the exposed group. In other words, given a causal effect, we know how many cases in the exposed group are attributable to the exposure ($RR > 1$) or prevented by the exposure ($RR < 1$). However, in public health we are also interested in how many cases in the total population are attributable to an exposure. This involves not only the effect of the exposure, but also the frequency of the exposure in the population. The population attributable fraction (PAF) is commonly used to express the impact of an exposure in a population. It can be calculated as $PAF = (\text{risk}_{\text{total population}} - \text{risk}_{\text{controls}}) / \text{risk}_{\text{total population}}$. Alternatively, if we have adjusted relative risks available we can incorporate the prevalence of the exposure (p_{exp}) into a useful equation.

$$PAF = \frac{(RR - 1)p_{\text{exp}}}{(RR - 1)p_{\text{exp}} + 1}$$

However, to calculate a risk, we need the number of events per population at risk, and in observational studies there are situations, where we have no sufficient information about the population of risk. The case control study is the stereotype for this situation, because controls are only a selected proportion of the risk population. Here the more general odds ratio can be used to describe an effect. The odds ratio is calculated as the ratio of the exposed odds *versus* the non-exposed odds of the outcome, which is identical to the ratio of odds of exposure in those with the outcome over the odds of exposure in those having not experienced the outcome. From a simple 2×2 table the difference between risk ratio and odds ratio can be easily seen.

| | | Outcome experienced | |
|---------|-----|---------------------|----|
| | | Yes | No |
| Exposed | Yes | a | b |
| | No | c | d |

$$\text{OR} = \frac{a/b}{c/d} = \frac{ad}{bc} \quad \text{RR} = \frac{a/(a+b)}{c/(c+d)}$$

Both for the odds ratio and the risk ratio we have to assume equal observation times for the comparison groups. If the outcome is not very frequent, the odds ratio has a comparable size as the risk ratio and can be used to approximate the relative risk. For frequent outcomes, however, the odds ratio overestimates the relative risk and must be interpreted with caution. Whenever possible the risk ratio should be used to describe an effect, exceptions are observational study designs where the population at risk remains undetermined. Noteworthy, the odds ratio is frequently reported in observational studies instead of the more adequate risk ratio, because odds ratios are a direct output from logistic regression models that are standard methods to adjust for confounders.

2.5 Interpreting an effect: bias, confounding, and sampling error

Whether such an effect reflects a true association cannot be verified easily. Rather we accept an association as true if we can exclude flawing factors. These sources of error are: (1) Bias, (2) confounding and (3) sampling error. If we have sufficient reasons to declare all these three factors as insufficient to distort our effect, we have a good indication that the effect is a true association. This is referred to as *internal validity* of a study and is strongly related to study design. We will now get into some more detail below and give more examples when we discuss the study design types in depth.

2.5.1 Bias

Bias can be seen as a systematic error contained in the study design, conduct or interpretation of a study. Whereas extensive lists of particular bias forms exist, there are two basic forms of bias:

- Selection bias
- Information bias

Selection bias occurs if study populations are selected in an erroneous way that comparison groups are not comparable. Depending on the study design this may be a problem of selecting cases, selecting controls, selecting unexposed groups or having no identical follow up between comparison groups.

Information bias occurs if measurements are different between comparison groups. Typically for case control studies this refers to a different measurement methods (interview for controls, chart review for cases) or measurement errors (different recall of distant items between cases and controls due to the disease under investigation) between

cases and controls, in cohort studies the major problem arises from measurement problems of the outcome. Blinding is generally a good feature to protect against information bias.

Bias is therefore usually a problem in the study design, and therefore the study methodology gives us the clue to whether we are faced with a biased effect. The more important question is, however, whether the potential bias matter. Sensitivity analyses are recommended to answer this question. We will discuss specific sources of bias with the study examples later.

2.5.2 Confounding

Confounding is a nuisance effect that distorts the association between a risk factor and an outcome by another factor. This factor is called a confounder, and it must be associated with the risk factor, must be associated with the outcome but must not be on the causal pathway between risk factor and outcome. Typically many confounders act simultaneously. Suppose that we find an association between carrying lighters in pockets and lung cancer. You would probably say that smoking is a good alternative explanatory factor for this association. Smoking will be associated with carrying lighters in pockets, and smoking is a well-established risk factor for lung cancer. Furthermore it is not reasonable to consider that people carry lighters, therefore smoke, which then results in lung cancer. Smoking is therefore a perfect candidate as a confounder. The usual methods to handle confounding are restriction, stratification, matching or multivariable modelling. Multivariable regression models are the contemporary tools used for adjustment, with exposure propensity scores as a specific application in the context of pharmacoepidemiology, though not without controversy [23]. Other calibration techniques e.g. by using external data are available [24]. If correlated observations like repeated measurements are incorporated in the analyses, more complex techniques like random effects models mixed models or generalized estimation equations are standard frequentistic methods, and also Bayesian methods are available. The causal pathway issue has gained some attention in the last years when the concept of non-lipid effects of statins was described. When the effect of statins on cardiovascular outcomes was examined, lipids could be seen as potential confounders. The problem here is the obvious causal pathway: we would consider at least in part that the statin effects are due to lipid modulations. If we now adjust the statin effect on lipid changes by using regression methods we get the “non-lipid” effects of the statins [25, 26]. The major source of uncertainty, however, comes from unmeasured confounders, which results in residual confounding – a shortcoming that can only be mastered by appropriate randomization and is therefore inherent in non-randomized observational research.

As confounding is always present in observational research, meaningfully adjusted effects should be looked at rather than crude unadjusted effects.

2.5.3 Sampling variation

Sampling variation or the play of chance is another error that may influence an association. Statistical methods can be used to describe the amount of uncertainty that is due to sampling error – an effect that follows a law of nature whenever we draw samples – as we do in every clinical study. The usual frequentistic way is the presentation of the 95% confidence intervals. These intervals provide us with a range where we can be 95% confident that the effect in the underlying population will be. If this confidence interval includes our no-effect level (e.g. one for a relative risk), we would say that the observed effect might be explained by chance alone. If the confidence interval does not include the no-effect level, we can say that this effect is beyond a chance finding. Other possibilities to quantify sampling error include the calculation of p -values. For details please refer to Chapter 12.

3 Overview of study design types

Figure 1 shows a systematic overview of study design types. Study types are differentiated based on certain design principles: studies using individual patient

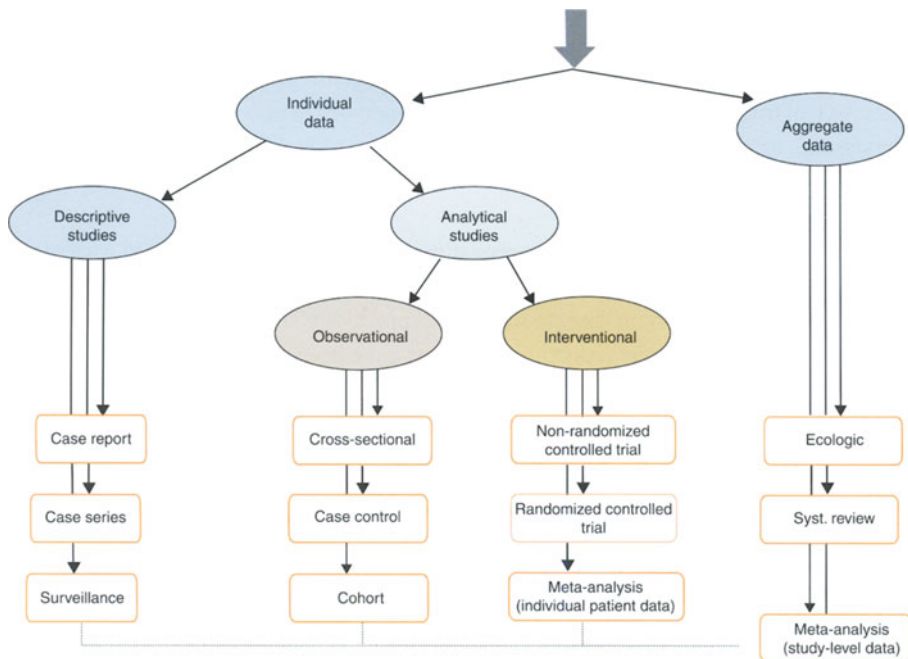


Fig. 1 Systematic overview of study design types

data *versus* aggregate data, descriptive *versus* analytical studies, and within the latter, observational *versus* experimental studies [27]. The following section will briefly discuss study design types used in pharmacoepidemiology with a focus on observational studies.

3.1 Descriptive studies

As the simplest form of descriptive studies, *case reports* and *case series* play an important role in detecting adverse drug reactions (ADR). Spontaneous reporting of cases of suspected ADR to pharmacovigilance systems have become the primary method of collecting post-marketing information on drug safety [28]. Spontaneous reporting systems depending on voluntary reporting from individual health care providers have been implemented in many countries (MedWatch, US; EU pharmacovigilance system; WHO Programme for International Drug Monitoring). Other sources are case reports/series of suspected ADR reported in the medical literature.

Suspicious about an ADR usually arise from individual or a cluster of unusual clinical manifestations observed in users of a drug. Spontaneous reports can be the initial step to identify ADR that are unexpected, or too rare or with long latency to be detected in premarketing trials. However, very few definite conclusions can be drawn from case reporting. Limitations are incomplete information on the numerator (all cases) due to underreporting and selective reporting (the most unusual cases). Moreover, lack of information on the denominator (all persons exposed to the drug) makes it impossible to determine the frequency of reactions. Finally, case reports cannot establish an association between a drug and a reaction because there is no comparison with non-users of the drug.

However, data from spontaneous reporting databases may be combined with other data bases containing information on exposed populations (drug sales, prescriptions) or background information on disease incidence (morbidity statistics). Recently, sophisticated uses of spontaneous reporting databases have been implemented, such as data mining using Bayesian algorithms [29]. Such systems search for unexpected occurrences and hidden patterns of associations. *Proportional reporting ratios* compare the proportion of a reported ADR for a specific drug to the proportion of reports for related drugs or all other drugs [30].

Typical descriptive studies are *surveillance studies* that describe the distribution of characteristics in a defined population [31]. As mentioned earlier, for descriptive studies a representative study sample or a population-based study is essential since the denominator for reported frequencies is the only frame of reference. Examples in pharmacoepidemiology are studies on drug utilization that collect information to estimate the number and characteristics of persons using a drug in a population, deriving data from prescriptions records or drug sales

(e.g. IMS Health). Other examples are studies describing disease incidences such as national morbidity or mortality statistics. Moreover, post-marketing surveillance studies on specific drugs are frequently purely descriptive as they lack a control group.

There is frequently an overlap between descriptive and analytical studies. For example, within the study population, drug utilization may be compared between subgroups based on demographic characteristics, e.g. age, morbidity, socio-economic status. Surveillance studies are frequently repeated at regular intervals and comparison over time allows analysing time trends. Similarly, there may be comparison between populations (hospitals, regions and countries).

Time trends or comparison between populations are usually not based on individual patient data but on aggregated data from population clusters. Such studies are called *analyses of secular trends* or *ecologic studies*. In the context of drugs, this could be analysis of drug utilization and disease morbidity in parallel over time or between populations and how these coincide. Coinciding trends may provide hints on possible associations, e.g. ADR. An example would be to study sales data for oral contraceptives and compare these to mortality from thromboembolism using vital statistics, over several years. However, such trends may be related to other factors that change at the same time. Since, in an ecologic study, the comparison is not based on individual person data, it is impossible to control for these factors. False conclusions about individual-level associations from ecologic studies are called *ecologic fallacy*.

3.2 Analytical study designs

Among the analytical study design types, the observational studies (cross-sectional, cohort, case-control studies) will be discussed in this chapter while experimental studies (randomized controlled trial) are discussed in Chapter 8.

Recently, the *STROBE guidelines* (STrengthening the Reporting of OBservational Studies in Epidemiology) have been published to set standards for reporting of observational studies [14, 17]. As observational studies are prone to bias and confounding, detailed reporting of all methodological details is essential to judge their validity. The guidelines are not intended as a prescription for designing or conducting studies nor as an instrument to evaluate the quality of observational research. However, the STROBE guidelines provide a checklist of study design items to be reported which is very useful as a reference when planning a study to make sure all issues are addressed. Similarly, the checklist may be considered when appraising an observational study. Whether observational study protocols should be registered prospectively in publicly accessible registers (like clinicaltrials.gov) is currently debated [32].

3.2.1 Cross-sectional study

A cross-sectional study assesses the presence of exposure and outcome in members of a study population at the same point in time and determines whether an association exists between being exposed and having the outcome [27]. This type of design is primarily applicable to prevailing exposures, e.g. genetic dispositions, and prevalent outcomes, i.e. chronic diseases, and does not allow assessing time-relationships and the incidence of outcomes. Cross-sectional studies are not ideal for pharmacoepidemiology since drug exposure is a time-dependent variable and drug effects occur over time.

3.2.2 Cohort study – principles and practical example

Cohorts are defined as groups of persons sharing certain demographic and clinical characteristics [33]. In the widest sense, persons may stem from the same background population but frequently cohorts are defined by presence of a certain disease. Within a cohort, those exposed to a defined risk factor (*exposed group*) are compared to those without the risk factor (*unexposed or control group*) and are followed forward in time looking for differences in the incidence of defined outcomes. Control groups may also be persons exposed to a different risk factor. In some instances, exposed and control subjects may stem from different populations that differ with respect to the exposure factor (e.g. workers at two different factories). In this situation, the control group needs to be assembled by matching on relevant demographic characteristics (*external controls*). Pharmacoepidemiologic cohort studies identify persons exposed to a specific drug and compare these to persons not exposed to that drug (or exposed to a different drug) who stem from the same population (cohort). Persons not exposed to the drug may be considered external controls, as they differ in many baseline characteristics (e.g. underlying disease) from those requiring the drug. Outcomes are measures of drug effectiveness, drug safety or cost-effectiveness.

All subjects of the cohort may enter the study at one point in time (closed cohort) or subjects may be allowed to enter at different time points within a defined study period (open cohort). In drug cohort studies, study-starting point should ideally be the time when an individual starts the drug. The study ends for the individual when the outcome is reached or when the subject is censored (when stopping the drug, after a fixed period, or when the study observation ends). At study start, all members of a cohort must be free of the outcome and are followed over time to compare the incidence of outcome between the two groups. The unexposed group is supposed to provide the background incidence and a significant difference in the incidence of outcome in the exposed group is inferred to be related to the exposure.

The cohort study design conceptually looks forward in time although the timing of how they are planned and conducted may vary. In the past, the terms prospective and retrospective cohort study have been used but the STROBE statement disadvises to use

these terms as they are not sufficiently informative [17]. A cohort study may be planned prospectively, the study population defined and data collection performed prospectively. Alternatively, the data may have been collected prospectively but are used for analysis of *post hoc* study questions. Finally, a study question may be posed retrospectively, and the data derived from a database or even collected retrospectively from medical records or patients. The STROBE guidelines advise to report the time sequence of study objective, defining the study population, and collection of data in detail since each of these items have their respective sources of bias.

Drug cohort studies most commonly use existing automated databases that may or may not have been generated for that purpose (administrative databases). Other types of cohort studies using prospective data collection are national intensive monitoring programmes (e.g. Prescription Event Monitoring in the UK) [34], pharmacy-based drug surveillance studies [36], and *ad hoc* drug cohort studies, i.e. data collection for a specific study purpose. Drug surveillance databases usually allow comparison between different drug exposures but do not necessarily have data on non-exposed groups. Post-marketing drug surveillance studies performed by pharmaceutical companies do not usually include non-exposed control groups, therefore cannot be considered cohort studies.

The main advantages of cohort studies over other observational study designs are that they can determine (i) the frequency of outcome in defined populations and (ii) the time-dependence of outcome (incidence risk, rate and hazard). Cohort studies can determine the natural course of disease, the influence of various risk factors, and can generate risk and prognosis scores. The comparison is based on one primary exposure factor but several outcomes can be studied in parallel. Cohort studies are applicable to relatively uncommon exposures, thus are useful for post-marketing drug surveillance studies. However, they are less useful for rare outcomes. Cohort studies are frequently very large and may be conducted over long periods. There are prominent examples of cohort studies in the literature that have identified major risk factors in population health, such as the Framingham Heart study, British Physicians' Health study, Nurses' Health study, General Practitioners' Oral Contraceptive study, etc. [35]

Cohort studies usually have good external validity. Because of their observational character and more pragmatic follow-up, they have less selection in recruitment and retention of subjects than experimental studies. Therefore, cohort studies may serve to validate the results of clinical trials. While clinical trials assess the *efficacy* of a medical intervention under somewhat artificial conditions, cohort studies allow assessing *effectiveness*, the effect of an intervention under real life conditions [11, 37]. Cohort studies can also serve to assess *efficiency*, i.e. cost-effectiveness of an intervention.

Cohort studies usually have better internal validity than other observational studies (case-control and cross-sectional) because of their "prospective" study concept, definition of the study population, and better control over the timing of events. Thus, in

general, an association found by a cohort study is more likely to be true than from a case-control study. However, cohort studies still have large potential for bias and confounding [38, 39].

Selection bias occurs through differential representation of subjects in the exposed and unexposed study group. Selection bias may occur during recruitment and follow-up of cohort studies. Selective recruitment can occur if the reason for referral of a patient, e.g. to a hospital, is related to drug exposure (*referral bias*). Selection occurs if the participants' decision to participate in the study is influenced by drug exposure status (*self-selection bias*). *Differential loss to follow-up* can occur, for instance, if patient dropout of the study is related to an ADR. Thus, the study may not detect or underestimate the frequency of the ADR. Inversely, patients responding well to a drug are more likely to remain in the study that could result in an overestimation of the drug effect.

Information bias may result from misclassification of outcome status if influenced by knowledge of exposure status. *Detection bias* may occur when exposed subjects have different procedures of follow-up, e.g. more frequent visits. *Diagnostic suspicion bias* occurs when exposure status influences the interpretation of diagnostic tests. The obvious solution is blinding outcome assessment to exposure status and standardization of assessment. However, this is problematic in retrospective data collection.

Confounding is also an inherent problem in cohort studies because exposed and non-exposed groups will always differ in other demographic and clinical variables. A form specific to drug studies is *confounding by indication* (channelling), as the indication (disease) for receiving a drug will be related to outcome, particularly when considering drug efficacy [40]. An example was that statins were believed to reduce the risk of Alzheimer's disease based on an observational study [41] which was disputed by a randomized trial [42]. The explanation for the findings of the observational study was that physicians were reluctant to prescribe statins to Alzheimer patients, therefore exposure to statins was associated with a lower frequency of Alzheimer's disease [43]. Adjusting for indication is difficult, as it is a multifactorial phenomenon, and it is inherently not present in non-users of the drug. Ways to deal with confounding in non-randomized studies are *matching* or *restriction* on important covariables. A more advanced way is the calculation of *propensity scores*, which are used to increase the comparability between treatment groups [44, 45]. Given that multiple factors influence the indication for receiving a drug, propensity scores express the probability of being treated given an individual's covariables. Propensity scores are estimated using logistic regression with exposure (treatment) as the dependent and covariables influencing treatment as the independent variables. The treatment effect can be estimated using propensity scores for (i) matching, (ii) stratification, and (iii) as covariable in regression analysis. By use of the propensity scores, the influence of all covariables used for its estimation is adjusted for. However, unmeasured confounding may remain.

3.2.3 Case-control study – principles and a practical example

Basically, the case control design starts with sampling a group of cases, individuals that have experienced the outcome of interest. Then the comparison group is established from people that are free from the outcome, who are called controls. In a next step the exposure is measured retrospectively in both cases and controls. The difference in exposure then constitutes the effect.

A prominent early example of this convincing design was the study by Doll and Hill on the effect of smoking on lung cancer published in 1950 [48]. At that time increasing attention was paid to risk factors for malignancies [49]. Specific malignancies are relatively rare outcomes that usually evolve after a long latency. In this situation prospective Cohort studies take a very long time and involve huge populations. Lung cancer is currently the most frequent malignancy in developed countries. The age adjusted annual incidence in the UK is 47 per 100,000 population. Accordingly, to detect only one case an average group of more than 2100 individuals needs to be followed up. For less frequent malignancies, like malignancies of the brain with an estimated annual incidence of 7 per 100,000 more than 14,000 individuals have to be examined to expect one case (cancerresearchuk.org). Because exposure and outcome are assessed at the same time, the case control design is very efficient, quick and usually much cheaper than cohort studies or interventional studies [52]. Accordingly, the case-control design is frequently used in infectious disease outbreak research. Sample size of cohort studies is very much depending on the frequency of the cases. As cases can be accessed directly within the population in a case control study, it is the perfect design to investigate rare diseases. Adverse drug reactions are therefore a sensible application for this design in pharmacoepidemiology [6, 53]. Recently also applications in pharmacoconomics are reported [10].

The disadvantage of the case control design is the enormous potential for several forms of bias. Advanced skills are necessary to perform and interpret meaningful case control studies. This fact is aggravated by the observation that novice researchers frequently perform case control studies, because they are so resource efficient.

Juurlink and co-workers have used the case control design to investigate the drug interaction between proton-pump inhibitors and clopidogrel [54]. We will now discuss details of the case control study design along this example.

The rationale for this research is the frequent co-medication of platelet aggregation inhibitors for ischemic heart disease and proton-pump inhibitors to reduce the risk of gastrointestinal side effects of antiplatelet therapy based on recommendations from accepted guidelines [55, 56]. On the other hand, there is some evidence suggesting a drug interaction between clopidogrel and proton pump inhibitors. Clopidogrel is a pro-drug, which is activated by hepatic cytochrome P450 isoenzymes, whereas some proton-pump inhibitors can inhibit the important cytochrome P450 2C19. The resulting reduction in antiplatelet activity of clopidogrel was assumed to cause adverse outcomes

particularly in patients after high-risk coronary interventions [57]. For a quick and meaningful investigation of this important health problem the researchers opted for the case control design.

3.2.3.1 The cases

Cases were patients who had myocardial re-infarction within 90 days after hospital discharge for an initial infarction. The cases included Ontario residents after myocardial infarction on clopidogrel in 2002–2007 who were aged 66 years or older and had died or were readmitted with myocardial infarction. Cases were identified from a database when having hospital admission ICD-codes I21 and I22. Linking four different national databases generated this database.

Accordingly all incident cases were detected, which prevents the exclusion of the very sick patients who are more likely to die early and being not represented in the study then. The inferior alternative would have been to include only prevalent cases after myocardial re-infarction who were for example cared for in cardiology clinics. Prevalent cases would not necessarily be representative for possible cases, and the risk factors examined would include not only those related to acquiring the disease, but also those associated with longer survival – which is in fact usually not the primary study question.

On the other hand people were identified by a diagnosis code from a database. The possibility of misclassification, such as examining cases as controls and *vice versa* is a matter of concern, because it entirely relies on a single code. Treating physicians without any knowledge about the study question usually do this coding. Therefore misclassification may occur, but will usually be non-differential and may counterweight the precision gained by the large numbers available in database studies. However, there is some evidence, that the coding process is satisfying in this region [58, 59]. Some particular outcome classification problems are foreseeable if diagnostic criteria undergo profound changes during a study. For instance a change in the clinical case definition from the WHO criteria to ESC/ACC criteria resulted in an increased prevalence of the diagnosis by more than 35% [60]. This means, that some individuals that were cases in the later period of the study would have been potential controls in the earlier phase. To some extent the degree of miscoding depends on the disease that constitutes the outcome [61, 62]. As a consequence mortality remains the most robust outcome, and morbidity outcomes must always be seen with caution.

Another issue is the selection of cases from the health related databases, because it only includes people that have ever received a health card. In countries with non-universal insurance coverage, the wealthier people are under-represented which hampers the generalisability of estimates. Likewise unemployed people may drop out in other health-insurance systems. Methodologically this is of concern, because socioeconomic status has a well-established association with many health outcomes [63–65].

However, in Canada the coverage is expected to be high, so this issue should not be too important for this example [7].

3.2.3.2 The controls and the source population

The next step in a case-control study is the selection of controls. An unbiased selection of controls requires that the controls are representative of the population that generates the cases. Therefore this is a good time to ask, what represents the source population. In our example, the population consisted of Ontario residents aged 66 years or older after a myocardial infarction, who received clopidogrel. People were excluded if they had clopidogrel before the index myocardial infarction, if they were cared for in long-term facilities or if they had proton-pump inhibitors for helicobacter eradication, because these indicate different conditions and may introduce unnecessary scatter.

In this study the source population could be sufficiently described by linking four different national databases. Information for every individual was available. Controls were then selected by random sampling from the source population, thus yielding a representative, i.e. unbiased group.

This condition is usually hard to achieve, in particular if the cases come from tertiary care hospitals. Clinical research is often performed in tertiary care hospitals, but usually it is difficult to define the source population. Some methods have been developed to acquire a somewhat unbiased control sample. One way to go is to access people by calling them randomly – a method known as random digit dialling. This is a good option if the source population is the general population, but excludes people who do not have a telephone or do not want to respond. These are typical sources of selection bias. Cases could also be asked to invite friends to participate as controls if they have not got the disease. This method is denoted proxy matching. Here, some typical risk factors like socioeconomic status, age, and sex are usually constant within these case-control pairs. That reduces confounding, but the pairs must not be matched on the risk factor of interest. It depends very much on the study question whether this method yields an unbiased and sensible sample. More frequently researchers use controls from other departments within a hospital, intending that the controls are free of the outcome. The problem with this approach is the assumption that the exposure factor distribution in the controls reflects the source population, which is usually not true and introduces severe selection bias. As an example if we wanted to know whether alcohol abuse induces liver cirrhosis, we could select cases from a hepatology clinic. We could acquire controls from the trauma department thinking that this is an entirely different discipline. If we find out that the proportion of alcohol abuse is not very different between cases and controls we would falsely conclude that alcohol abuse is not associated with liver cirrhosis. This is a typical example of selection bias, because the controls are not representative of the source population, but in fact selected to a trauma department according to the exposure factor alcohol abuse. Except from the study

example with a complete sampling frame of the source population, no method of control selection is perfect, and in doubt two methods should be used simultaneously to select controls. If the results from both methods are comparable, we will have more confidence in the robustness of the research [66].

3.2.3.3 Measurement of the main exposure factor

Juurlink et al. again used their database to identify individuals that have received proton pump inhibitors. They measured this risk factor identically for cases and controls from one reliable source. This is not always a simple task. For example, if hospitalized cases are compared to non-hospitalized controls, a chart review will only be possible for the cases, and controls might then be interviewed – an obvious source of information bias. But also if database information is used, entries on drug exposure may be different for in-patient periods and ambulatory care, because data collection modes may differ, sources of supply may differ and the dosing may differ. Juurlink mastered another frequent problem in case control studies by searching prescription databases, because they were independent of patients' recall. In fact in some situations it is apparent that cases will recall certain events much better than healthy controls that have not been concerned with a serious medical condition. This specific form of information bias is referred to as recall bias. Typical case-control studies measure the exposure factor in a retrospective manner, and unlike in our example have no control over the sequence from risk factor to the outcome, because the exposure factor is measured when the outcome is already present. Sometimes an early clinical outcome may be falsely taken as an exposure factor, and the wrong conclusion is referred to as reverse causality. For instance when meat consumption is erroneously examined as risk factor for gastric cancer, it may turn out that the cases with stomach cancer have lower meat consumption than healthy controls. This finding, however, is better explained as an early clinical symptom of the disease than as meat being protective against stomach cancer.

3.2.3.4 Handling potential confounders

Confounding is a central issue in observational clinical studies. Generally there are some methods available to handle confounders if sufficient information is available, like multivariable regression modelling. The major limitation is unmeasured residual confounding. In case-control studies the controls are selected, so it is appealing to select the controls along known confounders. The technical term for this procedure is matching. Matching is frequently used in this setting to handle confounding by creating case-control pairs with equal confounder levels. Thereby the influence of the matched variable is cancelled out within a pair. Matching can be very efficient if only a few variables (usually age and sex) are used, but may be logistically very complex if either the populations are small or the number of matching variables is high. Juurlink

et al. used four matching variables and had some problems to find the intended three matching controls per case despite the large available cohort. In some situations matching may be superior to simple multivariable adjustments. For instance when socioeconomic status is hard to measure correctly thus proxy matching adjusts for measurable and non-measurable factors at once. On the other hand, overmatching may occur if the matching variables are no strong confounders thus obscuring true effects. Noteworthy most matched designs require a matched analysis, because data are not any more independent. Conditional logistic regression models are typical applications. Despite all advantages of database-studies, important clinical information that may include confounding factors is usually not completely contained [67], as well as more complex information like multiple diseases whether related or not. In the actual study example some important factors like smoking status, blood pressure or over-the-counter aspirin could not be considered, because this information was not sufficiently available.

3.2.3.5 Analysis and results

In contrary to cohort studies, we compare cases to controls here, as usually reflected in a “characteristics of participants” table (Table 2). Crude (i.e. unadjusted) estimates of the risk factor differences can be seen here, as well as imbalances in other factors that may turn out as potential confounders. In many studies cases have more co-morbidities than controls. The proportion of cases *versus* controls only depends on the researcher choice, and usually does not reflect the incidence of the outcome in the population. It is therefore not directly possible to calculate risk ratios of the outcome in exposed relative to non-exposed like in cohort studies. The approach, however, is to compare the frequency of exposure in cases relative to controls. An odds ratio is an appropriate measure to describe such an association, and is therefore the standard output from case

Table 2 Association between exposure to proton pump inhibitors (PPI) and recurrent myocardial infarction among patients who started taking clopidogrel following index myocardial infarction

| Exposure to proton pump inhibitor | Cases (<i>n</i> = 734) | Controls (<i>n</i> = 2057) | Unadjusted odds ratio (95% confidence interval) | Adjusted odds ratio * (95% confidence interval) |
|--|----------------------------|--------------------------------|---|---|
| None | 448 (61.0) | 1317 (64.0) | 1.00 | 1.00 |
| Current PPI use (within last 30 days) | 194 (26.4) | 424 (20.6) | 1.32 (1.08–1.62) | 1.27 (1.03–1.57) |
| Pantoprazole | 46 (6.3) | 125 (6.1) | 1.06 (0.74–1.52) | 1.02 (0.70–1.47) |
| Other proton pump inhibitor | 148 (20.2) | 299 (14.5) | 1.43 (1.14–1.80) | 1.40 (1.10–1.77) |

Adapted from Ref. [54]

control studies. Multivariable logistic regression provides very flexible models to directly estimate odds ratios, to simultaneously adjust for confounders and allow for dependence in matched designs. They are also used to describe the amount of uncertainty due to sampling error, by providing confidence intervals.

From this example we see that 26% of cases were exposed to proton pump inhibitors compared to 21% of the controls. After multivariable modelling Juurlink et al. found in this sample that the odds of proton pump exposure were 1.27 times higher in the cases compared to controls. The confidence interval indicates that we can be 95% confident that this odds ratio will be between 1.03 and 1.57 in the population. This confidence interval did not include the null hypothesis (i.e. $OR = 1$ indicating no difference), therefore this effect is beyond what can be explained by chance alone. The easier way is to say that this is a significant effect.

Noteworthy not all proton pump inhibitors have the same effect on cytochrome P450 2C19 inhibition [68]. To investigate whether these differences in biological action translate to clinical effects, a stratified analysis was conducted. Expectedly pantoprazole, which has no reported cytochrome P450 2C19 inhibition, was not associated with recurrent myocardial infarction, whereas the other proton pump inhibitors were significantly associated with the outcome. Formally, a test for interaction should be used to test whether this difference in the effect is explained by chance alone.

3.2.3.6 Summary of case control studies

The case control study is a good method to assess rare outcomes or exposures with a long latency. Cases should represent typical cases, and controls should be representative for the source population that produces the cases. The difference in exposure is compared between cases and controls, expressed as an odds ratio for discrete exposures. Special attention should be drawn at the selection of controls and differences in exposure measurement between cases and controls. Reverse causality is the flawed interpretation of effects if early outcomes are assessed wrongly as risk factors. This is sometimes difficult to distinguish, as risk factor and outcome are assessed simultaneously.

3.2.4 Case-crossover studies

In a case-crossover study, each patient acts as his own control [69, 70]. The pattern of exposure is compared between the time when an outcome event occurred (event time) and control time. The main advantage is that between patient confounding is eliminated, because the comparison is within each patient. Case-crossover studies are suitable if the following criteria are fulfilled: (i) the exposure of interest must be transient (a drug taken intermittently), (ii) the outcome must be an acute event, and (iii) the risk associated with the exposure must be immediate and subside rapidly. If a patient experiences an outcome event he will be asked whether he has taken the drug

during a few hours before the event (risk period) and whether he had taken the drug, e.g. a week earlier (control period). In the analysis, the distribution of exposure during the risk period is compared to the control period. A challenge in this design is recall bias. Another disadvantage is that information on the timing when a drug was taken is not contained in administrative databases.

3.3 Meta-analysis of observational studies

Meta-analysis of randomized research is well developed and up-to-date methods are available [71]. Moreover, meta-analytic methods can be used for most observational study designs. The benefits of meta-analysis include a gain in precision, explicit description and handling of bias-risk and in detail examination of heterogeneity. However, Cochrane reviews were restricted to randomized studies for a long time, because observational research itself is very heterogeneous and in particular sources of bias are much more complex than in randomized studies. Nonetheless, recent advances in observational study meta-analysis methodology lead the Cochrane Collaboration to incorporate also non-randomized studies into their systematic reviews. Methodological issues of non-randomized studies are detailed in a whole chapter in the Cochrane handbook, and more importantly in the pharmacoepidemiologic context, a separate chapter is dedicated to adverse effects methodology. This regularly updated and enhanced information can be freely accessed from the internet (<http://www.cochrane-handbook.org/>).

Case Study (cohort study): The risk of venous thrombosis in users of hormonal contraception

Lidegaard et al. have used the cohort study design to assess the risk of venous thrombosis (VT) in current users of different types of hormonal contraception [46]. This study is discussed as a typical example of a population-based cohort study that used linkage of several registries of prescription, health and demographics. Although an association between combined oral contraceptives and VT had previously been shown, the *rationale* for the study was to determine the overall risk in a representative population, the risk in relation to the duration of use, in various combination regimens, various doses, and route of administration.

The *setting* was Denmark in the period of 1995–2005. *Study participants* were all Danish women aged 15–49 identified from the *Danish Central Person Registry*, but excluding women with malignant disease or a previous cardiovascular event, as identified by the *National Registry of Patients*. Periods of pregnancies, as identified from the *Abortion and Birth Registry* with pregnancy duration esti-

mated from gestational age, were excluded from the study observation period. The data were analyzed as time at risk (woman years). *Exposure* to contraceptives was obtained from the *National Registry of Medicinal Products Statistics* that contains all redeemed prescriptions on Danish citizens according to Anatomical Therapeutic Chemical (ATC) codes and the amount of drug in daily doses. Exposure was categorized according to time of usage (current use, previous use (during the study period), and never use), regimen (combined oral contraceptives, progestogen only, hormone releasing intrauterine devices), oestrogen dose (50, 30–40 and 20 µg), type of progestogen, and length of use of combined oral contraceptive users. Non-users (never and previous users) were used as reference group. *Outcome* was occurrence of a first deep vein thrombosis or pulmonary embolism, identified from the *National Registry of Patients* that contains discharge diagnoses from all Danish hospitals classified according to the International Classification of Diseases (ICD). The majority of diagnoses had been verified by ultrasonography or venography. Data on potential *confounders* was information on redeemed drugs for diabetes, heart disease, hypertension, and specifically diuretics, beta-blockers, calcium antagonists, ACE-inhibitors or angiotensin receptor-blockers, and lipid lowering drugs obtained from the *National Registry of Medicinal Products Statistics*; and information on individual's educational status from *Statistics Denmark*.

Results: In total, 10.4 million woman years were recorded of which 3.3 million woman years were during receipt of contraceptives. The crude absolute risk of VT in non-users was 3/10,000 and 6.3/10,000 in current users of oral contraceptives. The risk increased significantly with increasing age. The adjusted rate ratio for current use *versus* non-use was 2.8 (95%CI 2.7–3.0). The relative risk in users decreased with duration of use, decreasing dose of oestrogen, differed for various progestogens in combination products, but was similar to non-users for progestogen only contraceptives and hormone releasing intrauterine devices.

Discussion: There were several reasons to address the study questions by a population-based cohort study: (i) a representative sample was required to estimate the absolute risk of VT in contraceptive users; (ii) head-to-head comparisons of various contraceptives would be unfeasible in experimental studies; (iii) overall, VT is an infrequent outcome, but also occurs in non-users of contraceptives and contraceptives use only has a modest effect; thus a very large study was required; (iv) other factors are strong determinants of the risk of VT, e.g. age; thus representation of all age groups and adjustment was required. The main strengths of this study were its size and population-based design, resulting in high power and external validity. The study setting is unique through the linkage of several databases that provide complete nationwide data.

Thus, there was little room for selection bias, neither in recruitment nor loss to follow-up. In this way, the study could provide absolute risk estimates, and relative risk estimates for multiple aspects of contraceptive therapy. The study adjusted for calendar year to account for time trends in use of types of contraceptives and in diagnostic sensitivity for VT. There is some potential for observer bias regarding outcome assessment, as about 10% of diagnoses of VT were uncertain. Limitations of the study were that only few potential confounders were assessed. This is a typical problem of database studies that have to confine themselves to the data available in the database(s) or go through the cumbersome process of obtaining external data. The latter would have been impossible in a study of this size. Educational status of women was used as proxy for socio-economic status, which is common praxis. However, this does not fully reflect other factors such as life-style, health attitudes, etc. Two important factors have not been addressed that have documented influence on the risk of VT, namely family history or genetic predisposition for VT (e.g. the common Factor V Leiden mutation), and body mass index. These factors may have also been associated with exposure because physicians may have prescribed contraceptives with a lower perceived risk of VT (based on earlier studies) to women predisposed to VT. If women receiving lower risk contraceptives had a higher incidence of VT because of their predisposition, this would attenuate the risk estimated from this study. This is an example of confounding by indication or, in fact, “confounding by contraindication”.

Interestingly, a concurrent well-designed case-control study from Netherlands published at the same time came to quite similar conclusions [47]. However, the case-control study was limited to calculating odds ratios and did not assess time at risk data. Moreover, risk estimates from odds ratios were somewhat higher in that study underlining that case-control studies are prone to overestimating risks.

References

1. Medical Research Council (1948) Streptomycin treatment of pulmonary tuberculosis. *BMJ* 2: 769–782
2. Cochrane AL (1972) Effectiveness and Efficiency. Random Reflections on Health Services. Nuffield Provincial Hospitals Trust, London
3. Lewin L (1881) Die Nebenwirkungen der Arzneimittel. *Pharmakologisch-klinisches Handbuch*. Verlag August Hirschwald, Berlin
4. Meyler L (1952) Side Effects of Drugs. Elsevier, Amsterdam
5. www.rpsgb.org.uk/pdfs/museve3.pdf, accessed Jan 20, 2010
6. Jick H, Vessey MP (1978) Case-control studies of drug induced illness. *Am J Epidemiol* 107: 1–7

7. Schneeweiss S, Avorn J (2005) A review of uses of health care utilization databases for epidemiologic research on therapeutics. *J Clin Epidemiol* 58: 323–337
8. Hennessy S (2006) Use of health care databases in pharmacoepidemiology. *Basic Clin Pharmacol Toxicol* 98: 311–313
9. Tsang R, Colley L, Lynd LD (2009) Inadequate statistical power to detect clinically significant differences in adverse event rates in randomized controlled trials. *J Clin Epidemiol* 62: 609–616
10. Caro JJ, Huybrechts KF (2009) Case-control studies in pharmaco-economic research: an overview. *Pharmacoeconomics* 27: 627–634
11. Vandenbroucke JP (2004) When are observational studies as credible as randomized trials? *Lancet* 363: 1728–1731
12. <http://www.pharmacoepi.org/about/index.cfm>, accessed Jan 20, 2010
13. Aday L (1989) Deciding who will be in the sample. In: Aday L (ed.) *Designing and Conducting Health Surveys*. Jossey-Bass Inc, San Francisco
14. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP (2007) Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *BMJ* 335: 806–808
15. Friedman LM, Furberg C, DeMets DL (eds.) *Study population*. In: *Fundamentals of Clinical Trials*, 3rd edn. Springer, New York
16. Peters TJ, Eachus JJ (1995) Achieving equal probability of selection under various random sampling strategies. *Paediatr Perinat Epidemiol* 9(2): 219–224
17. Vandenbroucke JP, von Elm E, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ, Poole C, Schlesselman JJ, Egger M (2007) STROBE Initiative. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. *PLoS Med* 4(10): e297
18. Parkinson J, Davies S, Van Staa T (2007) The general practice research database: now and the future. In: Mann R, Andrews E (eds.) *Pharmacovigilance*, 2nd edn. Wiley, Chichester
19. Sturkenboom MCJM (2007) Other databases in Europe for the analytic evaluation of drug effects. In: Mann R, Andrews E (eds.) *Pharmacovigilance*, 2nd edn. Wiley, Chichester
20. Stürmer T, Glynn RJ, Rothman KJ, Avorn J, Schneeweiss S (2007) Adjustments for unmeasured confounders in pharmacoepidemiologic database studies using external information. *Med Care* 45(Suppl 2): S158–S165
21. Harpe SE (2009) Using secondary data sources for pharmacoepidemiology and outcomes research. *Pharmacotherapy* 29(2): 138–153
22. West SL, Savitz DA, Koch G, Strom BL, Guess HA, Hartzema A (1995) Recall accuracy for prescription medications: self-report compared with database information. *Am J Epidemiol* 142(10): 1103–1112
23. Austin PC (2008) The performance of different propensity-score methods for estimating relative risks. *J Clin Epidemiol* 61: 537–545
24. Schneeweiss S, Glynn RJ, Tsai EH, Avorn J, Solomon DH (2005) Adjusting for unmeasured confounders in pharmacoepidemiologic claims data using external information: The example of COX2 inhibitors and myocardial infarction. *Epidemiology* 16: 17–24
25. Robinson JG (2008) Models for describing relations among the various statin drugs, low-density lipoprotein cholesterol lowering, pleiotropic effects, and cardiovascular risk. *Am J Cardiol* 101: 1009–1015
26. Ludman A, Venugopal V, Yellon DM, Hausenloy DJ (2009) Statins and cardioprotection—more than just lipid lowering? *Pharmacol Ther* 122: 30–43.
27. Grimes DA, Schulz KF (2002) An overview of clinical research: the lay of the land. *Lancet* 359: 57–61
28. Härmark L, van Grootheest AC (2008) Pharmacovigilance: methods, recent developments and future perspectives. *Eur J Clin Pharmacol* 64: 743–752

29. Hauben M, Madigan D, Gerrits CM, et al. (2005) The role of data mining in pharmacovigilance. *Expert Opin Drug Safety* 4: 929–948
30. Szarfman A, Machado SG, O'Neill RT (2002) Use of screening algorithms and computer systems to efficiently signal higher-than expected combinations of drugs and events in the US FDA's spontaneous reports database. *Drug Safety* 25: 381–392
31. Grimes DA, Schulz KF (2002) Descriptive studies: what they can and cannot do. *Lancet* 359: 145–149
32. No Author listed (2010) Should protocols for observational research be registered? *Lancet* 375: 348, doi:10.1016/S0140-6736(10)60148-1
33. Grimes DA, Schulz KF (2002) Cohort studies: marching towards outcomes. *Lancet* 359: 342–345
34. Shakir SAW (2007) Prescription Event Monitoring in the UK. In: Mann R, Andrews E (eds.) *Pharmacovigilance*, 2nd edn. Wiley, Chichester
35. Dawber TR (1980) The Framingham study. *The Epidemiology of Atherosclerotic Disease*. Harvard University Press, Cambridge, MA
36. Borden EK, Lee JG (1982) A methodologic study of post-marketing drug evaluation using a pharmacy-based approach. *J Chronic Dis* 35(10): 803–816
37. Black N (1996) Why we need observational studies to evaluate the effectiveness of health care. *BMJ* 312(7040): 1215–1218
38. Grimes DA, Schulz KF (2002) Bias and causal associations in observational research. *Lancet* 359: 248–252
39. Mamdani M, Sykora K, Li P, Normand S-LT, Streiner DL, Austin PC, Rochon PA, Anderson GM (2005) Reader's guide to critical appraisal of cohort studies: assessing potential for confounding. *BMJ* 330: 960–962
40. Walker AM (1996) Confounding by indication. *Epidemiology* 7: 335–336
41. Wolozin B, Kellman W, Ruosseau P, Celesia GG, Siegel G (2000) Decreased prevalence of Alzheimer disease associated with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. *Arch Neurol* 57(10): 1439–1443
42. Shepherd J, Blauw GJ, Murphy MB, Bollen EL, Buckley BM, Cobbe SM, Ford I, Gaw A, Hyland M, Jukema JW, Kamper AM, Macfarlane PW, Meinders AE, Norrie J, Packard CJ, Perry IJ, Stott DJ, Sweeney BJ, Twomey C, Westendorp RG (2002) PROSPER study group. Pravastatin in elderly individuals at risk of vascular disease (PROSPER): a randomised controlled trial. *Lancet* 360(9346): 1623–1630
43. Glynn RJ, Schneeweiss S, Wang PS, Levin R, Avorn J (2006) Selective prescribing led to overestimation of the benefits of lipid-lowering drugs. *J Clin Epidemiol* 59(8): 819–828
44. Glynn RJ, Schneeweiss S, Stürmer T (2006) Indications for propensity scores and review of their use in pharmacoepidemiology. *Basic Clin Pharmacol Toxicol* 98(3): 253–259
45. Seeger JD, Kurth T, Walker AM (2007) Use of propensity score technique to account for exposure-related covariates. *Med Care* 45: S143–S148
46. Lidegaard Ø, Løkkegaard E, Svendsen AL, Agger C (2009) Hormonal contraception and risk of venous thromboembolism: national follow-up study. *BMJ* 339: b2890
47. Van Hylckama Vlieg A, Helmerhorst FM, Vandenbroucke JP, Doggen CJM, Rosendaal FR (2009) Effects of oestrogen dose and progestogen type on venous thrombotic risk associated with oral contraceptives: results of the MEGA case-control study. *BMJ* 339: b2921
48. Doll R, Hill AB (1950) Smoking and carcinoma of the lung: preliminary report. *BMJ* ii: 739–748
49. Cole P (1979) The evolving case-control study. *J Chronic Dis* 32: 15–27
50. <http://info.cancerresearchuk.org/cancerstats/types/lung/incidence/index.htm> (accessed Jan 14, 2010)
51. <http://info.cancerresearchuk.org/cancerstats/types/brain/incidence/index.htm> (accessed Jan 14, 2010)

52. Schulz KF, Grimes DA (2002) Case-control studies: research in reverse. *Lancet* 359: 431–434
53. Rodrigues LC, Smith PG (1999) Use of the case-control approach in vaccine evaluation: efficacy and adverse effects. *Epidemiol Rev* 21: 1–17
54. Juurlink DN, Gomes T, Ko DT, Szmitko PE, Austin PC, Tu JV, Henry DA, Kopp A, Mamdani MM (2009) A population-based study of the drug interaction between proton pump inhibitors and clopidogrel. *CMAJ* 180: 713–718
55. Bhatt DL, Scheiman J, Abraham NS, et al. (2008) ACCF/ACG/AHA 2008 expert consensus document on reducing the gastrointestinal risks of antiplatelet therapy and NSAID use: a report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents. *Circulation* 118: 1894–1909
56. Kushner FG, Hand M, Smith SC, et al. (2009) Focused Updates: ACC/AHA Guidelines for the Management of Patients With ST-Elevation Myocardial Infarction (Updating the 2004 Guideline and 2007 Focused Update) and ACC/AHA/SCAI Guidelines on Percutaneous Coronary Intervention (Updating the 2005 Guideline and 2007 Focused Update). *Circulation* 120: 2271–2306
57. Gilard M, Arnaud B, Cornily JC, et al. (2008) Influence of omeprazole on the antiplatelet action of clopidogrel associated with aspirin: the randomized, double-blind OCLA (Omeprazole CLOpidogrel Aspirin) study. *J Am Coll Cardiol* 51: 256–260
58. Austin PC, Daly PA, Tu JV (2002) A multicenter study of the coding accuracy of hospital discharge administrative data for patients admitted to cardiac care units in Ontario. *Am Heart J* 144: 290–296
59. Lee DS, Donovan L, Austin PC, et al. (2005) Comparison of coding of heart failure and comorbidities in administrative and clinical data for use in outcomes research. *Med Care* 43: 182–188
60. Kavsak PA, MacRae AR, Lustig V, Bhargava R, Vandersluis R, Palomaki GE, Yerna MY, Jaffe AS (2006) The impact of the ESC/ACC redefinition of myocardial infarction and new sensitive troponin assays on the frequency of acute myocardial infarction. *Am Heart J* 152: 118–125
61. Romano PS, Mark DH (1994) Bias in the coding of hospital discharge data and its implications for quality assessment. *Med Care* 32: 81–90
62. Kiyota Y, Schneeweiss S, Glynn RJ, Cannuscio CC, Avorn J, Solomon DH (2004) The accuracy of Medicare claims-based diagnosis of acute myocardial infarction: estimating positive predictive value based on review of hospital records. *Am Heart J* 148: 99–104
63. Carstairs V (2001) Socio-economic factors at area level and their relationship with health. In: Elliot P, Wakefield JC, Best NG, Briggs DJ (eds.) *Spatial Epidemiology, Methods and Applications*. Oxford University Press, Oxford
64. Eyler JM (1979) *Victorian Social Medicine: the Ideas and Methods of William Farr*. The Johns Hopkins University Press, Baltimore and London
65. Smith GD, Shipley M, Rose G (1990) The magnitude and causes of socio-economic differentials in mortality: further evidence from the Whitehall study. *J Epidemiol Comm Health* 44: 265–270
66. Ben-Shlomo Y, Markowe H, Shipley M, Marmot MG (1992) Stroke risk from alcohol consumption using different control groups. *Stroke* 23: 1093–1098
67. Lewis JD, Brensinger C (2004) Agreement between GPRD smoking data: a survey of general practitioners and a population-based survey. *Pharmacoepidemiol Drug Safety* 13: 437–441
68. Li XQ, Andersson TB, Ahlstrom M, et al. (2004) Comparison of inhibitory effects of the proton pump-inhibiting drugs omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole on human cytochrome P450 activities. *Drug Metab Dispos* 32: 821–827
69. Maclure M (1991) The case-crossover design: a method for studying transient effects on the risk of acute events. *Am J Epidemiol* 133: 144–153

70. Schneeweiss S, Stürmer T, Maclure M (1997) Case-crossover and case-time-control designs as alternatives in pharmacoepidemiologic research. *Pharmacoepidemiol Drug Safety* (Suppl 3): S51–S59
71. Higgins JPT, Green S (eds.) (2009) *Cochrane Handbook for Systematic Reviews of Interventions* Version 5.0.2 [updated September 2009]. The Cochrane Collaboration. Available from www.cochrane-handbook.org

Further reading

- Gordis L (2009) *Epidemiology*, 4th edn. Saunders, Philadelphia
- Hennekens CH, Buring JE, Mayrent SL (eds.) (1987) *Epidemiology in Medicine*. Lippincott Williams & Wilkins, Philadelphia
- Strom BL, Kimmel SE (eds.) (2006) *Textbook of Pharmacoepidemiology*. John Wiley, Sussex
- Etminan M, Samii A (2004) Pharmacoepidemiology I: a review of pharmacoepidemiologic study designs. *Pharmacotherapy* 24(8): 964–969
- Deeks JJ, Dines J, D'Amico R, Sowden AJ, Sakarovich C, Song F, Petticrew M, Altman DG (2003) Evaluating non-randomised intervention studies. *HTA* 7(27): iii–x, 1–173.

CHAPTER 10

Pharmacokinetics I: PK–PD approaches – antibiotic drug development

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Summary

The success of antibiotic therapy depends on complex interplay between the administered drug, its mechanism of action, concentration at site of infection, and complexity or severity of infection. Therapeutic response to an anti-infective agent or its pharmacological effect is often associated with high variability in clinical situations. Pharmacokinetics (PK) and pharmacodynamics (PD) contribute to a better understanding of the relationship between drug concentrations in biological fluids and its pharmacological effect. PK–PD studies can provide a means for exploring important pharmacological and toxicological properties of a drug in animals and humans. An integrated PK–PD approach, linking the exposure of a drug and the modulation of pharmacological targets, physiological pathways and ultimately disease systems, can be used to develop unified understanding of the data collected during different stages of drug discovery, which can also be applied in the drug development process. PK–PD relationships can be expressed by numerous mathematical models which have been increasingly demonstrated to be predictive of therapeutic outcomes during the development process. The PK–PD modelling and simulation approaches can streamline drug development and help make crucial decisions. These decisions include but not limited to planning clinical trials and designing optimal dosing strategies, both of which can be extremely costly and critical to the compound being developed if incorrect decisions are made. The purpose of this chapter is to discuss how PK–PD correlations and modelling and simulation process can be applied to drug development emphasizing antibiotic drug development.

Keywords: Pharmacokinetics, pharmacodynamics, antibiotics, protein binding, microdialysis, tissue penetration kill curves, *in vitro* models

1 PK–PD approach

Pharmacokinetic–pharmacodynamic (PK–PD) studies play an important role in drug development and drug evaluation. The primary objective of PK–PD modelling is to identify key properties of a drug *in vivo*, which allows the characterization and prediction of the time course of drug effects under physiological and pathological conditions. In the early pioneering studies, this was attempted by a rather empirical approach where the model comprised three components: a pharmacokinetic model, characterizing the time course of drug and metabolite concentrations in plasma; a pharmacodynamic model, characterizing the relationship between concentration and effect(s); a link model, which serves to account for the often observed delay of the effect relative to the plasma concentration (e.g. “effect compartment” model) [1, 2]. Such models can successfully be applied when the delay in effect is caused by drug distribution to extracellular targets by passive diffusion. However, target distribution kinetics may often be more complex, e.g. for drugs acting on intracellular targets or with a site of action in organs which are protected by specific barriers, e.g. the blood brain barrier. This requires that the role of specific transporters, which either increase or decrease the target-site concentration of the drug, is taken into account. Physiologically-based PK modelling concepts will be helpful to characterize and predict target-site distribution kinetics in such situations [2].

In recent years a far more mechanism-based approach is being pursued, which features specific expressions to characterize processes on the causal path between drug administration and effect. These include: target site distribution, target binding and activation, pharmacodynamic interactions (also with endogenous substances), transduction, homeostatic feedback mechanisms and the effects of the drug on disease progression. A key feature of this mechanism-based approach is the explicit distinction between drug-specific properties and biological system-specific properties [2, 3]. Drug-specific are those that describe the interaction between the drug and the biological system in terms of target affinity and target activation, whereas system-specific parameters describe the functioning of the biological system. The distinction between these two types of parameters appears to be crucial for the prediction of drug effects in humans from *in vitro* and animal experiments. Drug-specific properties like the *in vivo* target affinity and intrinsic efficacy can often be predicted on the basis of *in vitro* bioassays. The values for these properties or parameters often appear to be identical between species, implying that these may not require scaling when applied in inter-species extrapolation. Moreover, there is no or lesser need to take intra- and inter-individual variability in the values of these parameters into consideration. These observations on drug-specific properties have been made for small molecule drugs; it still is to be investigated if these hold for biologicals (proteins, antibodies) as well. On the other hand, biological system-specific parameters (e.g. the level of expression of the target protein or the rate constants of processes at the level of transduction) can only be estimated by *in vivo* systems analysis and usually their values

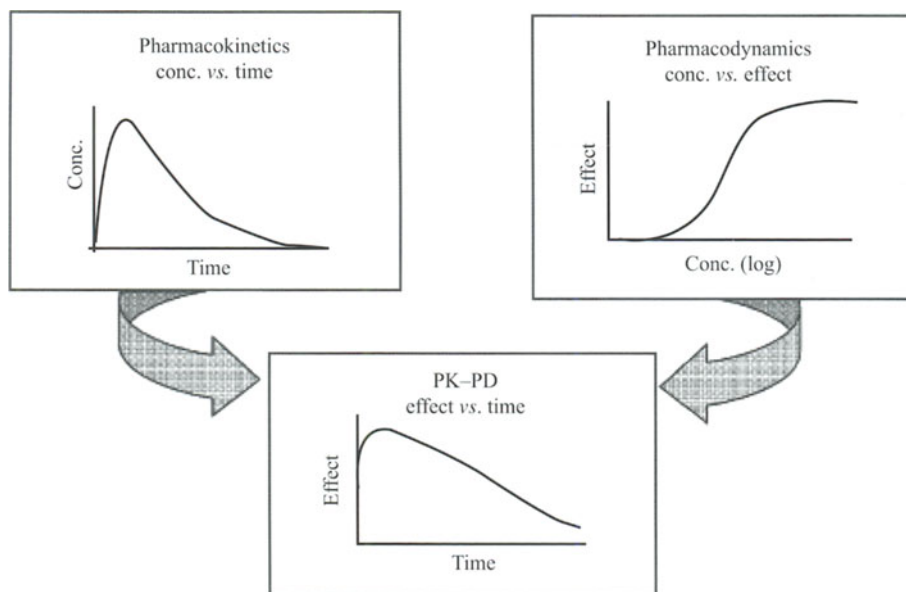


Fig. 1 Classical PK-PD modelling approach

will vary between species, individuals, disease states and other conditions. This implies that interspecies scaling of biological system-specific parameters is required and that intra- and inter-individual variability in these parameters must be taken into account.

In general, pharmacokinetics describes what the body does to the drug, the time course of the drug concentrations in plasma or tissue fluids. Pharmacodynamics describes what the drug does to the body, the pharmacological effects as a function of the drug concentration. A PK-PD model is a mathematical concept that links the pharmacokinetics and pharmacodynamics of a drug, and describes the time course of pharmacological effect of a given dose, which is very helpful to determine appropriate dosing regimens (Fig. 1). The optimal dosing regimens of antibiotics will have tremendous impact on therapeutic outcome and cost, as well as in reducing the emergence of drug resistance [4].

It has been widely accepted that only free (unbound) antibiotic concentrations in the interstitial fluid (ISF) at the target site are responsible for the antibacterial effect and may be more relevant in predicting therapeutic efficacy than total plasma concentrations. Since most infections occur not in plasma but in tissue sites (extracellular fluid), the ability of antibiotics to reach the target sites (tissue penetration) is a key determinant of clinical outcome. However, tissues are not homogenous compartments, and the distribution of drug molecules in plasma and tissues depends on their physiochemical properties (Fig. 2).

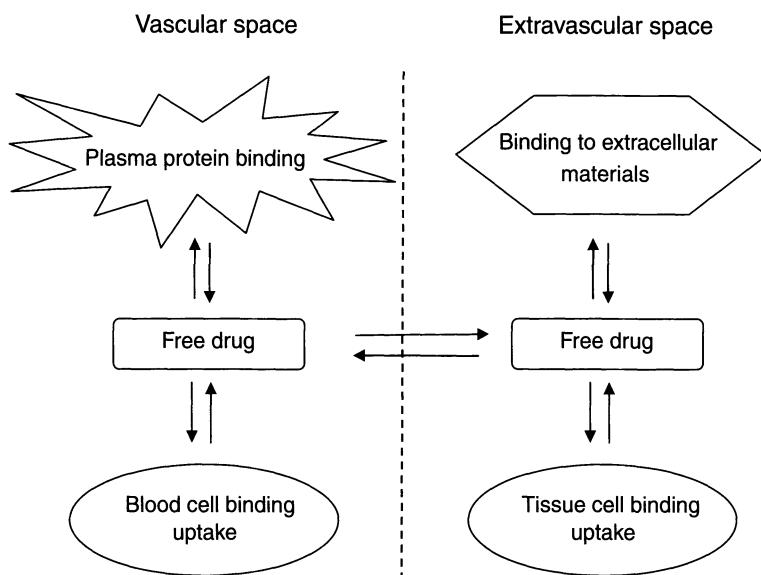


Fig. 2 Diagrammatic representation of (free) drug distribution into tissues

Once in plasma, some of the drug binds to plasma proteins or blood cells, or diffuses into the blood cells. Some drug also remains unbound (free fraction) in plasma and can move freely into other tissues. A similar scenario also occurs in the tissue, some drug molecules binding to the tissue proteins or the tissue cells and some staying unbound in the tissue fluid. The difference between the total plasma concentrations and the free tissue concentrations can be quite significant especially when the protein binding of the antibiotic is high [4]. Therefore, the total plasma concentration is not considered as an ideal measure for rational dosing of antibiotics and the unbound/free drug concentration at the infection site should be preferred.

Also, it is important to realize that the *in vitro* MIC values are determined in the presence of free antibiotic concentrations and the protein binding of the antibiotic is frequently not taken into account. An FDA guidance of 1997 stated that pharmacodynamic studies should include relating the concentrations at the site of action to the *in vitro* susceptibility of the target micro-organism [5]. To evaluate the antibacterial activity in this way, the free drug concentrations at the target tissue would be an appropriate PK index for rational dosing of antibiotics. Although MIC is a well-established PD parameter routinely determined in microbiology, it is not an ideal PD parameter. Antibacterial activity is a dynamic process, while MIC is only a threshold value, a one-point measurement with poor precision. Hence, MIC can only provide approximate information about the antibacterial effect of the antibiotics. Currently, a better PD approach, bacterial time-kill curves, can offer more detailed information about the antibacterial activity as a

function of both time and antibiotic concentration. A PK–PD model based on the free antibiotic concentrations at the target site and the time–kill curves may have better potential for rational dosing of antibiotics than parameters based on plasma concentrations and MICs.

2 Microdialysis for measuring free drug concentrations

In clinical pharmacology microdialysis (MD) is used as a tool to measure target site concentrations of antibiotics or other drugs in different tissues and organs and to

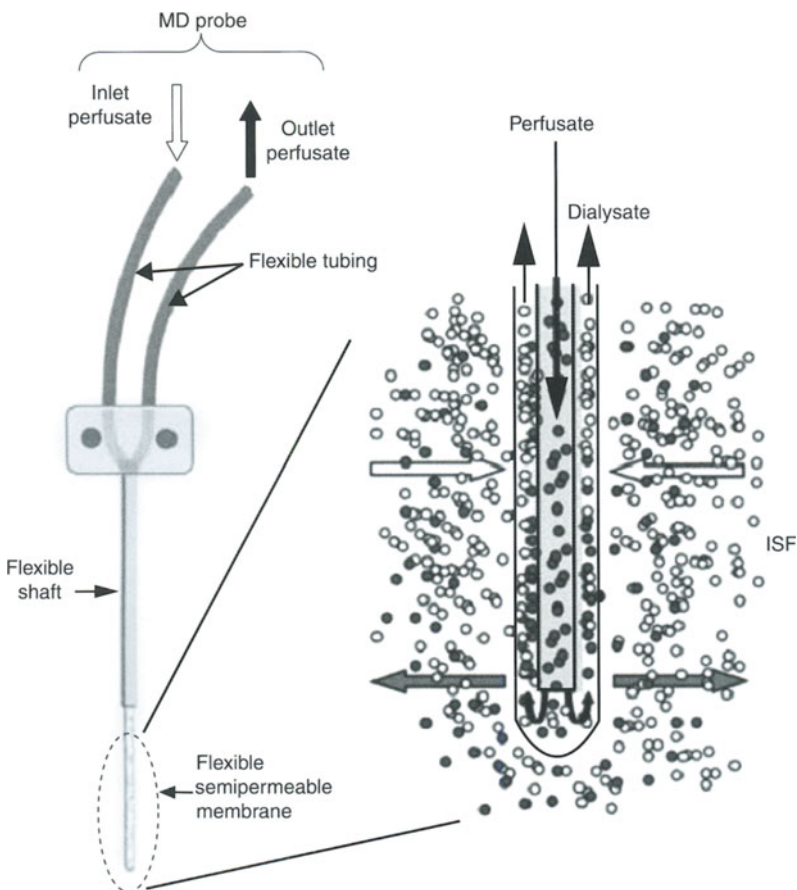


Fig. 3 Schematic diagram of a concentric microdialysis (MD) probe perfused with physiological fluid, Lactated Ringer's solution (●). Enlarged portion of the membrane shows diffusion of analyte (○) into the perfusate from the interstitial fluid (Picture modified from Refs. [6, 36])

subsequently relate target site PK to PD. Microdialysis is a practical and data-rich *in vivo* method, which is an extremely useful tool to investigate the PK–PD profiles of drug candidates. In this technique, a small, semi-permeable membrane (MD probe) is placed into the interstitial fluid (ISF) of the tissue of interest (Fig. 3). The probe is perfused with a physiological solution (e.g. Lactated Ringer's or saline) at a constant flow-rate of 0.1–5 $\mu\text{L}/\text{min}$ and at specified time intervals protein-free compound is collected for analysis [6, 36]. Sampling by MD is driven by diffusion of analytes across the dialysis membrane due to a concentration gradient from the external medium to the perfusate and is considered as a volume neutral process with little or no net transport of the external fluid into the MD probe [4, 6].

3 MD calibration methods

Since the MD probe is continuously perfused with fresh perfusate, a total equilibrium across the membrane cannot be established. However a steady-state rate of exchange across the MD membrane is rapidly reached. This steady-state exchange rate is described by the extraction efficiency (EE). The EE is the ratio between the loss/gain of analyte during its passage through the probe ($C_{\text{perfusate}} - C_{\text{dialysate}}$) and the difference in concentration between perfusate and the sample of interest such as tissue fluid, *in vitro* analyte, etc ($C_{\text{perfusate}} - C_{\text{sample}}$).

$$\text{EE} = \frac{C_p - C_d}{C_p - C_s}$$

where, C_p is the concentration of drug in the perfusate, C_d is the concentration of drug in the dialysate, and C_s is the concentration of drug in the sample (e.g. the extracellular fluid).

At steady-state, EE has the same value for all $C_{\text{perfusate}}$, no matter if the analyte is being enriched or depleted in the perfusate. For this reason, MD probes can be calibrated with either drug-containing perfusate or sample solutions [6, 7]. While various calibration techniques are available (e.g. low-flow rate method, zero-net flux method, extended zero-net flux method, etc.), retrodialysis by drug is the most commonly employed method in humans [8–10].

During retrodialysis, the probe is perfused with drug-containing perfusate (of known concentration) prior to or after drug administration, without the drug in the tissue. Since absence of drug in the tissue is required for retrodialysis, this calibration technique cannot be applied to endogenous compounds. Proper selection of an appropriate calibration method is critically important for the success of an MD experiment. Supportive *in vitro* experiments prior to use in animals or humans are therefore highly recommended. As the recovery determined *in vitro* might differ from the recovery in humans, it is important that recovery be determined in every *in vivo* experiment [7, 11].

Probe recovery is affected by many factors including MD flow rate (perfusion), temperature, probe membrane composition and surface area, nature of the dialyzed tissue (which precludes the use of calibration *in vitro* as a surrogate for calibration *in vivo*), physicochemical properties of the analyte of interest, and other factors that influence molecular diffusion characteristics [6, 7]. In general, the higher the perfusion flow rate, the lower the relative recovery. Higher temperatures and greater probe membrane areas usually result in increased recovery. The rate of solute clearance from the extracellular space is also an important determinant of recovery. Consequently, probe recovery may vary during an experiment if the rate of solute metabolism, cellular uptake or loss to blood from the extracellular space is perturbed.

MD is considered as a minimally invasive procedure with minimal tissue damage associated with the probe insertion. However, some tissue sites such as brain, lung, bone, heart, liver or the peritoneal cavity are not readily accessible to the MD procedure and require surgical implantation [13–20]. Another important aspect of MD technique is its dependency on perfusate flow rate and sensitivity of the analytical methods. As the analyte concentration decreases with increasing flow rate, highly sensitive analysis techniques may be required [12]. However, if very low-flow rates are used, the time resolution might be compromised. For this reason, flow rate and analytical procedures require extensive fine-tuning. MD has emerged

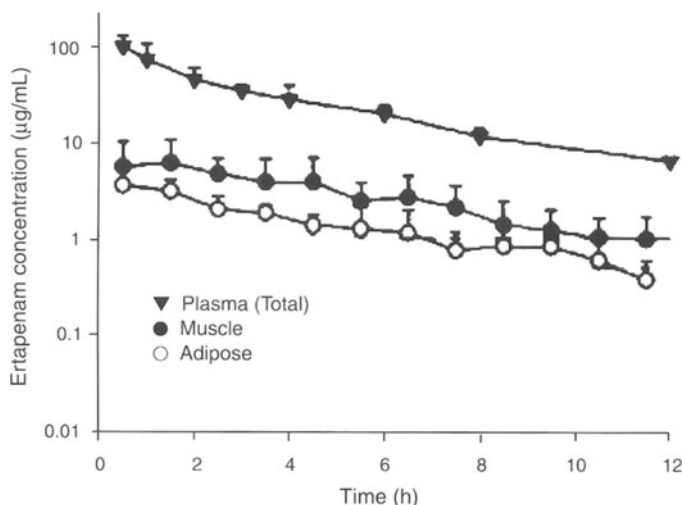


Fig. 4 Concentration of ertapenam (mean \pm SD) in plasma, muscle and adipose tissues determined from a clinical microdialysis study in healthy volunteers ($n = 6$) with 1 g intravenous infusion for 30 min (Picture modified from Ref. [7])

as the method of choice to monitor drug concentrations in the extracellular space. However if the site of action is located intracellularly, MD cannot measure that concentration directly. Even in these cases, the respective extracellular concentration resides closer to the site of interest than the respective plasma or blood concentrations [7].

The MD-sampling technique provides numerous advantages, most notable is its ability to provide a concentration–time profile of pharmacologically active antibiotic within the ISF and assess the penetration of drugs into the tissue of interest. A representative tissue distribution profile from a clinical microdialysis study is shown in Fig. 4. MD has been suggested as the most appropriate technique to be used in tissue distribution studies. Although MD is currently not required in drug development, it has been recommended by the FDA for the assessment of bioavailability and bioequivalence of topically applied generic drugs, especially of dermal formulations.

4 MD and tissue penetration of antibiotics

MD measures unbound, pharmacologically active drug concentrations in the ISF, which is the target site for many bacterial infections, and therefore the technique has led to a reappraisal of concepts of tissue-penetration by antimicrobial drugs [21–23]. MD data indicate that in healthy people interstitial concentrations of beta-lactams are in the range of free serum concentrations, whereas interstitial levels of quinolones and macrolides are considerably lower than those predicted from biopsies. For several conditions, notably septicaemia and septic shock, tissue concentrations of antibiotics such as piperacillin may be sub-inhibitory, even though effective concentrations are attained in serum.

5 Microdialysis and PK–PD

One of the necessary components to quantitatively determine the connections between drug concentrations in the blood (PK) and a drug response (PD) is the ability to measure the drug compound in the biophase or tissue that is closer to the actual site of action of the drug. MD has brought new opportunities to PK–PD research that will allow better understanding of exposure–response relationships and that will ultimately help to develop better drug products. MD allows direct access to the ECF of the tissues and therefore creates much more meaningful data than serum or plasma concentrations. This is probably most apparent in the field of anti-infective agents, because most infections are located in the ISF which is easily accessible to MD.

In this field, MD has been employed to measure drug concentrations in a variety of animal tissues including muscle, lung and middle ear fluid. The determination of these local concentrations allows one to assess if the administered dosing regimen is appropriate to obtain sufficiently high drug concentrations at the site of the infection. Integration of MD-derived tissue PK data to PK–PD kill curve approaches is an ideal approach to gain information on rational dosing of anti-infective agents.

6 Kill curves

Bacterial time-kill curves have been monitored in both *in vitro* kinetic models and animal infection models [4, 24]. It is more convenient and precise to simulate the human PK profiles of antibiotics with *in vitro* kinetic models than in animal models. The two main characteristics of *in vitro* PD models are drug exposure and bacterial concentration.

Two different *in vitro* models have been used frequently (Fig. 5): models with constant antibiotic concentrations simulating a steady-state situation and models with variable antibiotic concentrations achieved by dilution or diffusion to simulate multiple dosing, including one- and multiple-compartment models [4, 25, 26]. Constant drug exposure is achieved by not replacing or changing the medium, while changing drug concentrations are obtained in systems with flowing medium. A loss of bacteria due to the experimental setting, which is observed in some models, may have a substantial influence on the results. Thus, to define the loss of bacteria in *in vitro* models the terms open and closed are often suggested. Open models allow the exchange of bacteria with the environment and closed models have no bacterial exchange. As a result, an open model always has a flowing medium (i.e. changing drug exposure), whereas closed models can have an unchanging or flowing medium (i.e. constant or changing drug exposure).

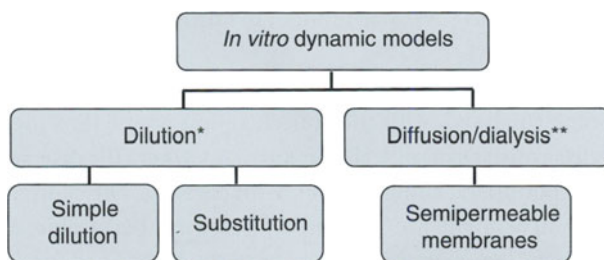


Fig. 5 Dynamic bacterial kill-curve: *in vitro* model review. **No bacterial loss, *May/may not have bacterial loss (Picture modified from Ref. [27])

7 Experimental settings of *in vitro* models

An *in vitro* model with its main component, the culture vessel, has to fulfill special requirements like temperature control, provide appropriate nutrients for bacterial growth and have facility for homogenous mixing of the bacterial suspension [27]. Numerous different *in vitro* models for antibiotics have been developed. Not all models are applicable for all purposes, and some can mimic only selected aspects and conditions, e.g. infections in specific compartments. The bacterial concentration (as the antibacterial effect) in the *in vitro* system is monitored over time under different antibiotic exposures by different methods like turbidimetry, microscope, viable counts etc. The bacterial concentration-time courses (time-kill curves) and derived PK–PD indices, such as area under the time-kill/bacterial curve, allow for detailed analysis of bacterial growth and death following antibiotic exposure [27].

In static *in vitro* models, bacteria should be suspended homogeneously in a culture vessel with constant antibiotic exposure in the medium. All conditions remain the same over the entire observation period. The bacterial growth without antibiotic can be limited by nutrition, space, aeration and toxic metabolites. The bacterial concentration changes in the vessel and can be studied over time [28, 29]. The working principle of dynamic models is more complex. The idea is to simulate the body clearance or half-life of the antibiotic and is realized in dynamic models by changing drug concentrations [28]. In dilution models the drug concentration in the culture vessel changes *via* substitution with fresh medium or by simple dilution. Substitution means to remove a defined volume from the *in vitro* model and supplement the same volume of fresh medium. In this case, both flow processes (in- and out-flow) are controlled. The volume in the culture vessel remains constant all the time. Simple dilution means to add a defined volume of medium to the culture vessel. Either medium is added to the input and the outflow is uncontrolled *via* overflow (or does not exist) or a pump removes medium from the culture vessel and fresh medium is sucked in from a reservoir. In both cases, the drug concentration in the culture vessel will be decreasing. The input of medium in dilution models can happen continuously or stepwise at regular intervals [29]. The input of the drug can mimic bolus, infusion or first order absorption [27].

Another method for achieving changing drug concentrations is *via* drug diffusion across a membrane (dialysis), with the concentration gradient as the driving force. The dialysis models consist of a central compartment, where the drug initially appears after dosing, and a peripheral compartment, with bacteria. The central compartment and peripheral compartment are separated by a semi-permeable membrane, i.e. permeable for drug and medium but not for bacteria. Fresh medium is continuously pumped from a reservoir into the central compartment and then into the waste. Thus, the medium in the peripheral compartment is continuously renewed by diffusion (from the central compartment), while the drug and bacteria can interact, but the

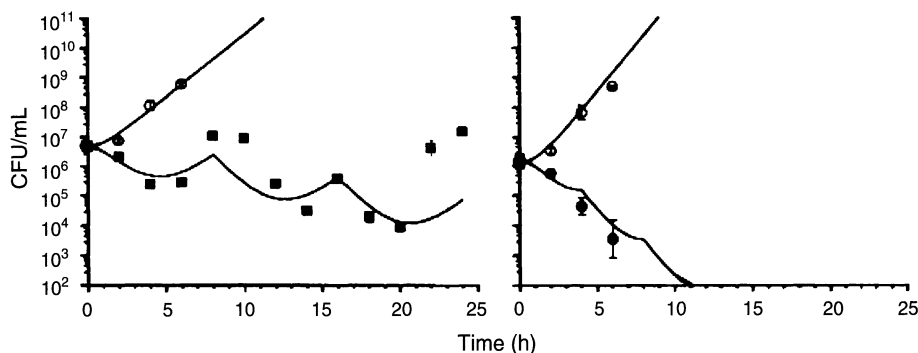


Fig. 6 The bacterial time kill curves of piperacillin in *E. coli* with the same daily dose using *in vitro* dilution model: 100 mg/mL (initial concentration) q8h (left) and 50 mg/mL (initial concentration) q4h (right). Values are mean \pm SD ($n = 4$) (Picture modified from Refs. [4, 32])

bacteria cannot leave this compartment. The circulation of medium in the peripheral compartment – counterflow towards the central compartment – can help to optimize the diffusion [30, 31].

The significant advantage of kill curve approach is that it is much easier to simulate different dosing regimens and their killing effect can be compared directly under the same conditions. Several antibiotics had been evaluated using kill curve approaches for their antibacterial activities under different dosing regimen simulations, such as piperacillin, piperacillin-tazobactam combinations and cefaclor [4, 32, 33]. The killing effect of piperacillin with simulated multiple doses in *Escherichia coli* was evaluated in a one-compartment dilution model. It is very clear that with the same total daily dose, the killing effect was more significant with shorter dosing intervals than with longer dosing intervals (Fig. 6). The results of time-kill curves of cefaclor with simulated modified-release doses have shown sufficient inhibition of bacterial growth of several relevant strains, which support the concept of sustained-release oral beta-lactam products [4].

In spite of their simplicity, static models play an important role for antibacterial PK-PD studies, but should be regarded as a starting point. For more complex PK designs, dynamic models will be more important. The dilution models have existed since the 1970s and have been intensively diversified [27, 34]. Almost at the same time, dialysis models have been introduced and, later, improved. Currently, the ratio of using dialysis or dilution models is balanced [27]. Recent developments combine the ideas of a one-compartment dilution model with filters and a two-compartment dialysis model, resulting in a computer-controlled semi-automated *in vitro* model for industrial purposes [27, 35]. Comprehensive understanding of the PD of antibiotics using various *in vitro* tools like kill curves facilitate the development of rational dosing strategies and improved therapeutic outcomes.

8 Conclusion

These days modelling and simulation approaches are fully integrated into the drug development process. Empirical, mechanistic or semi-mechanistic PK–PD models are progressively developed at various stages of the drug development process and are capable of iterative optimization of trial designs. For antibiotics, incorporating free concentrations from target sites as measured with microdialysis technique and PD information from dynamic kill curves instead of the traditional one-dimensional MIC parameter, into mechanistic/semi-mechanistic models would provide better predictability for evaluating effective doses and dosing strategies.

References

1. Sheiner LB, Stanski DR, Vozeh S, Miller RD, Ham J (1979) Simultaneous modelling of pharmacokinetics and pharmacodynamics: application to D-tubocurarine. *Clin Pharmacol Ther* 25: 358–371
2. Breimer DD (2008) PK/PD modelling and beyond: impact on drug development. *Pharm Res* 25: 2720–2722
3. Danhof M, de Jongh J, de Lange ECM, Pasqua OD, Ploeger BA, Voskuyl RA (2007) Mechanism-based pharmacokinetic-pharmacodynamic modelling: biophase distribution, receptor theory and dynamical systems analysis. *Annu Rev Pharmacol Toxicol* 47: 357–400
4. Liu P, Muller M, Derendorf H (2002) Rational dosing of antibiotics: the use of plasma concentrations versus tissue concentrations. *Int J Antimicrob Agents* 19: 285–290
5. FDA-CDER (1997) Guidance for industry – evaluating clinical studies of antimicrobials in the division of anti-infective drug products: FDA
6. Chaurasia CS, Muller M, Bashaw ED, et al. (2007) AAPS-FDA workshop white paper: microdialysis principles, application, and regulatory perspectives. *J Clin Pharmacol* 47: 589–603
7. Schmidt S, Banks R, Kumar V, Rand KH, Derendorf H (2008) Clinical microdialysis in skin and soft tissues: an update. *J Clin Pharmacol* 48: 351–364
8. Lonnroth P, Jansson PA, Smith U (1987) A microdialysis method allowing characterization of intercellular water space in humans. *Am J Physiol* 253: E228–E231
9. Olson RJ, Justice JB Jr. (1993) Quantitative microdialysis under transient conditions. *Anal Chem* 65: 1017–1022
10. Burkhardt O, Brunner M, Schmidt S, Grant M, Tang Y, Derendorf H (2006) Penetration of ertapenem into skeletal muscle and subcutaneous adipose tissue in healthy volunteers measured by in vivo microdialysis. *J Antimicrob Chemother* 58: 632–636
11. Stahl M, Bouw R, Jackson A, et al. (2002) Human microdialysis. *Curr Pharm Biotechnol* 3: 165–178
12. Davies MI, Cooper JD, Desmond SS, Lunte CE, Lunte SM (2000) Analytical considerations for microdialysis sampling. *Adv Drug Deliv Rev* 45: 169–188
13. Engstrom M, Polito A, Reinstrup P, et al. (2005) Intracerebral microdialysis in severe brain trauma: the importance of catheter location. *J Neurosurg* 102: 460–469
14. Ederoth P, Tunblad K, Bouw R, et al. (2004) Blood-brain barrier transport of morphine in patients with severe brain trauma. *Br J Clin Pharmacol* 57: 427–435
15. Herkner H, Muller MR, Kreischitz N, et al. (2002) Closed-chest microdialysis to measure antibiotic penetration into human lung tissue. *Am J Respir Crit Care Med* 165: 273–276

16. Tomaselli F, Maier A, Matzi V, et al. (2004) Penetration of meropenem into pneumonic human lung tissue as measured by in vivo microdialysis. *Antimicrob Agents Chemother* 48: 2228–2232
17. Thorsen K, Kristoffersson AO, Lerner UH, et al. (1996) In situ microdialysis in bone tissue. Stimulation of prostaglandin E2 release by weight-bearing mechanical loading. *J Clin Invest* 98: 2446–2449
18. Bahlmann L, Misfeld M, Klaus S, et al. (2004) Myocardial redox state during coronary artery bypass grafting assessed with microdialysis. *Intensive Care Med* 30: 889–894
19. Nowak G, Ungerstedt J, Wernerman J, et al. (2002) Clinical experience in continuous graft monitoring with microdialysis early after liver transplantation. *Br J Surg* 89: 1169–1175
20. Jansson K, Jansson M, Andersson M, et al. (2005) Normal values and differences between intraperitoneal and subcutaneous microdialysis in patients after non-complicated gastrointestinal surgery. *Scand J Clin Lab Invest* 65: 273–281
21. Muller M (2002) Science, medicine and the future: microdialysis. *BMJ* 324: 588–591
22. Muller M, Penadela A, Derendorf H (2004) Issues in pharmacokinetics and pharmacodynamics of anti-infective agents: distribution in tissue. *Antimicrob Agents Chemother* 48: 1441–1453
23. Brunner M, Derendorf H, Muller M (2005) Microdialysis for in vivo pharmacokinetic/pharmacodynamic characterization of anti-infective drugs. *Curr Opin Pharmacol* 5: 495–499
24. CPMP (1999) Points to consider on pharmacokinetics and pharmacodynamics in the development of antibacterial medicinal products. In: *Evaluation of Medicines for Human Use*. The European Agency for the Evaluation of Medicinal Products, London, UK, pp 1–7
25. Palmer SM, Kang SL, Cappelletty DM, Rybak MJ (1995) Bactericidal killing activities of cefepime, ceftazidime, cefotaxime, and ceftriaxone against *Staphylococcus aureus* and β -lactamase-producing strains of *Enterobacter aerogenes* and *Klebsiella pneumonia* in an in vitro infection model. *Antimicrob Agents Chemother* 39: 1764–1771
26. Firsov AA, Vostrov SN, Shevchenko AA, Portnoy YA, Zinner SH (1998) A new approach to in vitro comparisons of antibiotics in dynamic models: equivalent area under the curve/MIC breakpoints and equivalent doses of trovafloxacin and ciprofloxacin against bacteria of similar susceptibilities. *Antimicrob Agents Chemother* 42: 2841–2847
27. Gloede J, Scheerans C, Derendorf H, Kloft C (2010) In vitro pharmacodynamic models to determine the effect of antibacterial drugs. *J Antimicrob Chemother* 65: 186–201
28. Mueller M, de la Pena A, Derendorf H (2004) Issues in pharmacokinetics and pharmacodynamics of anti-infective agents: kill curves versus MIC. *Antimicrob Agents Chemother* 48: 369–377
29. Derendorf H, Hochhaus G (1995) *Handbook of Pharmacokinetic/Pharmacodynamic Correlation*. CRC Press Inc., Boca Raton, FL
30. Blaser J, Stone BB, Zinner SH (1985) Two compartment kinetic model with multiple artificial capillary units. *J Antimicrob Chemother* 15(Suppl A): 131–137
31. Mouton JW, den Hollander JG (1994) Killing of *Pseudomonas aeruginosa* during continuous and intermittent infusion of ceftazidime in an in vitro pharmacokinetic model. *Antimicrob Agents Chemother* 38: 931–936
32. Nolting A, Dalla Costa T, Rand KH, Derendorf H (1996) Pharmacokinetic/pharmacodynamic modelling of the antibiotic effect of piperacillin in vitro. *Pharm Res* 13: 91–96
33. Dalla Costa T, Nolting A, Rand K, Derendorf H (1997) Pharmacokinetic/pharmacodynamic modelling of the in vitro anti-infective effect of piperacillin/tazobactam combinations. *Int J Clin Pharmacol Ther* 35: 426–433
34. Grasso S, Meinardi G, de Carneri I, et al. (1978) New in vitro model to study the effect of antibiotic concentration and rate of elimination on antibacterial activity. *Antimicrob Agents Chemother* 13: 570–576
35. Wang L, Wismer MK, Racine F, et al. (2008) Development of an integrated semi-automated system for in vitro pharmacodynamic modelling. *J Antimicrob Chemother* 62: 1070–1077
36. Schmidt S, Barbour A, Sahre M, Rand KH, Derendorf H (2008) PK–PD: new insights for antibacterial and antiviral applications. *Curr Opin Pharmacol* 8: 549–556

CHAPTER 11

Pharmacokinetics II: ^{14}C -labelled microdosing in assessing drug pharmacokinetics at Phase-0

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Summary

Microdosing came onto the scene with the first publication of data in 2003. Since this time the number of compounds where the pharmacokinetics observed at a microdose compared to a therapeutic dose has grown steadily. Based on current data in the public domain, there are 23 drugs where microdose and therapeutic dose pharmacokinetics can be compared. Of these, 84% scale within a factor of 2 for oral administration and 100% for intravenous administration. Where pharmacokinetic non-linearity is seen, a growing understanding of the mechanisms involved are being applied to interpret the microdose data in the context of the selection of candidate drugs for further development. Inclusion of a ^{14}C isotopic tracer into the molecule enables sensitive AMS analysis to be used as well as obtaining an early indication of the drug's metabolism in humans.

1 Origins of ^{14}C -labelled tracers

The application of isotopic labels in tracing and quantifying the fate of a given chemical species within a biological system has a history going back over 50 years. One of the earliest examples was the elucidation of the photosynthetic pathway by Melvin Calvin for which he received the Nobel Prize in 1961. Untangling distinct metabolic pathways occurring within a living cell from the myriad of other reactions, all occurring simultaneously, was a formidable task. Calvin in his Nobel lecture described the problem thus:

Keywords: Isotopic tracer, Accelerator Mass Spectrometry, pharmacokinetics, dose linearity, metabolic linearity, propafenone, sumatriptan

“One of the principal difficulties in such an investigation in which the machinery which converts the CO_2 to carbohydrate and the substrate upon which it operates are made of the same atoms, namely carbon and its near relatives, is that ordinary analytical methods will not allow us to distinguish easily between the machinery and its substrate”.

Just prior to Calvin's experiments Martin Kamen and Samuel Ruben, discovered a new isotope of carbon (^{14}C) which had a relatively long half-life of 5760 years. Calvin, exploiting this newly discovered isotope, pulsed $^{14}\text{CO}_2$ into illuminated algal suspensions which were then solvent extracted. Over periods of time the extracts were analyzed using paper chromatography followed by exposure to radiographic film (state of the art at the time) to reveal a series of compounds into which the radioactive ^{14}C had been incorporated. By identifying each compound as it appeared, Calvin determined the sequence of events from the initial carbon fixation *via* $^{14}\text{CO}_2$ to the formation of sucrose. The ^{14}C isotope in CO_2 acted like a “beacon” incorporating itself into each compound in the pathway, distinguishing it from the plethora of other carbon-based substances present in the algal extracts. This original Nobel Prize winning research opened up a new age where ^{14}C began to be used to trace the biochemistry of ever-more complex organic molecules.

^{14}C is uniquely well placed as an isotopic biochemical tracer as it has a relatively long half-life, it can be directly incorporated into the molecular skeleton of organic compounds and the natural abundance is just $10^{-11}\%$ thereby having a very low background. Inclusion of a radioisotope into the molecular structure allows for direct quantification of the labelled compound, irrespective of its molecular structure. The radiotracer can be seen as offering a universal method of quantification and with its relative long half-life no correction for radio-decay is necessary with ^{14}C over the duration of a metabolic experiment. In addition, there are no major differences in the rates of chemical reactions with ^{14}C - or ^{12}C -compounds as the kinetic isotope effect (KIE) is relatively small. For lower atomic weight isotopes such as those of hydrogen and (^1H) and tritium (^3H) the KIE can be significant as ^3H is three times as heavy as hydrogen, consequently the $\text{C}-^3\text{H}$ bond has a lower zero-point energy than the $\text{C}-^1\text{H}$ bond and a higher activation energy is required for bond-cleavage. On the other hand, the KIE observed for carbon is comparatively small. For example, the CO_2 fixation rates by the enzyme ribose 1,5-bisphosphate carboxylase exhibits no more than approximately 2% difference when compared between carbon isotopes [1]. The biggest drawback of ^{14}C is that it is radioactive, emitting low energy β -radiation (E_{max} 156 keV) with a specific activity of 2.3 GBq/mmol, which restricts its administration to humans.

2 Administration of ^{14}C -drugs to humans

Candidate drug compounds are routinely synthesized with enriched levels of ^{14}C within the molecular structure and used to study their metabolic fate *in vitro*, in laboratory animals and humans. As stated above, because ^{14}C is radioactive there have traditionally been restrictions on administration to humans thereby limiting the possibilities for the study design [2]. The potential biological harm caused by ^{14}C -exposure is dependent upon the number of atomic disintegrations per unit time and the duration of exposure. The dosimetric quantity used for comparing the potential health effects of radiation to the human body is known as the Effective Dose Equivalent (measured in units of Severts, Sv) and it is on this that the regulatory authorities place limits. The amount of ^{14}C that can be administered to humans is calculated based on studies in the pigmented rat (known as dosimetry studies) which models the residence time of the radioactivity in a range of tissues [3]. Typically, it becomes increasingly difficult to obtain approval for a study using ^{14}C -drug in humans for a total radioactive exposure above 5 mSv. To put this into context, exposure to natural background radioactivity is around 2.5 mSv per year.

Drugs that remain in the body for prolonged periods of time due to for example, low plasma clearance or melanin binding, potentially lead to higher radioactive exposure than those drugs that are removed quickly. For slowly cleared drugs therefore, the amount of radioactivity (number of atomic disintegrations per unit time) has to be decreased so as not to exceed the regulatory exposure limits. Since the detection of the ^{14}C within the drug has traditionally relied on scintillation counting methods, then there will quickly become a point where reducing the amounts of radioactivity will lead to compromised sensitivity for the assay. Scintillation counting relies on detection of the low energy β -particle (i.e. an electron) *via* a classical scintillation event. Although widely used, the technique is not very sensitive as on average it takes 4.5×10^9 atoms of ^{14}C to generate 1 disintegration per minute (dpm) due to the half-life of this isotope. As a consequence, certain situations arise where the amount of ^{14}C -drug that can be administered to humans is too low to effectively conduct a study. It was at this point that Accelerator Mass Spectrometry (AMS) came onto the scene.

3 Accelerator Mass Spectrometry

AMS was first developed for archaeological radiocarbon dating in the mid-1970s [4]. AMS is an isotope ratio technique whereby carbon anions accelerated to high energies are passed through a low pressure gas or thin foil for electron stripping, thereby leading to a charge state change. The resulting high energy carbon cations are efficiently separated through a magnetic field and detected by gas ionization or solid state detectors (^{14}C) or Faraday cups (^{12}C and ^{13}C). Nitrogen (^{14}N) which would otherwise cause major

isobaric interference does not form a stable anion and therefore is removed in the process. Because AMS measures the actual number of ^{14}C atoms, rather than relatively infrequent decay events, it is extremely sensitive, being able to measure, as a general guide, around 2 atomole of ^{14}C , equivalent to approximately 0.0002 dpm. (The limits of detection in biological samples will be somewhat higher than this depending upon background ^{14}C levels.) AMS was first applied to drug development studies in the 1990s where there was a lack of sensitivity in the traditional scintillation counting methods due to the limited amounts of radioactivity that could be administered to human volunteers [5–9]. It very rapidly became apparent that because of the sensitivity of AMS the amounts of ^{14}C present in the drug administered could be reduced without adversely affecting the assay. Whereas traditionally, around 3.7 MBq (100 μCi) might be administered to human volunteers (for a drug with a half-life of a few hours and no significant tissue binding) if AMS was used then the dose could be reduced a thousandfold to 3.7 KBq (100 nCi). The human body, on average, contains approximately 3.7 KBq (100 nCi) of naturally occurring ^{14}C [10] and therefore the dose of ^{14}C -drug could be considered trivial. Regulatory authorities began to generally accept that these levels of ^{14}C represented an insignificant risk and relaxed the need to submit dosimetry data in support of the dose. Nowadays, there are some clinics that have a general agreement with the regulatory authority to administer up to 37 KBq (1 μCi) without compound specific approval.

3.1 The emergence of microdosing

In the 1990s a technique known as microdosing emerged [11] where both the mass of drug and amount of radioactivity administered to humans was kept very low ($\leq 100\text{ }\mu\text{g}$ and typically 200–1000 nCi). Because of the low levels of radioactivity used in these studies, the analytical method of choice was AMS. The method of analysis is however, not the focus of this chapter rather than the application of microdosing itself. AMS is used because it has sufficient sensitivity to measure ^{14}C at low levels but if other technologies become available, such as for example, Intracavity Optogalvanic Spectroscopy [12] then the application of the technique remains unchanged. It is also important to state that AMS and microdosing are not synonymous and that there are a range of pharmacokinetic and metabolism applications of this analytical technique, although beyond the scope of this chapter [13].

A microdose study is performed at a very early stage of drug development to obtain early pharmacokinetic data on a drug candidate in human volunteers. As its name implies, the dose administered in a microdose study is very small, the amount being defined by the regulatory authorities as 1/100th of the predicted pharmacologic dose or 100 μg whichever is the smaller [14]. These small doses are assumed to be inherently safer than pharmacologically active doses and therefore the regulatory authorities will approve human microdose studies based upon limited preclinical safety evaluation.

A microdose study would typically consist of 4–8 human volunteers administered a maximum of 100 µg of a candidate drug. To date microdose studies reported in the literature have used either the oral or intravenous routes of administration but in theory, any route could be applied. Following administration, samples of blood (plasma) and sometimes excreta are collected and analysed over time. Occasionally biopsies might also be obtained [15]. Since the dose administered is very low, so the drug concentrations in the samples collected are also low and therefore sensitive analytical methods are necessary in order to determine their concentrations. Where analysis involves just the unchanged parent drug (or sometimes specific metabolites) then LC-MS/MS has the advantage of not requiring the drug to be ^{14}C -labelled. On the other hand, the limits of quantification (LOQ) for most LC-MS/MS methods are typically 100 pg/mL occasionally achieving 10 pg/mL and rarely 1 pg/mL [16]. In situations where, for example, the drug has a low bioavailability or a high volume of distribution, then AMS analysis may be required on the grounds of sensitivity. As an example, assume a particular drug has a bioavailability of 50% and a volume of distribution of 200 L (clarithromycin would exemplify such a drug) then a 100 µg oral microdose would result in a maximum plasma concentration of 250 pg/mL and a LC-MS/MS assay with an LOQ of 100 pg/mL would hardly be adequate. Although LC-MS/MS analysis has been used in conjunction with microdose studies [17] inclusion of a ^{14}C tracer not only potentially lowers the LOQ into the low pg range but also has the advantage of allowing metabolic profiles to be generated. Perhaps surprisingly however, there are few examples of metabolic profiling in the literature from microdose studies, although some have recently appeared [18].

3.2 Application of microdosing

Microdose studies are typically applied in situations where the metabolism and pharmacokinetics are key to the choice of drug to be taken into full development. For example, drugs exhibiting a narrow therapeutic index where systemic concentrations have to be maintained within certain concentrations in order to avoid toxicity if the concentration is too high or a lack of efficacy if too low. The plasma half-life might be important in order to maintain a certain dosing regimen, such as once per day. Microdose studies are often conducted so that both an oral and an intravenous dose are administered to human volunteers in a cross-over design. The opportunity to administer the drug intravenously, albeit as a microdose, enables the absolute bioavailability of a drug candidate to be assessed and in conjunction with data from the oral route, data on whether limited bioavailability is due to absorption or first pass effects can be assessed. An example is shown in Fig. 1 showing data from a microdose study with sumatriptan. ^{14}C -sumatriptan was administered to 6 volunteers as an oral dose on one dosing occasion and an intravenous dose on another dosing occasion (www.EUMAPP.com). The dose was 100 µg, 200 nCi for both dose routes. Plasma samples collected over time were

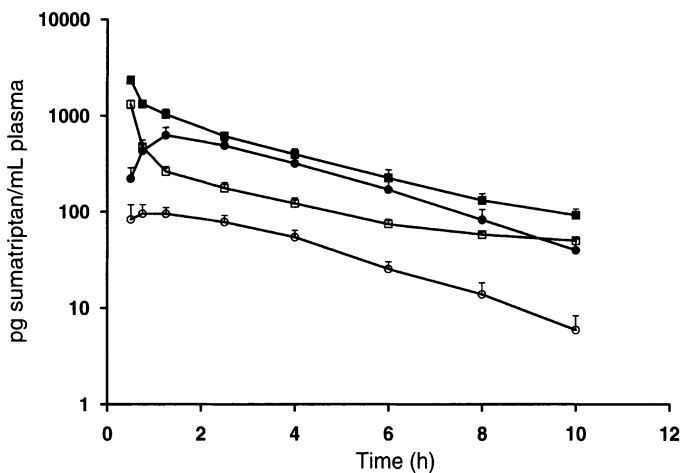


Fig. 1 Log-linear plot of plasma concentration vs. time following a single oral or single intravenous dose of $100\text{ }\mu\text{g}$ of ^{14}C -sumatriptan to human volunteers. Key: total ^{14}C of oral dose (●) unchanged parent drug for oral dose; (○) total ^{14}C for intravenous dose (■) and unchanged parent drug for intravenous dose (□). Error bars are + standard deviation, $n = 6$

analysed for unchanged parent drug and for the total ^{14}C concentration. The latter represents the total sum of parent sumatriptan plus any ^{14}C -metabolites. (Of course, the placement of the ^{14}C within the molecule (the indole ring in this case) should be such as to follow the core of the molecule.) Following an intravenous microdose of $100\text{ }\mu\text{g}$ ^{14}C -sumatriptan at the early sampling time point the plasma concentration of total ^{14}C was similar to parent drug. The concentration of total ^{14}C and parent drug then rapidly diverged with time, as sumatriptan was metabolized. Following an oral dose of ^{14}C -sumatriptan the concentration of total ^{14}C and parent were significantly different from the first plasma sampling. The lower concentration of parent sumatriptan compared to total ^{14}C reflected the high first pass metabolism of this drug. The absolute bioavailability of sumatriptan, calculated from the respective AUCs of unchanged parent drug for both the oral and IV microdose was approximately 20%. The first pass effect could be quantified by comparing the AUC for total ^{14}C with that of unchanged parent drug. For the oral dose, $\text{AUC}^{\text{parent}}/\text{AUC}^{\text{total}}$ was 0.17, thus showing only 17% of circulating ^{14}C was parent drug.

4 Pharmacokinetic linearity

When conducting a microdose study, the drug should have no pharmacologic effect. Inherent in the study design therefore, is the fact that the doses administered are

significantly lower than those that will be used clinically and therefore the question arises as to how the data obtained from a microdose might be applied to the higher, therapeutically-relevant doses. Although a very reasonable question, any answer should first be put into context around the objectives of a microdose study. A microdose study is not a replacement for metabolism and pharmacokinetic studies conducted at therapeutic dose levels. Microdose studies are used as a decision making tool as to whether the particular characteristics of a drug are suitable for further development. These characteristics may be specific pharmacokinetic parameters as described above, or questions concerning what the likely dose level and frequency are likely to be and if this is fitting with the intended therapy. For example, four doses a day may be acceptable for a drug treating an acute condition (e.g. an anti-infective) but might not be considered sustainable for chronic conditions (hypertension). The commonly held view, based on the currently widely adopted approach of allometric scaling of animal data to human pharmacokinetics, is that any prediction that is within a factor of two of the true value would be acceptable. Using this criterion, of the data currently in the public domain for 23 drugs, approximately 84% have scaled between a microdose and a therapeutic dose for oral administration and 100% for those given intravenously [19]. Detailed comparisons have been made in a number of reviews on microdosing and so will not be restated here [20, 21] other than to consider conditions under which non-linearity might appear.

Sumatriptan was given as an example above (also see Fig. 1). The bioavailability of sumatriptan determined from the microdose study was 20% and from a therapeutic dose it was approximately 8%. On the face it therefore, the prediction from the microdose was not particularly accurate (2.5-fold difference). As stated above however, microdose data must be viewed in the context that it is preliminary data used primarily in the selection of candidate drugs to go forward into full development. Clearly the microdose study shows sumatriptan has limited bioavailability due to both absorption and first pass metabolism. The latter information is particularly important as although formulation may remedy poor absorption, removal of the drug by first pass effects are far more difficult to deal with. A decision as to whether to take drug with limited bioavailability into development therefore, may be based more on the reasons why its bioavailability is limited as upon a precise measurement of the magnitude. In addition, the respective clearance values for the microdose and an absolute bioavailability study administering an oral dose of 50 mg were virtually identical at 46 and 50 L/h respectively. Sumatriptan exhibits metabolism-dependent elimination *via* cytosolic monoamine oxidase [22] and it is currently difficult to predict clearance and first pass loss in humans from *in vitro* data.

Clearly non-linearity in the pharmacokinetics will arise if saturation occurs at higher doses. Propafenone for example is known to show non-linear bioavailability due to saturable first pass metabolism. The principle enzyme involved in CYP 2D6 and higher doses (150 mg) propafenone can saturate this enzyme during oral

dosing. In contrast, midazolam undergoes high first pass metabolism *via* CYP 3A4, but at therapeutic oral doses of 5 mg, this enzyme is not saturated and therefore the pharmacokinetics scale very well from a microdose [23]. Non-linear pharmacokinetics can also arise due to target mediated disposition. The drug warfarin is one example as it exhibits non-linearity in the distribution phase of the drug-concentration time plots, prior to the point where a steady state is achieved due to high affinity binding onto a low capacity binding site, coupled to a low volume of distribution [23].

Both the design and interpretation of microdose experiments therefore, have to be placed into context of our growing understanding of where non-linear pharmacokinetics might arise. This is perhaps not surprising as it should be no different to any other type of experiment. For example, extrapolation of the rates and formation of metabolites in humans from laboratory animal data must be undertaken in the light of an understanding in species differences in metabolism. An example would be the handling of small organic acids by rat, dog and human. Renal elimination of such compounds is severely impaired in the dog compared to the rat and human [24]. Faced with conflicting rat and dog data only such knowledge enables the experimenter to take a valued view that the rat data are probably more relevant to human than the dog. In the lack of such knowledge, perhaps a microdose study might enable a better decision to be made using preliminary data in the human.

5 Microdosing and metabolism

Extracts of plasma and excreta from a microdose study can be analysed chromatographically to reveal the relative amounts of parent drug and metabolites over time. The presence of ^{14}C in the drug, providing it is a suitable position within the chemical structure, ensures that unexpected metabolites are still observed in the chromatographic profile. Historically, microdose studies have focused on the acquisition of data pertinent to the parent drug, rather than examining metabolism. More recently however, metabolic profile data have been obtained and an example is shown in Fig. 2 where two candidate drugs, IDX899 and IDX989 were administered as an oral microdose (100 μg , 100 nCi) to separate groups of four healthy male volunteers [18]. Plasma samples collected at 24 h from dosing were pooled by subject, extracted and analysed by HPLC and AMS. The profiles presented in Fig. 2, show that both compounds were well metabolized with relatively little parent drug present after 24 h. It has to be recognized of course that metabolism data acquired from microdosing studies are at low dose levels and therefore they may differ to those observed at higher therapeutic doses. Nevertheless, preliminary microdose metabolism data may give an indication on how well *in vitro* and animal profiles compare to the human and can flag potential issues of species specific metabolism.

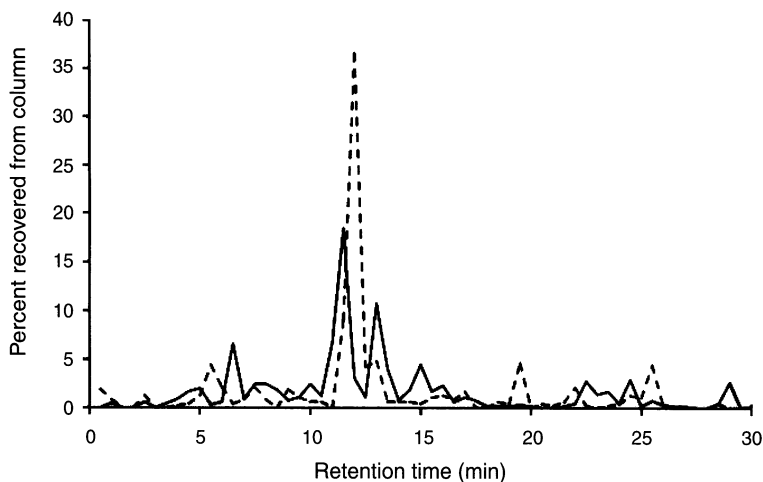


Fig. 2 Chromatogram showing the metabolic profiles of IDX989 (---) and IDX899 (—) in plasma 12 h from oral administration of 100 ug, 100 nCi of each candidate drug to 4 healthy male volunteers. The retention time of IDX989 was approximately 27 min and IDX899 26.5 min (From Ref. [18])

6 Conclusions

The utility of microdosing has grown over the past 10 years and experience now gives a better indication of how the technique can be applied. Microdosing can be used in situations where the pharmacokinetics or metabolism is an important factor in the selection of the drug for further development. For many drugs, where models exist that reliably predict the pharmacokinetics in humans, then microdosing probably offers little benefit but in situations where existing models prove to be unreliable or where there are significant species differences in the pharmacokinetics, then microdosing offers the opportunity of obtain data from the target species, namely human.

References

1. Saljoughian M, Williams PG (2000) Recent developments in tritium incorporation for radiotracer studies. *Curr Pharm Des* 6(10): 1029–1056
2. Lappin G, Temple S (2006) *Radiotracers in Drug Development*. Taylor and Francis CRC Press, FL, USA
3. Whitby B (2006) Quantitative whole body autoradiography. In: Lappin G, Temple S (eds.) *Radiotracers in Drug Development*. Taylor Francis CRC Press, FL, USA
4. Bennett CL, Beukens RP, Clover MR, Gove HE, Liebert RB, Litherland AE, Purser KH, Sondheim WE (1977) Radiocarbon dating using electrostatic accelerators: negative ions provide the key. *Science* 198(4316): 508–510

5. Turteltaub KW, Felton JS, Gledhill BL, Vogel JS, Southon JR, Caffee MW, Finkel RC, Nelson DE, Proctor ID, Davis JC (1990) Accelerator mass spectrometry in biomedical dosimetry: relationship between low-level exposure and covalent binding of heterocyclic amine carcinogens to DNA. *Proc Natl Acad Sci USA* 87(14): 5288–5292
6. Felton JS, Turteltaub KW, Gledhill BL, Vogel JS, Buonarati MH, Davis JC (1991) DNA dosimetry following carcinogen exposure using accelerator mass spectrometry and ^{32}P -postlabeling. *Prog Clin Biol Res* 372: 243–253
7. Turteltaub KW, Frantz CE, Creek MR, Vogel JS, Shen N, Fultz E (1993) DNA adducts in model systems and humans. *J Cell Biochem Suppl* 17F: 138–148
8. Vogel JS, Turteltaub KW, Finkel R, Nelson DE (1995) Accelerator mass spectrometry. *Anal Chem* 67(11): 353A–359A
9. Garner RC (2000) Accelerator mass spectrometry in pharmaceutical research and development – a new ultrasensitive analytical method for isotope measurement. *Curr Drug Metab* 1(2): 205–213
10. Lappin G, Garner RC (2003) Ultra-sensitive detection of radiolabelled drugs and their metabolites using accelerator mass spectrometry. In: Wilson I (ed.) *Handbook of Analytical Separations*. Elsevier, Amsterdam, pp. 331–349
11. Lappin G, Garner RC (2003) Big physics, small doses: the use of AMS and PET in human microdosing of development drugs. *Nat Rev Drug Discov* 2(3): 233–240
12. Murnick DE, Dogru O, Ilkmen E (2008) Intracavity optogalvanic spectroscopy. An analytical technique for ^{14}C analysis with subattomole sensitivity. *Anal Chem* 80(13): 4820–4824
13. Lappin G, Stevens L (2008) Biomedical accelerator mass spectrometry: recent applications in metabolism and pharmacokinetics. *Expert Opin Drug Metab Toxicol* 4(8): 1021–1033
14. ICH Topic M3: Note for Guidance on non-clinical safety pharmacology studies for human pharmaceuticals
15. Lappin G, Warrington S, Honeybourne D, Sanghera D, Dowen S, Lister N, Islam K, Lociuoro S (2007) Concentrations of AR-709 in plasma and key compartments of the lungs after microdosing. In: Poster Presented at the Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, USA
16. Lappin G, Wagner C, Langer O, Merbel VD (2009) New ultrasensitive detection technologies and techniques for use in microdosing studies. *Bioanalysis* 1(2): 357–366
17. Ni J, Ouyang H, Aiello M, Seto C, Borbridge L, Sakuma T, Ellis R, Welty D, Acheampong A (2008) Microdosing assessment to evaluate pharmacokinetics and drug metabolism in rats using liquid chromatography-tandem mass spectrometry. *Pharm Res* 25(7): 1572–1582
18. Zhou XJ, Garner RC, Nicholson S, Kissling CJ, Mayers D (2009) Microdose pharmacokinetics of IDX899 and IDX989, candidate HIV-1 non-nucleoside reverse transcriptase inhibitors, following oral and intravenous administration in healthy male subjects. *J Clin Pharmacol* 49(12): 1408–1416
19. Lappin G (2010) What are the next steps in microdosing? *Bioanalysis* 2(2): in press
20. Ings R (2009) Microdosing: a valuable tool for accelerating drug development and the role of bioanalytical methods in meeting the challenge. *Bioanalysis* 1(17): 1293–1305
21. Lappin G, Garner C (2008) The utility of microdosing over the past 5 years. *Expert Opin Drug Metab Toxicol* 4(12): 1499–1506
22. Diamond S (1995) The use of sumatriptan in patients on monoamine oxidase inhibitors. *Neurology* 45(6): 1039–1040
23. Lappin G, Kuhn W, Jochimsen R, Kneer J, Chaudhary A, Oosterhuis B, Drijfhout WJ, Rowland M, Garner RC (2006) Use of microdosing to predict pharmacokinetics at the therapeutic dose: experience with 5 drugs. *Clin Pharmacol Ther* 80(3): 203–215
24. Timchalk C (2004) Comparative inter-species pharmacokinetics of phenoxyacetic acid herbicides and related organic acids. evidence that the dog is not a relevant species for evaluation of human health risk. *Toxicology* 200(1): 1–19

CHAPTER 12

Epidemiology and bio statistics

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1 Introduction

Classically, epidemiology is defined as the study of the causes and the consequences of diseases occurring in a certain population. However, in the recent years the scope of epidemiological research has become much boarder, including the research for optimal treatment approaches in both acute and chronic diseases and the determination of number of patients affected by a disease, the latter being a cornerstone of public healthcare and preventive medicine. Epidemiological research provides a methodological basis for determination of factors involved in disease progression and risk.

2 Measures of the disease frequency

Epidemiologic disease frequency measures allow for the determination of the proportion of subjects suffering from a disease in a population and are therefore an important source of information for public healthcare. However, measures of disease frequency have in the recent years also gained importance in clinical pharmacology.

Imagine, for example seldom occurring severe side effects of a pharmacon. If for example 100 severe side effects are reported for a certain drug the magnitude of the problem needs to be considered. Should these 100 cases be cause for concern, should the treatment regimen be reconsidered? This question can only be answered based on the frequency of the side effect, considering the prescription rate of the drug and relating the number of occurring side effect to the total number of intakes. In addition, one may ask whether the drug induces an increased risk for side effects compared to other drugs in the same class or with the same indication? Again, the number of reported side effects tells little when the prescription rate of the two pharmacons is unknown.

Keywords: Epidemiology, prevalence, incidence, mortality, attributable risk, relative risk, correlation analysis, ecological studies

Indeed, the questions “how often does a disease occur and how well is a drug tolerated?” are key questions in epidemiological research. In the following we describe the measures of disease frequency that are most frequently used to answer these questions, the prevalence of a disease and the incidence of a disease.

2.1 Prevalence

The prevalence of a disease is a measure defined as the proportion of people currently having a certain disease. It is calculated as

$$\text{Prevalence} = \frac{\text{number of people suffering from a certain disease}}{\text{total number of people in a population}}$$

Usually, the prevalence is expressed as the prevalence ratio, describing the proportion of subjects suffering from a disease. Consequently, prevalence estimates are often expressed in percent. For example, several studies have investigated the prevalence of diabetes mellitus [1]. These studies estimated that the world wide prevalence for diabetes is 2.8% for the year 2000.

However, these data represent the prevalence among the whole population, regardless of other personal characteristics such as age or sex, geography or other possible confounding factors. To gain more information about the population, the prevalence can be further stratified. This means that the analysis of prevalence in the whole population

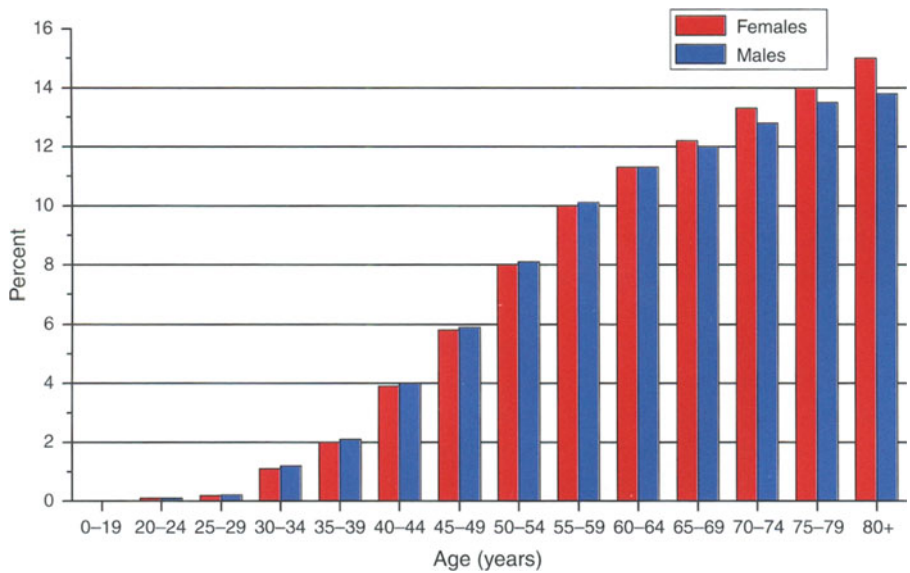


Fig. 1 Global diabetes prevalence by age and sex for 2000. Modified from Ref. [1]

can be further separated by building subgroups. For example the prevalence of diabetes can be stratified by race, sex, economic factors, such as income, or as shown in Fig. 1, by age. Obviously the prevalence of diabetes is not equally distributed via all age groups, but increases with increasing age. Accordingly, the prevalence is approximately 2% in the age group of 35–39, whereas it almost doubles when reaching the age of 50. These considerable differences can also be seen when stratifying for other factors.

As a further differentiation one can distinguish between the so called point prevalence and the period prevalence. The point prevalence gives a measure of the prevalence at a certain point of time, whereas the period prevalence describes the prevalence over a certain period of time. When the type of prevalence rate is not specified, usually point prevalence is meant. One must, however, mention that point prevalence in its strict sense cannot be investigated, because of the time required to perform a study. This is of little importance in diseases of chronic nature such as diabetes, but may be of major relevance in acute diseases with seasonal accumulation. In such diseases period prevalence is often given, specifying which fraction of the populations has the disease of interest over a specified period of time. Examples are annual prevalence rate and lifetime prevalence rate.

2.2 Incidence

In contrast to the prevalence, which describes the proportion of people currently suffering from a disease, the incidence describes the new cases of a disease that have developed over a certain time period. Again the incidence is described as a proportion. It is calculated as

$$\text{Incidence proportion} = \frac{\text{number of newcases of a disease}}{\text{number of subjects in the population}}$$

The incidence of a disease is of special importance because it gives an estimate of the risk to develop a disease within a specified period of time. For example, if from 10,000 persons 350 persons develop a disease over a 2 year period of time, the incidence is 3.5% for this time period. This incidence proportion is also known as cumulative incidence and defined as the number of *new* cases of disease occurring over a specified period of time in a population at risk *at the beginning of the interval*.

A slightly different approach is to calculate the incidence rate. The incidence rate reports the number of new cases of disease relating to a certain risk time. This risk time can, for example, be expressed as person years. In several publications, the incidence rate is also called the incidence density. It is calculated as

$$\text{Incidence rate} = \frac{\text{number of new cases of a disease}}{\text{person time at risk}}$$

The inclusion of the person time at risk gives a more realist measure of the incidence of a disease, especially if the time at risk is heterogeneously distributed among the

population under study. For example suppose that somebody wants to evaluate the incidence of a deadly car accident among a population. Although you will certainly end up with an incidence proportion if you calculate the number of deaths divided through the number of subjects in a population, this might only be a rough guess of the reality. Imagine that your population will include also a considerable proportion of people, who never – or very seldom – drive a car. Translated into medicine that would mean that there may be a number of patients who are not (or not always) exposed to a certain risk factor for the development of a disease. Therefore, the incidence rate, including the person time at risk, better reflects the reality than reporting only the incidence proportion.

2.3 Mortality

Mortality is defined as the number of deaths within a stated period of time divided by the number of persons at risk within a population. The so called total or crude mortality rate reflects deaths from all causes and is usually expressed as deaths per 1000. A disease-specific mortality indicates deaths caused by a certain disease and is often reported on the basis of 100,000 persons.

3 Relationship between prevalence, incidence and mortality

Of course, prevalence and incidence are not entirely independent from each other. The prevalence reflects how often certain diseases develop and how long they last. Or, to put it differently, the prevalence of a disease also includes the disease duration, rather than simply providing a measure for the risk. For example diabetes, has a relatively high prevalence when measured at a certain point of time, because it is a lifelong disease, although the incidence is relatively low.

In contrast, some forms of cancer have a low prevalence, because patients die fast. Prevalence is not only dependent on the rate of new occurring diseases, but also on the number of patients who die from the disease or who completely recover. As shown in Fig. 2, every new case enters a prevalence pool and remains there until death or recovery.

Consequently, when studying the etiology of a disease, it may be better to analyze both incidence and prevalence, since prevalence includes also information about the duration of a disease, rather than only providing a pure measure of risk. In particular, prevalence data does not consider patients that die before the prevalence study starts. In contrast, given that the disease prevalence indicates the number of patients currently suffering from a disease, prevalence data is important for planning of health services.

In addition, prevalence instead of incidence is often used to study rare chronic diseases, where it is difficult to accumulate large numbers of incident cases. However,

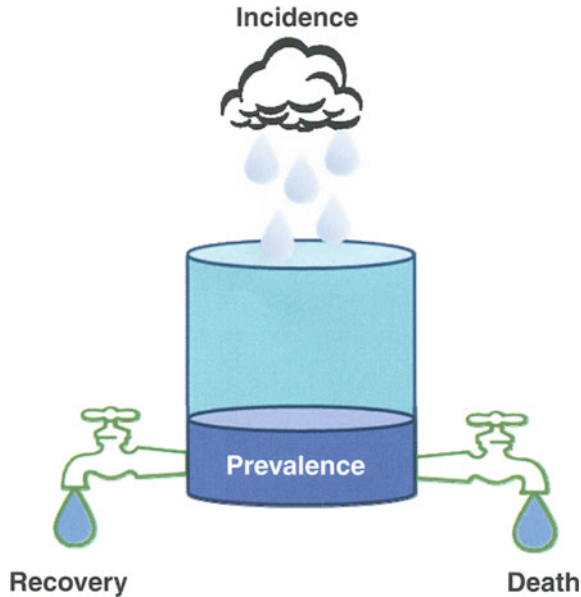


Fig. 2 Relationship between incidence and prevalence. Disease prevalence is dependent on the number of new occurring cases of the disease, but also on the number of patients who die or who recover

again, given that differences in prevalence can also be caused by different survival and recovery rates, the interpretation remains difficult.

4 Survival analysis

Survival is defined as the probability for staying alive for a specific period of time. The survival rate represents the percentage of people in a study or treatment group who are alive for a given period of time after diagnosis. Survival analysis attempts to answer the following questions: What is the fraction of a population or disease which will survive a certain period of time? Do particular circumstances or treatments increase or decrease the survival time?

For various severe diseases such as cancer, the 1-year or the 5-year survival time is estimated. For example, for pancreatic cancer, the median overall survival time under a combination chemotherapy regimen has been reported in one clinical trial reported to be 20.4 months [2]. Basically, the survival (S) can be calculated as

$$S = \frac{N - D}{N}$$

where N represents the number of newly diagnosed patients and D the number of deaths observed during a specific period of time. Imagine, for example 10 persons with lung disease being followed for 10 years. If 2 out of the 10 persons die during this time span, then the 10 year survival is $(10-2)/10 = 0.8$ or 80%. This number indicates the probability of surviving a specified length of time and is inversely related to the risk of death.

However, survival analysis does not necessarily have to focus on the death of a subject as clinical endpoint. In many clinical trials the clinical endpoint is not death, but for example aggravation of a disease or the occurrence of another critical clinical event. Even if the final outcome is not the time from entering the study to death, the terms survival time is used.

5 Censored data

One of the problems that may occur when performing survival analyses arises when patients are lost for follow up. The reasons for losing patients for follow up may be widespread including people moving away or non-compliance. Another bias may occur if a patient dies from a disease not related to the disease in question.

For all these cases, the missing data is called censored data. Censored data is of special importance in survival analyses because it may have a considerable impact on the outcome. If we consider the data of the above mentioned example of the 10 people suffering from lung disease we will find the following. If 20% (in our case 2 patients) of the sample is lost for follow up, two scenarios are possible: first, the 2 subjects have survived, resulting in 10 year survival of 80%. Alternatively, the two subjects could already be dead. This would

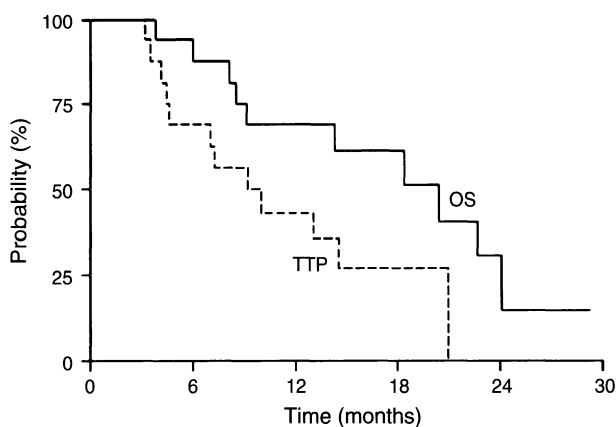


Fig. 3 Kaplan–Meier curves for the time to progression and overall survival in patients with pancreatic cancer. *TTP* Time to progression; *OS* overall survival

results in true a survival of 60%, leading to a serve overestimation of the survival rate. Consequently, several statistical approaches have been developed to overcome this problem. The most commonly used technique used to account for this problem is the Kaplan–Meier analysis. This statistical method takes censored data into account and allows for the correct interpretation of the results. Usually, such data is displayed in a so called Kaplan–Meier plot. A typical Kaplan–Meier plot is shown in Fig. 3.

6 Case fatality rate

The case fatality rate is defined as the percentage of people suffering from a disease, who die as a result of that illness within a given period of time. The case fatality rate is most frequently applied to a specific outbreak of an acute disease. In this case all patients have to be followed for an adequate period of time to include all attributable deaths but also all deaths not related to the disease. As an example, severe diseases such as Marburg haemorrhagic fever show case fatality rates of 80% and more [3].

7 Risk, relative risk and attributable risk

Risk, relative risk and attributable risk are important measures for epidemiological research. In particular, the risk is a measure of the probability of developing or dying from a disease during a certain period of time. For an exact definition and the use of this variables, please refer to the chapter “Observational studies”.

8 Epidemiologic study designs

Earlier in this book it has been emphasized that one crucial point to evaluate the effect of a drug or treatment is the random allocation to subjects. However, in epidemiologic research, where a researcher is investigating a possible association between the exposure to a risk factor and a subsequent disease, this approach is not feasible. It is ethically impossible to randomly expose subjects to possible hazardous factors such as radiation or dangerous environmental factors. Consequently, studies that focus on investigating an association between a risk factor exposure and a disease are mostly observational.

Additionally, clinical trials have a limited ability to detect infrequent events or events that result in common symptoms. This is mainly caused by the fact that clinical trials have usually strict inclusion and exclusion criteria, which allow for the exact determination of a certain drug effect, but often cannot be generalized to the entire population. Although the study designs that are used for epidemiological studies share a lot of properties with other clinical studies, certain differences can be pointed out. For a

detailed description of these studies designs please refer to the chapter “observational studies”.

9 Statistical measures

9.1 Why use statistics?

If you experience flue like symptoms, such as fatigue and headache you might consider taking aspirin. In most cases, this will lead to a relief of the symptoms. However, if you give aspirin to a large group of patients with the same symptoms, not all of them will experience the same effect. Unfortunately, this does not only hold true for this kind of drug. All kind of measurements done in humans rarely show the same results from one occasion to the next. In addition, symptoms and diseases cannot be simply regarded as present or not present. Whereas one patient may completely recover due to a pharmacological treatment, others will experience only a slight improvement of symptoms, no effect or an even a worsening of symptoms.

Although personal experience helps to get an idea whether a treatment may be helpful or not, our daily observation is not enough to quantify treatment effects. This holds true also for biological hazards and environmental risk factors. Although it is common sense that smoking considerably increases the risk of lung cancer, almost everyone of us knows somebody who has been smoking for decades without any visible signs of health impairment.

Statistics helps us to judge whether a new treatment is effective – and, if so – to estimate the effectiveness of the treatment, independent from personal feelings and expectations. Furthermore, statistics helps to ensure that data gained from a clinical study can be generalized for the rest of the population. Given that the field of biomedical statistics is wide and complex, the current chapter can only give a rough overview about the statistical methods used for clinical research. For a more detailed introduction into medical statistic the reader is referenced to the appropriate textbooks.

9.2 Variables

Variables assessed in epidemiological research can be divided into continuous, categorical, or binary. Continuous variables can take on an infinite number of values and are generally real numbers. Examples of continuous variables include serum concentrations of glucose and systemic blood pressure or body weight. Binary variables can take on only two values, Examples are sex (male or female), presence of a disease (yes or no) and regular intake of a certain medication (yes or no). Categorical variables can take on a few possible values. Examples include race, staging of a disease and marital status.

9.3 Presentation of variables

Normally too many data are collected in a clinical study to list individual outcomes in a table. Hence, numerous procedures have been proposed to display such data. In a histogram, observed values of a variable are displayed on the x -axis versus the relative frequency of these values on the y -axis.

In addition, a number of values are calculated to characterize the data. The most frequently calculated measure is the arithmetic mean, which is calculated by summing all of the observed values of a variable and then dividing by the total number of observations. The most common measure of the variability of the data is the standard deviation. For calculation of the standard deviation the difference between the individual value and the mean value is calculated for each variable. These differences are squared, summed up and divided by the total number of observations – 1. The obtained value is called variance and the standard deviation is calculated as the square root of the variance.

10 Population and sample

The population refers to all living people that are characterized by a specific characteristic. A sample is a subset of this population. Normally clinical trials are not performed by including the entire population. The aim of a study is, however to infer results from sample to the whole population. This is only possible if the selected sample is representative of the population. To select an adequate sample study participants should be drawn at random from a population (random sample). If a sample is randomly selected two factors determine how accurately a sample represents the population: sample size and variance. Obviously the higher the sample size the better is the population mirrored in the study.

10.1 Hypothesis testing and the p value

The most important question concerning medical statistics is to evaluate whether a hypothesis, defined during the planning phase of an experiment, is correct or has to be rejected. For this purpose, statistical tests are used. In the recent years, a couple of statistical tests have been introduced, all focusing on different statistical questions. In particular, most of the statistical tests try to answer the question whether a difference, which can be observed between two groups is accidental or reflects a true difference between groups or treatment. In the scientific literature, this is usually reported as “being statistically significant”.

In most of the published studies, a result is called statistically significant if the probability that the observed difference happened only by chance (without a really

difference between the groups) has a maximum of 5%. This is reflected by the term $p < 0.05$. In the last years, the p -value and being statistically significant has become more and more importance. In several disciplines, the p -value has become a sacred cow in the scientific community.

However, it has to be considered what a $p < 0.05$ really means and care has to be taken when interpreting such results. First, and most importantly, it has to be considered that even if a difference is statistically significant, it can be wrong. If we define the probability that our effect happens by chance as 5%, this also means that we have to face a risk of 5% to receive a positive test, even if there is not true effect. In statistics, this is also called a false positive result or a type I error. Or to put it differently, a 5% error probability also means, that, on average every 20th test is false positive. As discussed later in this chapter, this is of special importance, considering that in the most of the studies more than one statistical test is performed.

Another important point that needs to be stressed is that the 5% value is quite arbitrary, although it has become conventional in the medical literature in the last years. Consequently, the 5% value may not be appropriate for all studies. Imaging the following example: You are planning a picnic with your family on the next weekend. While you are loading your car, you hear the weather forecast in the radio: The forecast says that there is a probability of rain of 5% for the next weekend. Most certainly, this will not affect your holiday plans. Then the car mechanic from your local garage calls. Because of a fabrication defect of your new car, you have a probability to have a deadly car accident of 5%. I am sure you will have your car fixed before you leave for holidays. Of course this is a far-fetched example. However, it should demonstrate that 5% error probability may be acceptable for several questions, whereas it is not for others. In contrast, other studies and hypothesis may require a more strict (or less strict) error definition.

10.2 Post hoc analysis or “fishing for results”

As stated earlier in this book, one of the most important issues when planning a clinical study is to clearly define a hypothesis that is to be confirmed or rejected. Although it might be in the human nature to collect as much as data as possible, main outcome parameters have to be determined and statistical analysis has to be planned in advance and clearly described in the trial protocol. It is of special importance to separate the main outcome parameters from all other outcome parameters (such as safety variables or other additional outcome variables) in order to test the main hypothesis of the study. Otherwise the investigator may run into the danger to do *post hoc* statistical tests, i.e. tests that have not been foreseen in the original protocol, until one finds a statistical significant result.

In the scientific community, this post-hoc testing is usually called “fishing expedition” and should reflect the problem that you will certainly find a statistically significant result, if you only perform a sufficient number of tests.

This problem holds especially true for clinical studies that include post-hoc stratification or sub-group analysis. Obviously doctors are interested in the question whether a certain drug works better or worse in a particular group of patients with special characteristics. Although this is certainly a valid question, these subgroups need to be determined in advance, and the trial adequately planned. Most importantly, this has a major impact on the sample size calculation. The approach to do a post-hoc stratification of the original study group until one finds a statistically significant result, is not a valid approach.

It is important to state that this does not mean that additional outcome variables should not be analyzed in general. One has, however, to clearly differentiate between the main hypothesis that has been the original focus of the study, and additional hypothesis, that are to be explored afterwards. These additional hypothesis that have not been defined before study start may be presented in an explanatory manner or used hypothesis generating for a subsequent study specifically investigating this issue.

10.3 Multiple testing

As stated above, when performing a statistical test, we allow a certain error (in most of the cases set at 5%) that the test we are performing is false positive. That means we have a 5% risk that our test detects a difference between two groups, although no real difference exists. However, these assumptions hold only true if we perform one single test only. Obviously, if we carry out a large number of independent tests, each with a significance level of 5%, some of the tests will be significant, even in the absence of a real effect.

If we face the scientific literature we will find that in the most of the studies more than one independent statistical test is used. This has to be considered when interpreting the results of such trials. One solution to control for the type I error, is to use a statistical correction for multiple testing. Among others, the Bonferroni method has been introduced as a simple procedure to correct for multiple testing. The idea of the test is that if we conduct n tests at a significance level of α_{sig} , we consider the results as statistically significant only if the p value is less than α_{sig}/n . For example, if we consider to perform 5 significance tests at a significance level of 0.05, we would only declare a p value of 0.01 ($0.05/5$) or less as statistically significant.

10.4 Correlation analysis

Correlation or linear regression analysis is a statistical technique to assess the relationship between two variables. In correlation analysis the linear association between two variables is calculated. The strength of the association is reflected by the correlation coefficient. Consequently, the correlation analysis answers the questions whether there is an association between two variables.

For more complex questions, regression analysis has been introduced. In regression analysis the dependence of one variable on another is calculated. Basically a regression analysis is performed when it is believed that one variable is directly caused by another. The relationship can then be expressed by a regression equation.

10.5 Association and causation

An important error that is often made in the interpretation of clinical studies is to assume that simply because two variables are associated, one causes the other. This holds especially true for observational studies, where the possibility of unknown confounding variables can never be ruled out. The assessment whether an association is really linked to a distinct cause is sometimes difficult and mainly based on the interpretation of the researcher.

One of the most important points when interpreting an observed association and a possible cause is to evaluate whether there is a plausible biological hypothesis underlying the observed association. In addition, several attempts have been made to find objective criteria to determine a causal relationship. Nowadays the “Hill’s Criteria of Causation” or sometimes referred to as the “Bradford Hill criteria” are normally used to judge the causative relation between two variables [4]. Originally introduced by Austin Bradford Hill (1897–1991), a British medical statistician, Hill’s Criteria form the basis of modern research to assess scientifically valid causal connections between potential risk factors and diseases. The most important criteria are described as follows.

10.5.1 Association strength

The stronger the association between possible cause and disease, the more likely is that the relation is causal. If a disease risk is for instance strongly correlated to the exposure time of a potential hazard, the causal relationship is extremely likely.

10.5.2 Temporal relationship

The temporal relationship is the only knock out criterion. Obviously, the exposure has to precede the disease. If for example smoking is believed to cause lung cancer, then it is clear that exposure to cigarette smoke must always precede the occurrence of the disease.

10.5.3 Dose-response relationship

If available, a dose relationship between a drug or a potential hazard and a clinical outcome is a very strong hint for a valid causal relationship. However, it is important to notice that the absence of a dose-response relationship does not necessarily rule out the possibility of a causal relationship.

10.5.4 Constancy

The association between potential hazard and outcome has to be consistent in all studies, even when using different statistical approaches or different designs. The more experiments show an association, the more likely is a causal relationship.

10.5.5 Plausibility

As stated above, an important point is that the potential causal relationship is plausible with respect to the current knowledge and the scientific understanding of the disease and the risk factor. Or to put it differently, there is the need for a theoretical basis as an explanation. Although one may find, by chance, an association between the number of soled cars in the western countries between 1900 and new and the number of people wearing green t-shirts, it does not necessarily mean that one is caused by the other. One has, however, to consider that the current scientific understand of diseases may not be correct or complete, and may possibly be reconsidered based on findings of epidemiological studies.

10.5.6 Experiment

Experimental data can provide evidence to confirm or reject the hypothesis. However, this approach is not feasible for all diseases and conditions.

10.5.7 Specificity

In this context, specificity means that one single cause results in one single condition. Although, if found, this strengthens the probability that the found association is a causal, relationship, the absence of specificity does not exclude a causal relationship. In keeping with our daily live experience, diseases are often influenced by multiple factors. Thus, it is unlikely to find a single cause producing a specific disease.

Case Study: Multiple post-hoc comparisons in the “Second International Study of Infarct Survival (ISIS-2)”

One of the most prominent examples for inappropriate post-hoc comparisons has been published several years ago based on data of the so called ISIS-2 (Second International Study of Infarct Survival) study. Originally, the ISIS-2 study was designed to investigate the effect of either streptokinase treatment or daily administration of aspirin in a population scale study comprising more than

17,000 patients with suspected acute myocardial infarction [5]. The data of the study revealed that treatment with both aspirin and streptokinase was highly beneficial for the patients. Moreover, the combination of streptokinase with aspirin was significantly better than either one of the agents alone.

In addition, the authors report in their study also the outcome of a large number of post-hoc comparisons, focusing on the effect of the treatment on certain sub-groups such as sex, history of diabetes and others. However, on a closer look, this table looks surprising. As the first result, the authors present the odds ratio for subjects born under the astrological birth sign Gemini and Libra. In particular, for subjects born under stars Gemini and Libra, treatment with aspirin was not superior to placebo. This – on the first glance – funny presentation has a serious background: The authors were asked to include the results of the post-hoc comparisons in their final publication because of the potential clinical importance of these results. Being aware of the fact the multiple post-hoc comparisons are problematic, the authors agreed only provided that the first items that were shown in the table are the results of the star sign analysis. This was done simply to demonstrate that the interpretation of all of the post-hoc comparisons has to be done with caution.

References

1. Wild S, Roglic G, Green A, et al. (2004) Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 27: 1047–1053
2. Nakai Y, Isayama H, Sasaki T, et al. (2009) A pilot study for combination chemotherapy using gemcitabine and S-1 for advanced pancreatic cancer. *Oncology* 77: 300–303
3. Bausch DG, Nichol ST, Muyembe-Tamfum JJ, et al. (2006) Marburg hemorrhagic fever associated with multiple genetic lineages of virus. *N Engl J Med* 355: 909–919
4. Hill AB (1965) The environment and disease: association or causation? *Proc R Soc Med* 58: 295–300
5. ISIS-2 Collaborative Group (1988) Randomised trial of intravenous streptokinase, oral aspirin, both, or neither among 17,187 cases of suspected acute myocardial infarction: ISIS-2. ISIS-2 (Second International Study of Infarct Survival) Collaborative Group. *Lancet* 2(8607): 349–360

CHAPTER 13

Placebo effects and placebo control in clinical trials

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The history of placebo goes back several centuries. These “dummy pills” have been used by healers and physicians worldwide, ignored by the official medical community [1]. In 1931, Amberson et al. introduced the concept of experimental randomization to medical research via a study on tuberculosis treatment. They randomized 24 tuberculosis patients into two groups, one group receiving sanocrysin for treatment, the other group distilled water. The randomization was performed by flipping a coin [2]. Substances or medical procedures should be considered within a complex psychosocial context that may influence the therapeutic outcome [3]. A placebo can be any clinical intervention including gestures, words, devices, pills and surgery. In context with surgery the term sham is sometimes used to describe such a placebo intervention [4]. To dissect this psychosocial effect and to reject the specific action of the therapy, a dummy treatment, the placebo, is given which makes the patient believe to be effectively treated. The response to this treatment, the placebo effect, is also known under such terms as expectancy effect, context effect and meaning response. The real placebo effect is a psychobiological phenomenon that can be the result of different mechanisms including the anticipation of clinical benefit and Pavlovian conditioning [3]. Various studies suggest that there are physical aspects influencing people’s perceptions e.g. the colour and size of the pills. Others report that capsules are experienced to have stronger effects than tablets. Injections trigger a stronger placebo response than oral medication and surgery elicits probably the highest rates in placebo response [4]. It has been reported that placebos may improve subjective and objective outcomes in up to 30–40% of patients with a wide range of clinical conditions, considering that the placebo effect cannot be distinguished from the natural course of the disease, regression to the mean and the effects of other factors. In general, the presence of pain and anxiety, the involvement of immunobio-

Keywords: Placebo, control groups, ethics, surgery, irreversible harm

chemical processes and the autonomic nervous system are supposed to respond expediently to placebo, whereas chronic degenerative diseases, hyperacute illnesses like heart attacks and hereditary diseases are anticipated to resist [1].

Nowadays, the gold standard in clinical trial design is the double-blind, randomized, two-armed placebo controlled study [3] but since this first placebo-controlled trial in 1931, there has been a controversy regarding the appropriate use of placebo in clinical trials, especially when patients randomly assigned to receive placebo have forgone effective treatments [5–7]. Eventually this controversy has led to the initiation of active-control trials, where a new intervention is compared to an established one.

Conceptually the randomized, controlled trial (RCT) is not a form of individualized medical therapy; it is a scientific tool for evaluating treatments in groups of research participants, with the aim of improving the care of patients in the future. From the standpoint of research logic, RCTs generally do not intend to promote the best medical interests of enrolled subjects, but may even expose them to risks that are not outweighed by benefits. It is important that patient volunteers understand that they are enrolled in a study that may produce clinical benefits, but on the other hand may fail to produce benefits or even cause medical disadvantage. Thus, clinical research involves an inherent tension between the ethical values of pursuing rigorous science and protecting participants from harm [8].

To avoid exploiting research subjects, clinical trials must satisfy several ethical requirements. Accordingly, the use of placebo in clinical trials must be evaluated in terms of the ethical principles appropriate to clinical research, which are not identical to the ethical principles of clinical practice [9]. Clinical trials are unethical if they are not designed to answer valuable scientific questions with the use of valid research methods. In addition to having scientific merit, clinical trials must present a favourable risk–benefit ratio: the risks to participants must be minimized and justifiable by the potential value of the scientific knowledge to be gained from the study and care for future patients.

1 The recent debate about research ethics in placebo controlled trials (PCTs)

To harmonize attitudes towards ethical aspects of clinical research a number of ethical codes has been established and promoted. Perhaps the best known of these is the Declaration of Helsinki (DOH). The World Medical Association (WMA) was established in 1947, after the Second World War and today is an organization of 85 national medical associations representing roughly eight million physicians. A major revision and reorganization, specifically addressing the use of placebo (Article 29), was completed in Edinburgh, Scotland, in 2000 [10]. This revision states that “The benefits, risks,

burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists (www.wma.net). Unfortunately this wording has brought confusion to the scientific community and has led to two apparently contradictory conceptions about how to conduct a clinical trial [8]. At a four-day council-meeting in Ferney-Voltaire, France, the WMA agreed that there are circumstances which would legitimate the use of placebo although a proven therapy is available. If, for obligatory and scientifically correct methodological reasons the use of placebo is necessary to determine the impact or safety of a prophylactic, diagnostic or therapeutic method, or where such a method is investigated for a minor condition and patients who receive placebo are not subject to any additional risk of serious or irreversible harm, such a use of placebo may be legitimate [11]. The new version of the DOH (Seoul 2008) addressed important points like emphasizing the need for providing access to research to otherwise underrepresented populations, a clear differentiation between what should go in the protocol and what is required of the research ethics committee, registration of clinical trials, a clear discrimination between health professionals and scientists and researcher's justification of their request for exemption from the consent requirement to the research ethics committee (WMA news). However, the article about placebo (Article 32) was not changed.

2 Placebo vs. active control

One view is that placebo-controlled trials are still necessary (Placebo orthodoxy). Advocates of PCTs argue that without a placebo group to ensure validity, the finding that there is no difference between the investigational and the standard intervention can be misleading or uninterpretable [12]. On the other hand, proponents of active controls contend that whenever an effective intervention for a given condition exists, it must be used in the control group (*active-control orthodoxy*) [13]. Furthermore, they argue that placebo controls are inappropriate because the clinically relevant question is not whether a new drug is better than Placebo or nothing, but whether it is better than standard treatments. The aim of an active control trial is to show that the new intervention is more effective (superiority) or not worse (non-inferiority) than an old one. In these cases, a new intervention could have less side-effects or it could be cheaper than standard treatment. Advocates of active controls criticize placebo orthodoxy for placing the demands of science ahead of the rights and well-being of study participants. However, the ethical standpoint of active-control orthodoxy is also far from ideal. Most important, trials with active controls may expose more patients to harm than placebo-controlled trials. Equivalence trials, which evaluate the hypothesis that one drug is equivalent to another, typically require larger samples to achieve sufficient power,

because the delta, or difference between the rates of response to the two drugs, is likely to be smaller than that between the rates of response to an investigational treatment and placebo [14].

3 The issue of “assay sensitivity”

The assay sensitivity of a clinical trial is defined as the ability to distinguish an effective treatment from a less effective or ineffective treatment. There are different requisites for assay sensitivity depending on whether trials intend to show differences between treatments (superiority trials) or intend to show non-inferiority (www.ich.org). A trial planned to demonstrate superiority of a test treatment compared with control lacking assay sensitivity will fail to show superiority of the test treatment and will fail to lead to a conclusion of efficacy. On the other hand, a trial designed to demonstrate efficacy by showing non-inferiority of a test treatment to an active control lacking assay sensitivity may find that the ineffective treatment is non-inferior and so could lead to an erroneous conclusion of efficacy (www.ich.org). An important aspect that needs to be considered in PCTs and even more so in active control trials is that results of badly executed trials can create the illusion of efficacy. This aspect is based on to the concept of assay sensitivity. Assay sensitivity establishes a trials ability to demonstrate between-intervention differences and is pertinent to all trial designs. Poor assay sensitivity can result in type I (false conclusion of efficacy) or type II errors (false conclusion of no efficacy). In contrast to a PCT, where a type II error is usually less important, a false conclusion of “no difference” is the type of error one wants to avoid in an active-control trial [15]. In 1999 assay sensitivity was analyzed by the ICH, which offers, issued as ICH E10, a list of eight factors that can comprise assay sensitivity (ICH).

4 Placebo controlled trial: ethical or not?

There is no simple answer to a complex problem. However, we believe that neither of the absolute positions (placebo *vs.* active-control orthodoxy) is tenable. From our perspective, the basis of a decision on whether or not a PCT is justifiable strongly depends on the particular research scenario:

Scenario 1: If an effective, life-saving or at least life-prolonging intervention exists, and if patients assigned to placebo would substantially more likely suffer serious harm than those assigned to receive the investigational intervention, a PCT should not be conducted. As an example, the efficacy of streptokinase in reducing morbidity and mortality after myocardial infarction made it unethical to conduct PCTs with tissue plasminogen activator, due to the fact that patients in the placebo control group have no access to a very beneficial medical intervention [16].

Scenario 2: On the other hand it is obvious that for diseases for which no proven therapy exists, are not serious and if there is only a minimal chance for patients receiving placebo to suffer harm or severe discomfort, a PCT seems justified. For instance, a PCT of a new antifungal for treatment of onychomycosis would meet these requirements. Also in case of otitis media an RCT might be justifiable as the discomfort associated with otitis media typically does not severely impair health. Most important, there is evidence that otitis media is a self-limiting disease and resolves spontaneously in most cases. Further “standard” antibiotics provide small benefit and can cause adverse reactions [17]. The risk associated with these trials are similar to those in epidemiologic studies in which blood samples are obtained solely for research purposes and in pharmacokinetic studies in healthy volunteers in which there is no benefit to the participants.

Scenario 3: In most situations, however, the way to go is not clear-cut because a treatment known to be effective is at hand and there is some potential of harm to subjects receiving placebo. The decision on whether or not a PCT would be justified must be based on an evidence-driven discussion on where in the spectrum of possible scenarios a given situation is located (Fig. 1). An interesting case that illustrates the struggle of researchers and the scientific community over the ethical responsibility of

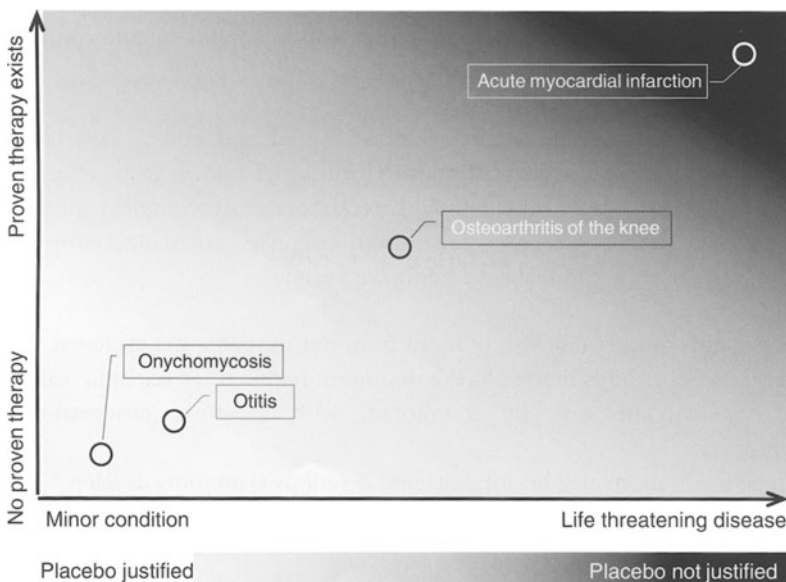


Fig. 1 When to use placebo. In most cases, the decision, whether a placebo controlled trial is justified or not, is not as easy as in myocardial infarction (white symbol) or in otitis media (black symbol)

researchers in PCTs is a publication on the use of “placebo surgery”, which is presented in detail below.

In this context, it is important to recognize that PCTs and active-controlled trials have distinct objectives, and each type of trial may have a role in a sequential approach in evaluating new therapeutic interventions.

5 Criteria for justification of placebo

As cited above, the WMA mentions placebo control in Article 32 of the current version of the DOH: “. . . However, a placebo controlled trial may be ethically acceptable, even if proven therapy is available, . . . where for sound methodological reasons use is necessary to determine the efficacy . . . , or . . . for a minor condition and the patient will not be subject . . . to serious or irreversible harm.” (www.wma.net).

Therefore a PCT has a sound scientific rationale if the following criteria are met:

- there is a high placebo-response rate
- the disease is typically characterized by a waxing-and-waning course, frequent spontaneous remissions, or both
- existing therapies are only partly effective or have very serious side effects
- the low frequency of the condition means that an equivalence trial would have to be so large that it would reasonably prevent adequate enrollment and completion of the study.

Although PCTs that meet these methodological and ethical criteria may be justifiable even though the participants forgo therapies known to be effective, they remain worrisome. Consequently, standard precautions must be implemented for these trials. When such a trial is proposed, the institutional review board must ensure that the following safeguards are available to minimize harm:

- participants at increased risk of harm from non-response are excluded
- the placebo period is limited to the minimum required for scientific validity
- subjects will be carefully monitored, with inpatient observation when appropriate
- rescue medications will be administered if serious symptoms develop
- there are explicit and specific criteria for the withdrawal of subjects who have adverse events.

In addition, the investigators should clearly disclose the rationale for using placebo, explain that subjects in the placebo group will not receive standard effective treatments, and state the risks associated with forgoing such treatments.

Case Study: Placebo Surgery

Here we describe an interesting case study about the first use of “placebo surgery” in a clinical trial [18]. Arthroscopic lavage or debridement is the method of choice to relieve the pain of osteoarthritis of the knee when medical therapy fails. In the USA more than 650,000 arthroscopies are performed each year producing enormous costs. In uncontrolled studies about 50% of the patients reported relief from pain, but the physiological basis of this pain relief is not clear.

A randomized, placebo-controlled trial was performed to assess the efficacy of arthroscopic surgery of the knee in alleviating pain and improving function in patients with osteoarthritis. The patients as well as the assessors of outcome were blinded to the treatment assignments. Patients enrolled in the trial were recruited from the Houston Veterans Affairs Medical Centre from October 1995 through September 1998. Inclusion criteria were: 75 years old or younger, osteoarthritis of the knee as defined by the American College of Rheumatology, at least moderate knee pain on average (minimum 4 on a visual-analogue scale ranging from 0 to 10) despite maximum medical treatment for at least six months, no arthroscopy of the knee during the previous two years. A radiological examination was performed to assess the severity of osteoarthritis in the study knee (that knee with the greater pain-induced limitation of function) and to grade it on a 0 to 4 scale. Three compartments (medial, lateral and patellofemoral) were scored and added together to generate a severity grade of 0 to 12. Exclusion criteria were: severity grade of 9 or higher, severe deformity, serious medical problems. The informed consent included writing in their chart “On entering this study, I realize that I may receive only placebo surgery. I further realize that this means that I will not have surgery on my knee joint. The placebo surgery will not benefit my knee arthritis.” In the end, 180 patients participated in the trial. Participants were divided into three groups according to the severity of osteoarthritis (grade 1–3, grade 4–6 and grade 7, 8). A stratified randomization with fixed blocks of six was used, 60 patients were assigned to the placebo group, 61 to the lavage group and 59 to the debridement group. The treatment assignments were sealed in sequentially numbered, stratum-specific envelopes and given to the research assistant, who handed the envelope to the surgeon, after the patient was in the operating theatre. The patient was not informed about the treatment assignment. One orthopedic surgeon performed all procedures. Patients in the debridement group and the lavage group received standard general anesthesia with endotracheal intubation. Participants in the placebo group received a short-acting intravenous tranquilizer and an opioid and spontaneously breathed oxygen-enriched air. In the lavage group the knee joint was lavaged with at least 10 L of fluid; anything that could be

removed through arthroscopic cannulas was flushed. Only an unstable tear in the meniscus was removed and the meniscus smoothed, but no other debridement was performed. In the debridement group the joint was lavaged with at least 10 L of fluid, rough cartilage was shaved, loose debris removed and all torn or degenerated meniscal fragments were trimmed. The remaining meniscus was smoothed, but no abrasion arthroplasty or microfracture was performed. In the placebo group a standard arthroscopic debridement procedure was simulated. Therefore the knee was prepped and draped and three 1-cm incisions were made in the skin, but no instrument entered the transactions for arthroscopy. End-point data were collected 2 weeks, 6 weeks, 3 months, 6 months, 12 months, 18 months and 24 months after the procedure. The primary end-point was pain in the study knee 24 months after the procedure, assessed by a 12-item self-reported Knee-Specific Pain Scale (KSPS), ranging from 0 to 100 (high score indicating more pain), created for this study. Five secondary efficacy end points were used: two additional assessments of pain and three assessments of function at all time points. General arthritis pain, not specifically in the study knee, was assessed by means of the four-item pain subscale of the Arthritis Impact Measurement Scale (AIMS2-P), higher scores indicating more pain. General body pain was assessed with the 2-item subscale of the Medical Outcomes Study 36-item Short-Form General Health Survey (higher scores indicating less pain). Two more self-reported measures of physical function were used: the 5-item walking-bending subscale from AIMS2 (AIMS2-WB, transformed into scales from 0 to 100, higher scores indicating more limited function) and the 10-item physical function subscale from the SF-36-P (transformed into scales from 0 to 100, higher scores indicating better function). For objective measurement the Physical Function Scale (PFS) was used to record the amount of time in seconds that a patient requires to walk 30 m and to climb up and down a flight of stairs as quickly as possible (longer times indicating poorer function). The trial was designed to have 90% power with two-sided type I error of 0.04 to detect a moderate effect size between the placebo group and the combined arthroscopic-treatment groups in term of body-pain as measured by the SF-36-P at two years, with an enrollment of 180 patients and 16 or fewer lost to follow up. All statistical tests compared the treatment groups in term of the values at each visit rather than analyzing the changes from the base line. The prespecified analytic strategy was to test at all time points if arthroscopic procedures are superior compared with placebo procedure, but lacking evidence of superiority, testing for equivalence was performed. The calculation of the minimal important differences was performed in two different ways: the change ratings of patients (the same, somewhat better/worse, much better/worse before surgery) and the standard error of measurement. For each scale the hypothesis that the placebo

procedure is equivalent to the arthroscopic procedures was tested. The results after one year and after two years show that there is no difference in knee pain and in arthritis pain between the placebo group and either the lavage group or the debridement group. There was also no significant difference between the placebo group and either the lavage group or the debridement group in the self-reported ability to walk and bend at one year. The results for objectively measured walking and stair climbing were poorer in the debridement group than in the placebo group after two weeks and one year, showing a trend toward worse functioning at two years.

Summarized Mosley's study provides strong evidence that arthroscopic lavage with or without debridement is not better than and seems to be equivalent to a placebo procedure in relieving pain.

There were many criticisms about this study and especially about generalizability (only one surgeon performed all procedures; most patients enrolled were male, although most patients with knee osteoarthritis are women; the equivalence analysis was underpowered; improper scales were used) [19]. Moreover, there is another problem: How can the use of "placebo surgery" be justified in this case?

Even opponents of sham surgery acknowledge that the double-blinded, randomized, placebo-controlled study is the so-called gold standard in research design and a PCT is ethically correct if (1) the risks are minimized, (2) risks which are not offset by potential benefits are limited and (3) the informed consent includes information about the planned procedure, the potential risks and benefits for the subject and the knowledge of the subject being a volunteer.

References

1. Papakostas YG, Daras MD (2001) Placebos, placebo effect, and the response to the healing situation: the evolution of a concept. *Epilepsia* 42(12): 1614–1625
2. Amberson JB Jr, McMahon BT, Pinner A (1931) clinical trial of sanocrysin in pulmonary tuberculosis. *Am Rev Tuberc* 24: 401–435
3. Colloca L, Benedetti F (2005) Placebos and painkillers: is mind as real as matter? *Nat Rev Neurosci* 6(7): 545–552 (Review)
4. Oken BS (2008) Placebo effects: clinical aspects and neurobiology. *Brain* 131(Pt 11): 2812–2823 (Epub 2008 Jun 21. Review)
5. Lasagna L, Mosteller F, von Felsinger JM, Beecher HK (1954) A study of the placebo response. *Am J Med* 16: 770–779
6. Way WL (1984) Placebo controls. *N Engl J Med* 311: 413–414
7. Emanuel EJ, Miller FG (2001) The ethics of placebo-controlled trials – a middle ground. *New Engl J Med* 345: 915–919
8. Miller FG, Rosenstein DL, DeRenzo EG (1998) Professional integrity in clinical research. *JAMA* 280: 1449–1454

9. Emanuel EJ, Wendler D, Grady C (2000) What makes clinical research ethical? *JAMA* 283: 2701–2711.
10. Puri KS, Suresh KR, Gogtay NJ, Thattai UM (2009) Declaration of Helsinki, 2008: implications for stakeholders in research. *J Postgrad Med* 55(2): 131–134
11. Saunders J, Wainwright P (2003) Risk, Helsinki 2000 and the use of placebo in medical research. *Clin Med* 3(5): 435–439 (Review)
12. Temple R, Ellenberg SS (2000) Placebo-controlled trials and active-control trials in the evaluation of new treatments. I. Ethical and scientific issues. *Ann Intern Med* 133: 455–463
13. Freedman B (1990) Placebo-controlled trials and the logic of clinical purpose. *IRB* 12: 1–6.
14. Leon AC (2000) Placebo protects subjects from nonresponse: a paradox of power. *Arch Gen Psychiatry* 57: 329–330
15. Urquhart J (2001) Demonstrating effectiveness in a post-placebo era. *Clin Pharmacol Ther* 70: 115–120
16. Brody BA (1997) When are placebo-controlled trials no longer appropriate? *Control Clin Trials* 18: 602–612
17. Glasziou PP, Del Mar CB, Sanders SL, Hayem M (2002) Antibiotics for acute otitis media in children (Cochrane Review). In: *The Cochrane Library*, Issue 3, Oxford
18. Moseley JB, O'Malley K, Petersen NJ, Menke TJ, Brody BA, Kuykendall DH, Hollingsworth JC, Ashton CM, Wray NP (2002) A controlled trial of arthroscopic surgery for osteoarthritis of the knee. *N Engl J Med* 347(2): 81–88
19. Maravic M, Landais P (2003) Arthroscopy for knee osteoarthritis. *Joint Bone Spine* 70(6): 404–406 (Review no abstract available)

SECTION 3

Tools in Clinical Pharmacology

CHAPTER 14

Tools in clinical pharmacology – imaging techniques

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1 Introduction

Biomedical imaging has been changing the way medicine is practiced ever since. Imaging techniques used in clinical pharmacology can be categorized as either functional or anatomical modalities. Functional modalities are capable of visualizing biological processes within organs or tissues at a molecular level. Positron emission tomography (PET) and single photon emission computed tomography (SPECT) are well established tools, whereas optical imaging (fluorescence) is a novel and promising one. Structural morphology of organs or tissues can be investigated with anatomical techniques such as magnetic resonance imaging (MRI), X-ray computed tomography (CT) or ultrasound imaging. Furthermore there is the up-and-coming possibility to combine different imaging modalities such as PET/CT, SPECT/CT, and MRI/PET in order to match functional to anatomical information.

In clinical studies, imaging endpoints might be closer to the cause of disease than rather non-specific physiological measures, such as vital signs, or biomarkers (distinctive biological or biologically derived indicators). Such endpoints allow accurate quantification of disease effects or some associated correlate and so potentially disease-modifying drug effects can be detected earlier than with conventional methods. Currently, nuclear imaging techniques are the most advanced and widely used imaging modalities for this type of assessment [1, 2]. In the discipline of clinical pharmacology, imaging modalities can provide important information in the following areas:

Keywords: Imaging, drug development, positron emission tomography, single photon emission computed tomography, optical imaging, magnetic resonance imaging, computed tomography, ultrasound imaging, aprepitant, dose finding study

- Pharmacokinetic information such as absorption, distribution, metabolism and elimination including delivery and residence time of radiolabelled drug candidates to specific tissues targeted for treatment.
- *In vivo* drug action at the desired pharmacological target site, including dose-target site occupancy relationships (e.g. dose finding studies).
- Pharmacological effects of a drug on *in vivo* biochemistry and physiology, drug-induced functional changes (e.g. blood flow and metabolism).
- Monitoring of disease progression.
- Monitoring of biomarkers.

Drug development is a lengthy, high-risk and costly process. Currently it takes about 10–15 years and almost US\$1 billion to bring a new drug to market. Recent data states that about 75% of the costs of drug development are due to failures mainly in the early stages of development [3]. Furthermore, 40% of exits from Phase I trials are caused by inappropriate pharmacokinetics of the test compound [4]. From an economical point of view shortening the process of drug discovery and development would be a major contribute in reducing this substantial cost. Regulatory authorities acted in order to advance exploratory investigational new drug studies in humans. In 2003, the European Agency for the Evaluation of Medicinal Products (EMA) published concept and position paper on nonclinical safety studies needed to support human clinical trials with a single dose of a pharmacologically active compound using microdose techniques [5]. According to this paper a microdose is defined as “less than 1/100th of the dose calculated to yield a pharmacological effect of the test substance based on primary pharmacodynamic data obtained *in vitro* or *in vivo* and at a maximum dose of $\leq 100\mu\text{g}$ ”. The feasibility of performing clinical microdose studies critically depends on the availability of ultrasensitive analytical methods that are capable of detecting minute drug amounts in plasma and tissue samples, such as accelerator mass spectrometry (AMS) or PET. For PET imaging, drugs labelled at high specific activity are commonly used, so that the mass of unlabelled drug associated with a PET tracer is usually low enough to satisfy the definition of a microdose. Microdose studies, also referred to as human phase 0, aim at describing a preliminary absorption, distribution, metabolism, and excretion (ADME) profile of a new compound in humans. The availability of such data at an early stage along the path of pharmaceutical development is crucial for decision making if a drug compound has potential for further clinical development [6].

In March 2004, the US Food and Drug Administration (FDA) denounced in the Critical Path Report the “slowdown, instead of the expected acceleration, in innovative medical therapies reaching patients” [7]. Molecular imaging is the major imaging technique used in clinical drug research and development. As already mentioned, PET and SPECT can be used to gain insights into the pharmacokinetics, bioactivity and dosing of drugs. In the following, a short overview of different imaging modalities that are currently used in clinical research is given.

2 Positron emission tomography (PET)

For PET imaging, so-called radiotracers are used, i.e. molecules labelled with short-lived positron emitting radioisotopes, such as ^{15}O ($t_{1/2}$, 2 min), ^{13}N ($t_{1/2}$, 10 min), ^{11}C ($t_{1/2}$, 20 min), ^{68}Ga ($t_{1/2}$, 68 min) and ^{18}F ($t_{1/2}$, 110 min). Typically, radiotracers are injected intravenously and their distribution within the body over time is monitored by a PET camera. The principle of PET is illustrated in Fig. 1. The positron which is emitted by the radioisotope annihilates with an electron and the mass of both particles is transformed into two γ -rays, which are emitted in directions 180° apart. This coincidence event is detected by a detector ring, which allows localization and quantification of the radiolabelled compound in the living organism. The sensitivity of PET for the detection

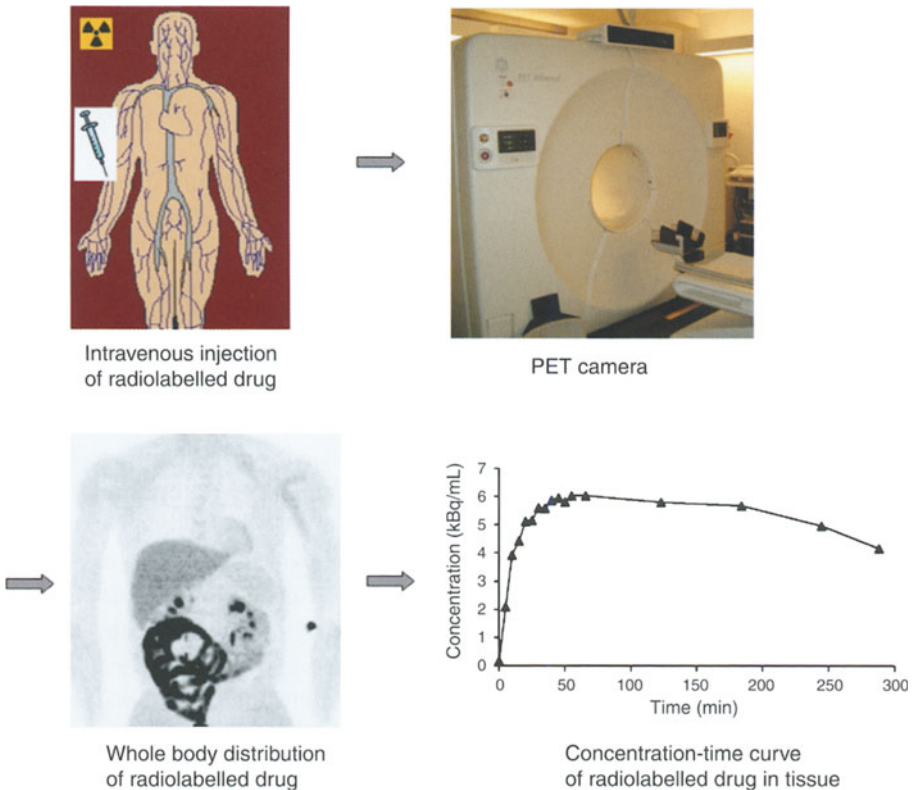


Fig. 1 Use of PET for pharmacokinetic measurements. Radiolabelled drug is intravenously injected and its distribution and pharmacokinetics measured non-invasively by PET imaging. Analysis of serial PET images taken over time provides concentration-time curves of the drug of interest (e.g. in tissue targeted for therapeutic treatment). The shown example image and curve represent the radiolabelled antibiotic agent [^{18}F]ciprofloxacin (see Ref. [14])

of mass is very high (10^{-11} – 10^{-12} mol/L), which allows administration of very small (μg), non-pharmacological drug quantities. The spatial resolution of PET depends on the size of the single detector component, varying between 2 and 8 mm³ in clinical imaging systems. Due to the short physical half-lives of PET radioisotopes an on-site cyclotron and a PET radiochemistry laboratory are mandatory.

One of the main advantages of PET is the quantitative nature of the technique allowing assessment of drug concentration in different tissues and organs. PET can be considered as a non-invasive technique, except that radiotracer is injected intravenously and that arterial blood sampling is commonly employed for parent drug and metabolite analysis. The use of ¹¹C as a radioisotope allows for the labelling of drug molecules without changing their chemical structures thereby conserving the physical and biochemical properties of the compound of interest. The short physical half-lives of PET radioisotopes result in favourable radiation dosimetry. A typically administered activity of ¹⁸F-tracer of 400 MBq given intravenously corresponds to a total effective dose of about 5 mSv, the same amount of ¹¹C-tracer corresponds to about 2 mSv. Therefore, the radiation exposure of one PET scan is approximately in the same order as the level of natural background irradiation (1–2 mSv/year).

Regulatory authorities have recently proposed a reduced preclinical safety testing package, when microdose quantities of drugs are administered to humans [5, 8]. This now offers the possibility for a straightforward translation of PET studies with radiolabelled drug candidates (“PET microdosing”) to investigations in humans [9].

One limitation of PET is the low spatial resolution compared with anatomical imaging modalities such as CT and MRI. However, this can be mitigated by PET-CT or PET-MRI combinations. Another limitation is that the short radioactive half-lives of PET radioisotopes only allow for short sampling periods giving inaccurate estimates of pharmacokinetic parameters, in particular for drugs which have long terminal-elimination half-lives. PET measures total radioactivity concentrations in tissue. If a radiotracer undergoes extensive metabolism, the interpretation of the PET data might be confounded by the presence of radiolabelled metabolites which contribute to the measured PET signal in tissue. The issue of dose-linearity is often discussed as a limitation of the microdosing concept, as there is concern that pharmacokinetic data determined after the administration of a microdose might fail to predict pharmacokinetic data of the drug observed at therapeutic doses. To address the issue of dose linearity and its implications in microdosing, an evaluation project known as the “CREAM trial” (CREAM = Consortium for Resourcing and Evaluating AMS Microdosing) was set up. In this trial, the pharmacokinetic properties of both a microdose and a pharmacological dose were examined for five substances whose human metabolism was difficult to predict by means of animal or *in vitro* models (warfarin, ZK253, diazepam, midazolam and erythromycin) [10]. Of the 5 drugs studied, microdose-pharmacokinetic data reflected pharmacological-dose pharmacokinetics for midazolam, diazepam and ZK253. Warfarin was not dose-linear in the

distribution phase and erythromycin, being acid labile, did not yield any result for the oral microdose [6, 10].

Different approaches exist to using PET in drug development. In the first approach, the drug of interest is directly radiolabelled and injected intravenously in order to assess its distribution to different body tissues and its target tissue pharmacokinetics *in vivo* (see Fig. 1) [11]. In a second approach, a validated PET tracer is used which is not identical to the studied drug and which allows for quantifying parameters related to expression of the pharmacological target (receptor protein, enzyme, transporter protein, etc.) of the drug of interest. Typically a baseline PET scan and a series of PET scans after administration of different doses of the investigated drug are performed, which allows for studying the displacement of the PET tracer by the drug *in vivo*. An example for this approach is given in Fig. 2. This paradigm has proven very valuable in measuring the degree of occupancy of the pharmacological target by different doses of a drug and has greatly aided in identifying starting doses for clinical trials [12, 13]. It has also been useful for assessing treatment response, in particular in oncology, by using metabolism tracers such as [^{18}F]fluorodeoxyglucose or proliferation markers such as [^{18}F]fluorothymidine.

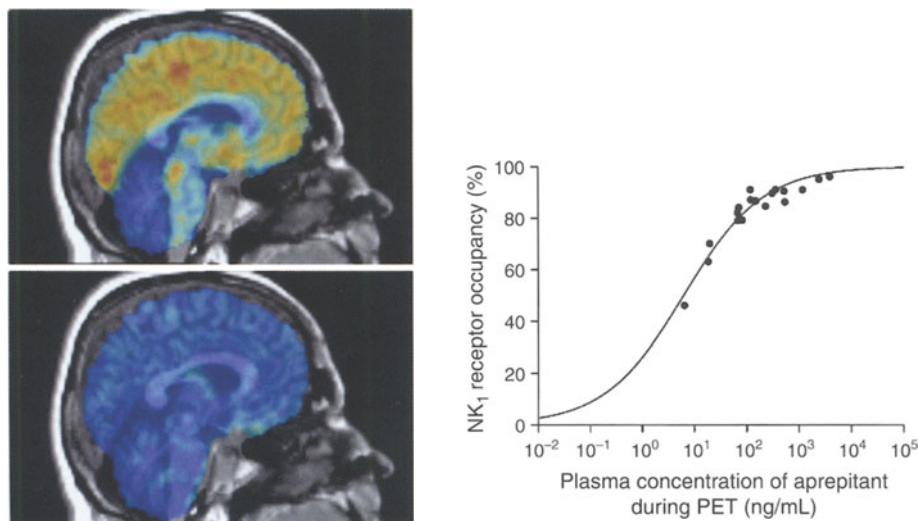


Fig. 2 Use of PET for receptor occupancy studies (reprinted with permission from Ref. [27]). Different doses of unlabelled drug (aprepitant) are given and binding of a validated PET tracer ([^{18}F]SPA-RQ) to drug target receptor in brain (neurokinin 1 receptor) is measured with PET. Upper panel (left) shows baseline scan without administration of unlabelled drug and lower panel (left) shows PET scan after drug administration resulting in reduced binding of PET tracer. Intensity of PET signal following different doses of unlabelled drug relative to baseline scan (receptor occupancy) is related to plasma concentration of unlabelled drug to provide concentration-effect curve (right)

PET can also be combined with other modalities than imaging modalities in clinical pharmacology such as clinical microdialysis to assess intracellular drug pharmacokinetics *in vivo*. PET yields a combined signal comprising the intracellular, the extracellular, and the intravascular fraction of a radiolabelled drug and its metabolites. With microdialysis unbound extracellular drug concentrations are measured. The combination of these two techniques leads to knowledge of intracellular rather than extracellular or total drug concentrations as recently exemplified by studying the tissue distribution of the radiolabelled broad-spectrum antibiotic [^{18}F] ciprofloxacin in humans [14].

3 Single photon emission computed tomography (SPECT)

In SPECT imaging, radioisotopes are used that emit one or more γ -rays of characteristic energies. In contrast to PET imaging, this allows two or more compounds with different radioisotopes (e.g. $^{99\text{m}}\text{Tc}$: $t_{1/2}$, 6.1 h; ^{123}I , $t_{1/2}$, 13.3 h or ^{111}In , $t_{1/2}$, 2.8 days) to be measured simultaneously within the same study. By rotating the gamma camera around the subject the localization and distribution of the labelled compound is recorded. Advantages of SPECT over PET include lower costs, potential for imaging with different radioisotopes simultaneously, the commercial availability of many molecular probes already in clinical use and the long half-lives of the employed radioisotopes allowing long distance transportation of the radioisotope or even the readily prepared radiotracer between site of production and administration to the subject. A limitation of SPECT is that most SPECT radioisotopes (except for radioiodine) require the introduction of chelating moieties into the molecule of interest. This structural modification can profoundly alter the physical or biochemical properties of a small molecule thereby greatly limiting the applicability of SPECT in studying drug disposition and pharmacokinetics. Other disadvantages of SPECT compared to PET include the lower sensitivity and spatial resolution and the more complex attenuation correction, giving in most cases only semiquantitative measures of drug tissue concentrations. SPECT has been used in clinical studies for assessing tumour metabolism and angiogenesis in oncology [14], myocardial perfusion and activity in cardiology, for perfusion and ventilation measurements in pulmonology, transporter occupancy in psychiatry [16] and lesion size and activity in multiple sclerosis in neurology [17].

4 Optical imaging

Optical imaging is a promising new imaging technique for potential use in clinical pharmacology, which is based on the detection of light emitted from cells or tissues.

The two most often used optical imaging approaches rely on fluorescence or bioluminescence as a source of light. Bioluminescence imaging requires genetic engineering of cells or tissues to image with a reporter gene that encodes one of a number of light-generating enzymes (luciferases). For *in vivo* fluorescence imaging fluorescent proteins or dyes are used, which need external excitation for light emission. Optical imaging techniques can visualize a variety of cellular and molecular processes *in vivo* including protein interactions, protein degradation, and protease activity. The lower limits of detection for optical imaging reach a sensitivity of up to femtomolar concentrations of an optical reporter or contrast agent. Compared to other imaging modalities, the costs of optical imaging devices are lower. However, due to scattering and absorption of light exact spatial localization and quantification of signal intensities are difficult to achieve. Fluorescence imaging is entering initial clinical testing in areas such as breast imaging and endoscopy. For example, diffuse optical spectroscopy of hemoglobin and deoxyhemoglobin in breast tumours shows promise as a biomarker for effective neoadjuvant chemotherapy in cancer patients [18, 19].

5 Magnetic resonance imaging (MRI)

Magnetic resonance imaging (MRI) was developed from knowledge gained in the study of nuclear magnetic resonance. Certain nuclei, such as hydrogen or phosphorous, have magnetic properties and possess angular momentum or “spin” and are detectable by MR. When these nuclei are exposed to a high static magnetic field (a typical MRI magnet is approximately 20,000 times the strength of the earth’s magnetic field) the magnetic moments of these protons align with the direction of the field. An electromagnetic field is then briefly turned on, causing the protons to alter their alignment relative to the field. When this field is turned off the protons return to the original magnetization alignment and these changes create the signal detected by the scanner. Contrast agents such as gadolinium compounds or iron oxide nanoparticles have become available recently. Injected intravenously they are used for blood vessel discrimination or for differentiation of tumour and scar tissue. For improved visualization of the gastrointestinal tract, MRI contrast agents are taken orally. MRI is widely used as an anatomical imaging modality in oncology, cardiology, orthopaedics, neurology and many more [20]. The related technique magnetic resonance spectroscopy (MRS) is a functional imaging modality, which allows for measuring a chemical entity (e.g. endogenous compounds such as neurotransmitter metabolites, drug molecules) in a specific tissue or organ section of the human body. MRS has found applications for measuring drug tissue levels *in vivo* [21] and as fMRI in psychiatry for drug trials for example in depression or anxiety disease patients [22].

6 Computed tomography (CT)

The absorption by the body of X-rays emitted from a focused X-ray source rotating around a subject placed in the centre of the CT scanner is used in computer tomography. High-resolution topographic anatomical images are reconstructed through a set of back calculations with a spatial resolution of less than 1 mm. CT is not a molecular imaging technique *per se*. However, CT provides a high quality anatomical framework for molecular imaging. In combination with molecular imaging techniques such as PET, PET–CT imaging has become a standard for functional and molecular imaging at the clinical level. CT is widely used as anatomical modality e.g. for disease monitoring, staging and grading in oncology, vessel diameter dimensions, plaque composition and heart contraction function in cardiology and in neurology for assessment of brain infarctions [17].

7 Ultrasound imaging

For ultrasound imaging high-frequency sound waves (1–40 MHz), which are emitted from a transducer and the echoes returning from the tissue are analysed to build up an image. Because resolution improves with frequency, while penetration decreases with frequency, the choice of ultrasound frequency is a trade-off between resolution and penetration depth. Ultrasound is a relatively cheap and easy accessible imaging modality without the use of ionising radiation. Ultrasound contrast agents (gas microbubbles) can be used to improve image quality by introducing a material with different acoustic properties from that of the scanned tissue. Ultrasound is for instance used in clinical trials in cardiology (cardiac contraction function, plaque and intima thickness, neo-angiogenesis) [23] and oncology (tumour size and extent, tumour perfusion) [24, 25]. Furthermore placement in- or outside of the target tissue (e.g. muscle tissue, subcutaneous adipose tissue) of devices such as microdialysis probes can be detected [26].

Case Study: Using PET in drug development – aprepitant

Aprepitant is an antiemetic substance that belongs to a class of drugs called substance P antagonists. The compound mediates its effect by blocking the neurokinin 1 (NK1) receptor. Aprepitant is manufactured by Merck & Co. and used for treatment and prevention of chemotherapy induced or postoperative nausea and vomiting. Based on autoradiographic studies in monkey and human brains showing a high expression of NK1 receptors in certain brain regions and

clinical findings of reduced incidence of chemotherapy-induced nausea and vomiting, it was decided to use PET to establish a correlation between dose, receptor occupancy and the observed clinical effect (dose-response relationship) [27]. To evaluate the plasma concentration-occupancy relationship, aprepitant dosed orally at 10, 30, 100 or 300 mg, or a placebo was administered to healthy volunteers ($n = 16$) once daily for 14 consecutive days [12]. The ratio of striatal/cerebellar NK1 receptor binding (striatum is a high receptor density region and cerebellum is a reference region lacking NK1 receptors) of the radiotracer [^{18}F]SPA-RQ was used to calculate trough receptor occupancy 24 h after the last dose of aprepitant. Blood samples for aprepitant plasma concentration measurements were taken. Brain NK1 receptor occupancy increased after oral aprepitant dosing in both a plasma concentration-related and a dose-related fashion (see Fig. 2). High ($\geq 90\%$) receptor occupancy was achieved at doses of 100 mg/day or greater. The plasma concentrations of aprepitant that achieved 50 and 90% occupancy were estimated as approximately 10 ng/mL and approximately 100 ng/mL, respectively. The presented study involved only a small number of subjects and a limited range of doses, however, there was a good correlation between the degree of receptor occupancy and plasma concentrations over the range achieved by clinically effective doses of aprepitant (Fig. 2). The description of this relationship was valuable for the development of aprepitant for central nervous system indications, because it helped to guide dose selection. This approach is especially valuable for speeding up clinical development where errors in dose selection can have a major impact by prolonging drug development timelines, as well as in trials that produce negative results, because the PET data can confirm that target site occupancies were achieved [12].

In another PET trial, the concept of NK1 receptor antagonism as an antidepressant mechanism was not supported. By clinical scores, a superior antidepressant efficacy of the comparator substance paroxetine and the absence of an effect for aprepitant have been assessed, despite sufficient target site receptor occupancy measured with PET and [^{18}F]SPA-RQ [27]. This study showed that the NK1 receptor is functionally not relevant with respect to the desired clinical end point indicating that it may not be productive to develop other molecules of this pharmacological class for this indication [29].

References

1. Farde L (1996) The advantage of using positron emission tomography in drug research. *Trends Neurosci* 19: 211–214
2. Zimmer L (2008) Can positron emission tomography facilitate paediatric drug development? *Fundam Clin Pharmacol* 22: 595–598

3. Goodall S, Ringel M, Tollman P (2004) Rising to the productivity challenge: a strategic framework for Biopharma. Boston Consulting Group Report, pp 1–12
4. DiMasi JA (2001) Risks in new drug development: approval success rates for investigational drugs. *Clin Pharmacol Ther* 69: 297–307
5. European Medicines Agency. Committee for Medicinal Products for Human Use (CHMP) (2004) Position paper on non-clinical safety studies to support clinical trials with a single microdose. <http://www.ema.europa.eu/pdfs/human/swp/259902en.pdf>
6. Bauer M, Wagner CC, Langer O (2008) Microdosing studies in humans: the role of positron emission tomography. *Drugs R D* 9: 73–81
7. Food and Drug Administration (2004). Innovation, Stagnation, Challenge and Opportunity on the Critical Path to New Medicinal Products. Critical Path Report. Rockville, MD
8. Food and Drug Administration. Center for Drug Evaluation and Research (CDER) (2006) Guidance for Industry, Investigators, and Reviewers. Exploratory IND Studies. <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm078933.pdf>
9. Bergström M, Grahnén A, Långström B (2003) Positron emission tomography microdosing: a new concept with application in tracer and early clinical drug development. *Eur J Clin Pharmacol* 59: 357–366
10. Lappin G, Kuhn W, Jochemsen R, et al. (2006) Use of microdosing to predict pharmacokinetics at the therapeutic dose: experience with 5 drugs. *Clin Pharmacol Ther* 80: 203–215
11. Bauer M, Langer O, Dal-Bianco P, et al. (2006) A positron emission tomography microdosing study with a potential anti-amyloid drug in healthy volunteers and patients with Alzheimer's disease. *Clin Pharmacol Ther* 80: 216–227
12. Bergström M, Hargreaves RJ, Burns HD, et al. (2004) Human positron emission tomography studies of brain neurokinin 1 receptor occupancy by aprepitant. *Biol Psychiatry* 15: 1007–1012
13. Tauscher J, Kielbasa W, Iyengar S, et al. (2010) Development of the 2nd generation neurokinin-1 receptor antagonist LY686017 for social anxiety disorder. *Eur Neuropsychopharmacol* 20: 80–87
14. Langer O, Karch R, Müller U, et al. (2005) Combined PET and microdialysis for in vivo assessment of intracellular drug pharmacokinetics in humans. *J Nucl Med* 46: 1835–1841
15. Perini R, Choe R, Yodanis AG, et al. (2008) Non-invasive assessment of tumor neovasculature: techniques and clinical applications. *Cancer Metastasis Rev* 27: 615–630
16. Klein N, Sacher J, Geiss-Granada T, et al. (2006) In vivo imaging of serotonin transporter occupancy by means of SPECT and [123 I]ADAM in healthy subjects administered different doses of escitalopram or citalopram. *Psychopharmacology* 188: 263–272
17. Willmann JK, van Bruggen N, Dinkelborg LM, Gambhir SS (2008) Molecular imaging in drug development. *Nat Rev Drug Discov* 7: 591–607
18. Cerussi A, Hsiang D, Shah N, et al. (2007) Predicting response to breast cancer neoadjuvant chemotherapy using diffuse optical spectroscopy. *Proc Natl Acad Sci USA* 104: 4014–4019
19. Luker GD, Luker KE (2008) Optical imaging: current applications and future directions. *J Nucl Med* 49: 1–4
20. Martinelli V, Radaelli M, Straffi L, et al. (2009) Mitoxantrone: benefits and risks in multiple sclerosis patients. *Neurol Sci* 30: S167–S170
21. Wolf W, Waluch V, Presant CA (1998) Non-invasive ^{19}F -NMR of 5-fluorouracil in pharmacokinetics and pharmacodynamic studies. *NMR Biomed* 11: 380–387
22. Cortese BM, Phan KL (2005) The role of glutamate in anxiety and related disorders. *CNS Spectr* 10: 820–830
23. Siebelink HM, Scholte AJ, Van de Veire NR, et al. (2009) Value of contrast echocardiography for left ventricular thrombus detection postinfarction and impact on antithrombotic therapy. *Coron Artery Dis* 20: 462–466
24. Goertz DE, Yu JL, Kerbel RS, et al. (2002) High-frequency Doppler ultrasound monitors the effects of antivascular therapy on tumor blood flow. *Cancer Res* 62: 6371–6375

25. Bertolotto M, Pozzato G, Crocè LS, et al. (2006) Blood flow changes in hepatocellular carcinoma after the administration of thalidomide assessed by reperfusion kinetics during microbubble infusion: preliminary results. *Invest Radiol* 41: 15–21
26. Müller M, Mascher H, Kikuta C, et al. (1997) Diclofenac concentrations in defined tissue layers after topical administration. *Clin Pharmacol Ther* 62: 293–299
27. Hargreaves R (2002) Imaging substance P receptors (NK1) in the living human brain using positron emission tomography. *J Clin Psychiatry* 63: 18–24
28. Keller M, Montgomery S, Ball W, et al. (2006) Lack of efficacy of the substance p (neurokinin1 receptor) antagonist aprepitant in the treatment of major depressive disorder. *Biol Psychiatry* 59: 216–223
29. Uppoor RS, Mummaneni P, Cooper E, et al. (2008) The use of imaging in the early development of neuropharmacological drugs: a survey of approved NDAs. *Clin Pharmacol Ther* 84: 69–74

CHAPTER 15

Current concepts of pharmacogenetics, pharmacogenomics, and the “druggable” genome

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Summary

The “post-genome” era we live in holds the great promise that our steadily growing knowledge on the genetics of inter-individual drug response variability can be translated into clinical practice. According to the current intriguing concepts of pharmacogenetics and pharmacogenomics, genetic information of individuals will be used to avoid “trial and error” scenarios during medication. Based on evidence from genomic testing, medicine is expected to evolve from the “one dose fits all” strategy to patient-tailored therapy, which is guided by individualized drug selection and dose optimization: a promising perspective for patient, industries, and health care providers. The scientific knowledge fueling this vision of a genomic medicine is rapidly expanding and validated brilliant examples already exist of how the outcome of a genomic test dictates specific therapies. However, major challenges still lie ahead until genomic medicine will become routine clinical practice. In this chapter, important facts of the principles in genomic medicine are summarized, providing insight into ways how genetic information of an individual can be used to improve drug safety and efficacy, and further can help to select optimal drugs and streamline the process of drug discovery and development.

Keywords: Human genome, single-nucleotide polymorphism (SNP), pharmacogenetics, pharmacogenomics, druggable genome, cytochrome P-450 (CYP) family (*CYP2D6*, *CYP2C9*), thiopurine methyltransferase (*TPMT*), uridine diphosphate glucuronyltransferase 1A1 (*UGT1A1*), dihydropyrimidine dehydrogenase (*DPYD*), trastuzumab (Herceptin), gene expression signatures, MammaPrint, abacavir

1 Genetic variation in the human genome: biological basis of pharmacogenetics

It has long been known that there exists substantial genetic variation among different individuals. But with the completion of the reference human genome sequence in 2003, the discovery of human genome sequence variants has just begun to explode [39, 81]. The human genome consists of 23 chromosomes containing a total of 3.27×10^9 base pairs of sequence information that includes $\sim 23,500$ protein encoding genes (Genome Reference Consortium, February 2009; <http://www.ensembl.org/>). The total mRNA content of a human cell, the transcriptome, however is estimated to consist of more than $\sim 150,000$ different gene transcripts due to a remarkable increase in genome complexity introduced during the process of gene expression. Compared to the relatively low total gene count, which is more than 5-fold lower than originally anticipated, the higher number of transcripts mainly results from alternative splicing events during mRNA maturation. Owing to a multitude of post-translational modifications of proteins, such as phosphorylation or glycosylation, the human transcriptome is expected to serve as template for the biosynthesis of an even higher number of different proteins.

Except for monozygotic twins, humans differ on average in every 100^{th} – 1000^{th} base pair. Several mechanisms are known to give rise to *de novo* DNA sequence aberrations (“mutations”), such as spontaneous chemical reactions (e.g. cytosine deamination), DNA damage (e.g. by radiation or oxygen radicals), or DNA replication errors. Representing a major source of genetic variation, single nucleotide changes constitute the most common sequence alteration within the human genome. They are called single-nucleotide polymorphisms (SNPs), although the vast majority is bi-allelic rather than polymorphic. The human genome is scattered with many millions of such SNPs, which constitutes a dynamic molecular basis for inter-individual variation of inherited traits. An arbitrary rule defines human genes genetically “polymorphic” if at a given locus a variant occurs with a frequency of $>1\%$ in one or more populations. To date more than ~ 25 million human SNPs have been identified, ~ 10 million of which have been validated as common SNPs with a minor allele frequency higher than 5% [14]. In addition, countless rare variants – so-called “private SNPs” – exist, many of which have been discovered through completion of the first three genomes of single individuals [43, 83, 86]. The number is steadily growing and is expected to be subject to substantial reconsideration after completion of the “1000 Genomes Project” (<http://www.1000genomes.org>), especially with respect to more complex sources of variation such as inversions, indels, and copy number variations (CNV) [29].

While the majority of SNPs are located in intergenic regions, SNPs affecting gene sequences include two major categories: (i) perigenic SNPs located either within promoter, intron, or downstream untranslated regions affecting e.g. the transcriptional activity, stability, or correct splicing of the mRNA copy, (ii) coding-region SNPs affecting exon sequences with the potential to alter the amino acid sequence or correct

length of the encoded gene product. To date, more than 230,000 non-synonymous coding SNPs are known (HapMap release 27; <http://hapmap.ncbi.nlm.nih.gov/>) but it is estimated that literally each of the ~500,000 exons encoded by the human genome harbors at least two SNPs.

2 The promise of pharmacogenetics, pharmacogenomics, and genomic medicine

Pharmacogenetics emerged as a discipline in the 1950s, when sensitivity to the antimalarial drug primaquine had been related to deficiency of glucose 6-phosphate dehydrogenase (G6PD) [2, 51]. Today it is known that the *G6PD* gene locus belongs to the most polymorphic genetic loci in the human genome. Pharmacogenetics is best defined as the discipline based on the identification and usage of such genetic variation aiming at explaining and predicting the variable drug response in individuals [65, 66, 84, 85]. Pharmacogenetics therefore usually focuses on polymorphisms in single or few genes encoding drug-metabolizing enzymes, drug targets, drug receptors, drug transporters, as well as disease-modifying genes that have been linked to drug effects [11]. There is a rich and continually growing list of pharmacogenetically important polymorphisms found in such genes. Most variant alleles are associated with reduced activity of an encoded protein, but there are also examples of variants, which confer enhanced activity, such as gene duplications or copy number variations. Because a pharmacogenetically important polymorphism is a stable genetic variable, an associated assay represents a typical “once-in-a-lifetime” DNA-based gene test, which is in general performed by a PCR-based genotyping method.

In contrast, the more holistic pharmacogenomic approach assesses the “whole genome” aiming at analyzing a large multitude of genes – up to many thousands – in parallel, which today is mainly achieved by addressing the highly dynamic variables of the transcriptome. Pharmacogenomics is therefore best defined as discipline that uses genome technology to study the relationship between drug effects and all relevant genes. Advanced technology such as GeneChip arrays can be deployed to study the total gene expression output of cells or tissues in a single experiment, which allows discovering e.g. gene expression changes in response to different drugs and/or doses. A pharmacogenomic test would represent e.g. an assay capturing a gene expression profile of tumour cells, suitable to predict response to an anti-cancer therapy. As follows, pharmacogenetics and pharmacogenomics can both be regarded as “genomic medicine” tools for personalized medicine, which employ information from the individual’s genome to guide medical decision making with the vision of individualized risk predictions and treatment decisions [17]. Figure 1 shows an illustration of different applications of the underlying concept and Table 1 summarizes important examples, which are elaborated within this chapter.

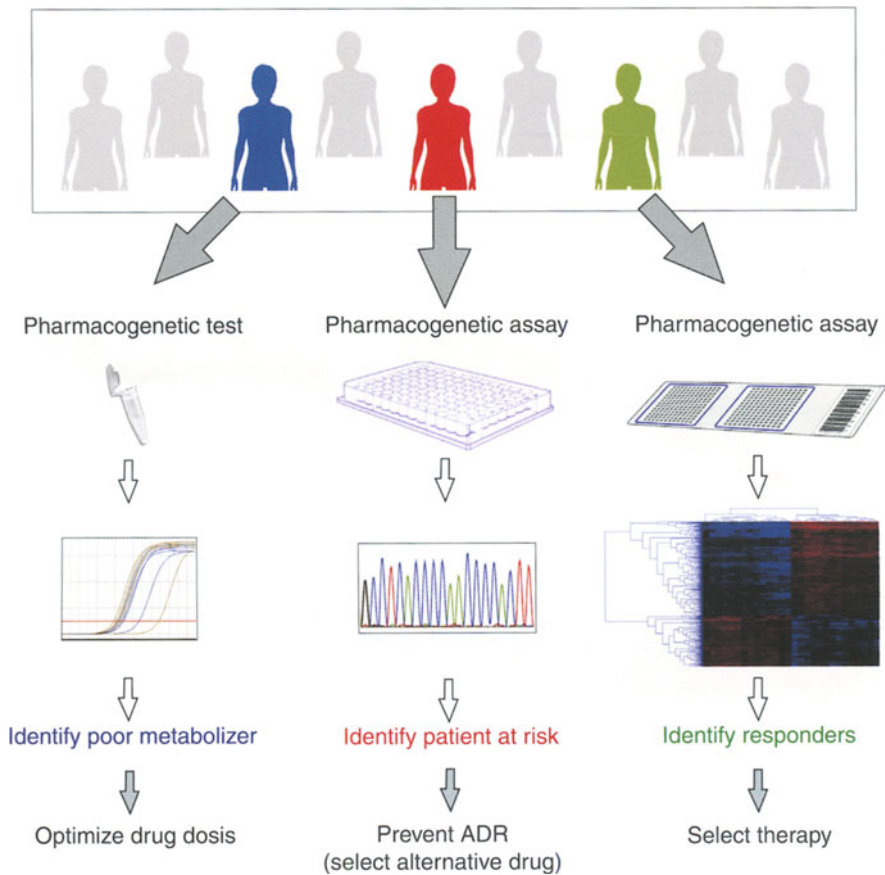


Fig. 1 Applications of the “Genomic Medicine” concept. Pharmacogenetics- and pharmacogenomics-based assays employ information from the individual’s genome to guide individualized treatment decisions and risk predictions. The illustration summarizes three possible applications, showing how genetic information of an individual can be used to optimize drug dose (left), improve drug safety (middle), or can help to select the optimal therapy (right). A relatively simple pharmacogenetic test (left), e.g. PCR-based genotyping of a common variant in a drug metabolizing gene such as *CYP2C9* or *CYP2D6*, can be used to identify patients (shown in blue), who are carriers of genotypes conferring reduced enzyme activity (“poor metabolizers”). This information can be used to optimize dose requirement. In such cases where more than few common variants account for interindividual variability in drug response, such as the *DPYD* gene, a re-sequencing approach of relevant genes will detect aberrations associated with e.g. adverse drug reactions (ADR). Thus, a pharmacogenetic assay (middle) can be used to identify patients at risk of an ADR (shown in red), which could be prevented by selection of an alternative drug. Pharmacogenomics uses genome technology, such as microarrays, whose readout allows e.g. to relate gene expression changes in tumour tissue to therapy response, such as the MammaPrint gene expression signature that predicts response to adjuvant therapy in breast cancer patients. A pharmacogenomic assay (right) therefore is suitable to identify patients, who will likely benefit from therapy (shown in green)

Table 1 Selected examples of pharmacogenomic assays with clinical relevance

| Genomic marker/class | Molecular phenotype | Clinical phenotype | Clinical utility | Example |
|----------------------------------|----------------------------|---|--|--|
| <i>Gene polymorphism</i> | | | | |
| Metabolism (Phase I reaction) | low activity | poor metabolizer | lower drug dose | CYP2C9 (warfarin) |
| Drug target | altered drug binding | variation in dose requirement | optimize drug dose | VKORC1 (warfarin) |
| Drug transporter | increased expression | multidrug resistance | identify non-responders | ABCB1 (antiepileptics) |
| Organic anion transporter | reduced function | reduced drug uptake | identify non-responders/risk of ADR | SLCO1B1 (statins) |
| Metabolism (Phase II reaction) | reduced function | reduced elimination | identify patients at risk of ADR | UGT1A1 (irinotecan) |
| Drug-related pathway | deficiency | severe toxicity | identify patients at risk of severe toxicity | TPMT (6-mercaptopurine) |
| | specific gain of function | hypersensitivity | identify patients at risk of ADR | HLA-B* 5701 (abacavir) |
| <i>Gene duplication</i> | | | | |
| Metabolism (Phase I reaction) | hyperfunctional | ultrarapid metabolizer | increase drug dose lower dose of prodrugs | CYP2D6 (antidepressants) CYP2D6 (codeine) |
| <i>SNP profile</i> | | | | |
| Drug target/receptor | variable | therapy response | individualize drug therapy | AGT, APOB, ADRA2A |
| <i>Protein biomarker</i> | | | | |
| Somatic cancer phenotype | overexpression | drug response | identify patient subgroup for treatment | HER2/neu (trastuzumab) |
| <i>Gene mutation</i> | | | | |
| Somatic cancer mutation | pro-apoptotic deficiency | increased drug response | identify patient subgroup for treatment | EGFR (gefitinib) |
| Germline mutation | | inherited disease | select patients for clinical study | antisense oligos (trials) |
| <i>Gene expression signature</i> | | | | |
| Somatic cancer phenotype | altered expression profile | response to therapy tumour sub-group | prediction of prognosis/therapy response classification | MammaPrint lymphomas |

ADR Adverse drug reaction

3 Genetic variants affecting pharmacokinetics

Most human drug-metabolizing enzymes, which are responsible for modification of functional groups (phase I reactions) or for conjugation with endogenous substituents (phase II reactions), exhibit common genetic polymorphisms with clinical relevance. Notably, these polymorphisms are most likely the evolutionary result of adaptation to selective pressure, probably mediated by challenges through food alkaloids or plant toxins. Consequently, the frequency of almost all of these polymorphisms differs substantially among ethnic groups. One reason for the relatively high frequency of variation may be that some enzymes are redundant and thus dispensable for life. Therefore, inherited differences in drug-metabolizing enzymes frequently follow monogenic traits, brought about by inactivating mutations in enzymes apparently without critical endogenous substrates. Molecular mechanisms of inactivation include “loss-of-function” mutations, such as nonsense mutations or frameshift mutations causing premature termination of translation, splice site mutations, or even complete gene deletions, and further non-synonymous missense mutations leading to reduced catalytic activity or protein stability. Usually these inactivating mutations affect single proteins and lead to extreme phenotypes characterized by excessive plasma concentrations, particularly in the case of drugs with a narrow therapeutic index. On the other hand, gene duplications resulting in a hyperfunctional phenotype are also known.

The cytochrome P-450 (CYP) family, a group of more than 50 heme-thiolate monooxygenase enzymes (<http://www.icgeb.org/~p450srv/450.html>) that function in the oxidative metabolism of a high number of natural compounds (such as steroids, fatty acids, prostaglandins, or leukotrienes), as well as drugs, carcinogens and mutagens, constitutes the most important class of metabolizing enzymes with high genetic variability [30, 32]. Only some members of this family, such as CYP1A1, CYP2E1, or CYP3A4 (a key enzyme involved in drug metabolism) are relatively high conserved. In contrast, to date more than 50 alleles of the *CYP2D6* gene have been detected and characterized, one of which inactivates enzyme function in about 7% of Europeans and affects the metabolism of many commonly prescribed drugs [19]. Another example of a clinically relevant polymorphism affects the *CYP2C19* gene and occurs predominantly in Asians. This mutation renders omeprazole therapy and eradication of *Helicobacter pylori* much more effective in Japanese as compared to Caucasians [74]. Apart from their role in metabolism, drug-metabolizing enzymes may also act as activators of prodrugs. This is the case for a number of opioids, such as codeine, which is converted to morphine by CYP2D6. Carriers of nonfunctional *CYP2D6* alleles may exhibit varying degrees of codeine resistance. In contrast, *CYP2D6* gene duplications lead to enhanced activation of codeine and corresponding side effects [16]. More recently, also gene promoter methylation- or microRNA-mediated regulation of the expression of some CYP genes has been described [18, 32].

Drug distribution may be affected by membrane transporters, such as P-glycoprotein (P-gp), the product of the *ABCB1* gene (formerly called *MDR-1*). *ABCB1* was originally identified by its overexpression as “multidrug resistance” gene in various tumours. Subsequently, *ABCB1* was shown to be also expressed in various human tissues involved in gastrointestinal absorption and bile excretion. In mice, disruption of *ABCB1* gene copies was associated with increased bioavailability and reduced urinary clearance, a finding which was reproduced in humans after administration of P-gp inhibitors. An *ABCB1* polymorphism affects absorption of digoxin and response to antiepileptics [22, 72]. Many other members of the ATP-binding cassette (ABC) drug transporter gene family are involved in intrinsic and acquired drug resistance of cancer cells, such as over-expression of *ABCC6* in 5-fluorouracil resistance of colon cancer [69].

It is not unlikely that there exists considerable genetic variation in genes encoding organic anion transporters, similar to the polymorphic CYP gene family. For instance, a common genetic variant of the *SLCO1B1* gene encoding organic anion-transporting polypeptide 1B1 has been recently shown to reduce the hepatic uptake of many statins, increasing the risk of statin-induced myopathy [57, 82]. In addition the same gene has been shown to harbor two SNPs that are important determinants of pharmacokinetics and clinical effects of methotrexate [75].

4 Pharmacogenetic testing for optimizing drug dose

The assessment of allelic variants of the *CYP2D6* and *CYP2C9* genes is deemed a well-established example of how pharmacogenetics could influence drug dose selection in clinical routine. While *CYP2D6* metabolizes ~25% of clinically important drugs and affects the pharmacokinetics of ~50% of the drugs in clinical use [30, 32], *CYP2C9* metabolizes 10–20% of commonly prescribed drugs [38]. Both genes have been studied extensively and represent paradigms of how predictive genotyping could be applied for dose selection and adjustment during pharmacotherapy.

The many allelic variants of the *CYP2D6* genes confer poor, intermediate, efficient or ultrarapid metabolizer phenotypes. Thus, genetic testing of *CYP2D6* variant genotypes provides a perfect means to identify subjects carrying e.g. duplicated gene copies. Carriers of such hyper-functional alleles will likely metabolize drugs more rapidly, causing therapeutic failure due to low drug plasma levels with commonly prescribed drug doses. In contrast, carriers of alleles conferring low enzyme activity will metabolize drugs more slowly, indicating lower drug dose requirements. In addition, prodrugs activated by *CYP2D6* will have a smaller therapeutic effect in such individuals. The clinical power of *CYP2D6* genotyping has been demonstrated for instance in predicting plasma clearance of antidepressants and neuroleptics that depend on conversion by *CYP2D6*.

Six common *CYP2C9* genotypes can be correlated either to normal, reduced, or very low enzyme activity. The low activity-conferring *CYP2C9* alleles cause a reduction in metabolism of warfarin, a vitamin K antagonist, and can lead to elevated warfarin response, as demonstrated by increased warfarin plasma levels, decreased clearance, and increased frequency of bleeding in clinical studies [1]. Pharmacogenetic testing may therefore be a perfect opportunity to identify patients who are at risk for warfarin-associated bleeding and require lower initiation and maintenance doses of warfarin. Another gene identified as a predictor of warfarin dosing is *VKORC1*, coding for the warfarin target protein vitamin K epoxide reductase complex protein 1 [35]. The clinical pharmacology advisory panel to the United States Food and Drug Administration (FDA) acknowledged the importance of genotyping *CYP2C9* and *VKORC1* during initial phases of warfarin therapy, and the drug label was amended accordingly in 2007.

Since 2004 a diagnostic test based on the GeneChip technology platform of Affymetrix Inc. (Santa Clara, CA, USA), which was developed and launched by Roche Diagnostics (Indianapolis, IN, USA), is available to assess the major allelic variants in the *CYP2D6* and *CYP2C19* genes. Although the product had been approved as *In-Vitro* Diagnostic Device (IVD) by regulatory authorities both in the United States and the European Union, it has not been commonly used as routine clinical assay, probably due to lacking decision making guidelines for its clinical application. Because the product lacked important other targets such as *CYP2C9*, the need for a more complete panel to be used in diagnostic procedures and research applications quickly arose. From the technological perspective, highly parallel testing of a large number of important variants indeed has become feasible. As an example, the DMET Plus Premier Pack (Affymetrix) has been developed, which includes GeneChip arrays, reagents, and analysis software to assess the genotype of nearly 2000 different drug metabolism markers in as many as 225 genes. By employing this highly advanced system, an additional – hitherto undetected – variant associated with the above mentioned warfarin dosing variability has been discovered recently [4]. Apart from its current powerful use as a discovery tool, this system further enables large-scale simultaneous measurements of combined effects of multiple polymorphisms in several drug-metabolizing enzyme and drug transporter genes, perhaps as a diagnostic device in near future [10].

5 Pharmacogenetic testing to prevent adverse drug reactions

Due to well described relationships between specific genes and drug toxicity, pharmacogenetics has been repeatedly proposed as powerful diagnostic and predictive tool for preventing adverse drug reactions [31, 55, 58]. It was estimated that adverse drug reactions account for 10% of all hospital admissions and constitute a leading cause of death [40]. Besides their importance in routine drug therapy, adverse drug reactions

moreover constitute perhaps the most important reason for failure in the drug development process.

As an example, genotyping of the *TPMT*, *UGT1A1* and *DPYD* genes has been suggested to ensure safer cancer therapies [46]. Intolerance to 6-mercaptopurine due to thiopurine methyltransferase (TPMT) deficiency, a standard drug used in treatment of acute lymphoblastic leukemia (ALL), represents an important genetic determination of a phase II reaction. TPMT inactivates the cytotoxic agent 6-mercaptopurine, a prodrug whose active metabolites (thioguanine nucleotides) kill proliferating cells by inhibiting DNA and RNA synthesis. Inherited interindividual variability of TPMT activity represents a major risk factor of severe 6-mercaptopurine toxicity in ALL patients. Three different variant alleles of the *TPMT* gene account for the vast majority of TPMT deficiency. Consequently, it has been conclusively shown that testing for the common TPMT variant alleles reliably identifies patients at risk of severe toxicity, thus enabling genotype-guided individualized clinical management [12]. By deploying a full treatment protocol with other chemotherapeutic drugs, extreme 6-mercaptopurine intolerance (and even fatal cases of bone marrow aplasia) can be avoided in patients carrying the risk genotypes. Similarly, reducing the 6-mercaptopurine dose allows to maintain high thioguanine nucleotide levels in patients carrying heterozygous genotypes that confer milder TPMT deficiency.

Irinotecan, a chemotherapeutic drug used to treat advanced colorectal cancer, is a prodrug that is converted into an active DNA topoisomerase I inhibitor, which then is eliminated via conversion to a hydrophilic metabolite through enzymatic conjugation with glucuronic acid. Reduced glucuronidation due to allelic variants in the *UGT1A1* gene encoding the uridine diphosphate glucuronyltransferase 1A1 causes an increased risk of irinotecan toxicity during therapy of cancer patients, clinically leading to severe diarrhea and/or neutropenia. Clinical studies successfully demonstrated an association between common *UGT1A1* polymorphisms and the risk of irinotecan toxicity in patients receiving irinotecan [34]. The common European *UGT1A1**28 allele was further significantly associated with grade IV neutropenia in a prospective trial [33].

The chemotherapeutic drug 5-fluorouracil has been used to treat cancers for more than 50 years. The enzyme dihydropyrimidine dehydrogenase (DPD) is an important enzyme in the catabolism of 5-fluorouracil. Genetic variation within the *DPYD* gene has been associated with reduced catalytic activity of DPD, which can cause life-threatening toxicity following exposure to 5-fluorouracil [41]. However, the existence of many different rare *DPYD* alleles related to compromised 5-fluorouracil metabolism severely complicates genetic testing. Combined with substantial phenotypic variability, this led to the proposal of a phenotyping assay rather than genotyping in order to predict toxicity in patients receiving 5-fluorouracil or its prodrug capecitabine. This is probably the reason for the fact that the FDA still has not taken action to recommend genetic *DPYD* testing for 5-

fluorouracil. In contrast, pharmacogenetic testing of *TPMT* and *UGT1A1* had been supported, leading to appropriate changes to the labels for 6-mercaptopurine and for irinotecan in 2004 and 2005, respectively.

6 Genetics of pharmacodynamics

A significant number of patients, with estimates ranging from 30 to 60%, treated with various drugs do not respond to treatment [30, 66]. The presence of non-responsiveness is usually detected clinically and the reasons for the lack of drug effects often remain “idiopathic”. Numerous ongoing pharmacogenetic studies hold great promise to change this situation in future. Aiming at “patient tailored” therapies, variation in genes encoding drug targets or key components of pathways is of primary interest. However, inherited differences in molecules determining pharmacodynamics frequently turned out to follow polygenic traits. The underlying genetic mutations often affect regulation of gene expression rather than inactivating the encoded protein function, such as promoter, 3'-untranslated regions, deep intronic or even intragenic polymorphisms. To date, several publications on pharmacogenetics of drug targets underline the importance of inherited determinants of drug response and help to start elucidating the possibly responsible mechanisms. The following selected examples illustrate how this knowledge could be ultimately translated into predictive tests to assist drug selection.

During antihypertensive therapy in patients with left ventricular hypertrophy, SNPs in the angiotensinogen gene (*AGT*) and the apolipoprotein B (*APOB*) predicted the change in left ventricular mass in response to irbesartan, while a SNP in the α 2A-adrenoreceptor gene (*ADRA2A*) was associated with response to the β 1-adrenoreceptor blocker atenolol [44]. Another SNP within the *APOB* gene was also associated with the blood pressure response to irbesartan but not to atenolol [45]. The predictive power of these SNPs could therefore be potentially deployed for the genotype-guided selection of either an angiotensin II type 1 receptor antagonist or beta-blockade based strategy in antihypertensive therapy. A further example of how a genotype could predict response to a specific pharmacotherapy has been suggested for cholesterol reduction therapy using pravastatin. The gene encoding HMG-CoA reductase, the target of pravastatin, harbors two common SNPs in linkage disequilibrium, which are significantly associated with smaller reductions in cholesterol serum levels in heterozygous carriers and thus reduce efficacy of pravastatin therapy [5]. Genotyping of the *HMGCR* gene could help selecting patients suitable for additional or alternative therapeutic strategies in cholesterol reduction [9]. Another example is given by a common variation in the platelet receptor P2RY12, which has been shown to constitute a significant determinant of the interindividual variability in clopidogrel treatment in patients with coronary artery disease [67].

7 The predictive power of pharmacogenomics

Despite the important advances mentioned above, a predictive testing regime that is based on a single-gene or single-SNP strategy might fail in some constellations, due to the polygenic nature of many drug effects. Therefore, approaches interrogating multiple genes, or even the whole genome or transcriptome have been developed. Through overcoming the limits of tests focusing on single or a few genes, the pharmacogenomic-based identification of non-responders further has set the scene to change the way pharmaceutical industry is developing and marketing drugs. Pharmaceutical companies will probably abandon the “chemical blockbuster” strategy in order to adopt the “biological individualized” model of drug development. Indeed, a number of important examples exist, which demonstrate validated approaches of deploying predictive biomarkers for stratification of patients to achieve safer and/or more efficacious therapy [76]. On a similar path, regulative authorities like the FDA are more likely to grant provisional approval on the basis of a surrogate/biomarker measure with clinical benefit in a single uncontrolled trial [15, 42]. This will also force industry to define subpopulations of patients who are likely responders.

A perfect example for this scenario is given by trastuzumab (Herceptin) therapy, a monoclonal antibody specifically targeting breast cancer cells overexpressing HER2/neu [26]. An obligatory diagnostic test has been developed to identify breast cancer patients likely to benefit from this therapeutic protein. In fact, trastuzumab is marketed solely for a small subset of patients and is approved for the adjuvant treatment of HER2/neu-overexpressing breast cancer. Given the low prevalence of matching breast cancer types ($\sim 10\%$), it has been suggested that without using the HER2/neu biomarker in clinical development, the drug would not have been successfully developed. Although the HER2 biomarker assay represents a protein-based rather than a genetic or genomic assay, this example provides insight into the ongoing evolution of drug development.

Other genetic diagnostic tests predicting response to cancer therapeutics exist, such as gefitinib therapy, which selectively inhibits the tyrosine kinase domain inhibitor of epidermal growth factor receptor (EGFR). Gefitinib is indicated for the treatment of locally advanced or metastatic non-small cell lung cancer. Somatic mutations in the EGFR tyrosine kinase domain are responsible for activating anti-apoptotic pathways, thus conferring increased sensitivity to gefitinib therapy [73].

The specific gene sequence of an individual patient can also guide individualized therapy in the setting of rare monogenetic inherited diseases. Many of these incurable diseases are caused by single gene mutations. Novel therapeutic options are currently tested in clinical trials, such as PTC124 [36], a compound that allows reading through premature translation termination codons, or therapeutic oligonucleotides, which have been designed to induce exon skipping in order to restore reading frames that have been disrupted by mutations, such as the antisense oligonucleotide PRO051 [79] or the

morpholino AVI-4658 [37]. These therapies aim at healing a genetic lesion rather than the disease itself [56]. Therefore, PTC124 therapy could e.g. apply for a Duchenne muscular dystrophy patient and for a patient suffering cystic fibrosis as well, given that in both cases the disease causing lesion is a nonsense mutation creating a premature stop codon. The patient's individual gene sequence therefore will dictate inclusion into the appropriate clinical trial and hopefully indicate the proper therapeutic option in future [21].

Another great potential for genomic testing lies in diagnosis and prognosis for chemotherapy of cancer, where predictive biomarkers can be applied to select patients who will benefit from specific drug treatments. Expression profiles that have been developed in large-scale whole-genome studies are now being used routinely to identify subclasses of previously hard to distinguish tumours, such as the distinction between Burkitt's lymphoma and diffuse large-B-cell lymphoma [7]. In addition, brilliant genomic approaches that go beyond disease classification have been developed for the prediction of prognosis and response to cancer therapy [17, 63]. Nearly 80% of breast cancer patients undergo adjuvant therapies, designed to destroy remaining cancer cells and prevent metastatic spread. According to two landmark studies published in 2002, patterns of gene activity within breast cancer cells significantly predicted the aggressiveness of the cancer and the clinical outcome [77, 78]. This gene expression signature outperformed all standard diagnostic criteria in predicting metastasis and overall survival and subsequently was successfully validated in large clinical studies for its ability to predict the need of adjuvant chemotherapy after surgical intervention in breast cancer [3]. A molecular diagnostic test based on this gene expression signature is marketed as "MammaPrint" (Agendia BV, Amsterdam, Netherlands), which has been approved by the FDA in 2007 and is starting to be used in routine clinical oncology for the genome-guided risk stratification and prognosis in breast cancer treatment. A similar opportunity exists to predict the therapeutic response to tamoxifen in patients with estrogen-receptor positive node-negative breast cancer ("Oncotype DX") [8, 49, 60], or prognosis in early stage non-small cell lung cancer [64].

Gene expression signatures have also been developed to predict resistance to four common drugs used to treat acute lymphoblastic leukemia in children [23, 24]. Notably, the set of expression signatures largely consists of gene transcripts that have not been associated with drug resistance before, a finding that is reproduced also *in vitro* for many anticancer drugs, such as 5-fluorouracil [69]. Thus, the expression levels of many unknown marker genes might not only predict resistance but also help to elucidate hitherto unknown molecular mechanisms of drug resistance. More recently, also microRNAs (miRNAs) have been shown to serve as good predictors in a variety of settings, such as progression and prognosis of cancers, neurological disorders, muscular hypertrophy, cardiovascular diseases and Type II diabetes [52]. The interrogation of the transcriptome of peripheral blood mononuclear cells or even plasma-based miRNAs represent a further possibility to measure dynamic gene expression data, e.g. to address

inflammation-related states [70, 71, 80]. In addition, epigenome-related alterations such as DNA methylation or histone modification status that lead to differential gene expression can be perfectly captured by expression profiling assays [18].

Owing to next-generation sequencing technologies, like 454 (454 Life Sciences, Branford, CT, USA), SOLiD (Applied Biosystems, Foster City, CA, USA), or Solexa (Illumina, San Diego, CA, USA), sequencing costs have been dramatically reduced during the past 5 years. The rapidly evolving data capacity together with significant methodological advancements will not only accelerate the rate of sequencing whole genomes, but also enable the generation of data capturing more complex information of the genome, such as exon-level gene expression, methylation, or protein binding regions. Thus, data such as output from cancer genome sequencing projects [50] likely will generate additional useful knowledge to be applied in genomic medicine. It is also foreseeable that in the near future “metabolomics” might complement genomics- and proteomics-based strategies on the way to individualized drug therapy [54].

8 The “druggable” genome

The sequencing of the human genome has also paved the way for novel strategies in drug development, creating the intriguing concept of the “druggable” genome [59]. The druggable genome is composed of the subset of the 23,500 genes in the human genome, which encode proteins able to bind small-molecule therapeutic agents. Known drug targets represent ~130 protein families and fall into six major gene families: G-protein coupled receptors, serine/threonine and tyrosine protein kinases, zinc metallopeptidases, serine proteases, nuclear hormone receptors and phosphodiesterases. Accordingly, early estimates – which actually were based on a total gene count of 30,000 – showed that the druggable genome is composed of ~3000 human genes (i.e. roughly 10% of the genome), which encode a protein able to bind a drug-like molecule [25]. A fraction of ~25–50% of the druggable genome was proposed to express potential drug targets, which was recently confirmed by a bioinformatics approaches showing that around 20% of the human proteome might represent a potential target for small-molecule drug design in medicinal chemistry [62]. Therefore, these estimates are seemingly rather stable and only a minor part should be missing as the current build of the human genome covers 99% of the genome. Future strategies will develop more refined genome annotations and extend the druggable genome concept from targets for small-molecule drug design to an expanded version covering also potential targets for “biologicals” or RNA interference [68]. Moreover, extensive bioinformatics efforts are ongoing to generate datasets of predicted protein targets to be used in virtual high throughput screening and to address issues like toxicity prediction or modeling of the three-dimensional characteristics of active sites in the predicted druggable protein families [87].

9 Challenges that lie ahead

The growing list of drug labels with changes related to genetic testing, such as for warfarin, abacavir, 6-mercaptopurine or irinotecan clearly represent milestones in pharmacogenetics. At present, the path of the FDA is highly promising [15]: product labels either directly recommend a genetic test or refer to a known association of a genetic variation with drug response or safety for more than 30 genes (<http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm>). The euphoria associated with completion of the human genome project and impressive investments by the pharmaceutical industry in pharmacogenomic approaches of drug discovery have triggered high expectations. Next-generation sequencing technologies, enabling ambiguous projects such as the “1000 Genomes Project”, set the scene for the vision even of personal genome sequencing for clinical purpose. Additionally, rapidly evolving DNA array technology platforms, high-throughput screening systems, and advanced bioinformatics will allow the tailoring of therapeutic agents targeted for specific subgroups of the population. However, considering the complexity of the data behind, it is expected that genomics will be widely underused for many years, because much work must be completed before this knowledge can be translated into daily practice [17, 53, 55]. On the path to full clinical adoption of genomic inventions, clinicians and health care providers need established and proven infrastructure for the appropriate use of genomic data. Researchers, technology providers, the diagnostic industry, and the regulatory authorities as well are challenged to develop effective methods and guidelines that assist the practicing clinicians to understand how genomic tests are incorporated into current models of health care and risk assessment [42]. Strict incorporation of pharmacogenetic testing into health care will further require major changes in regulatory and reimbursement policies to cover additional costs. Infrastructure for legislative protections for privacy of genomic data will create even additional costs.

On the scientific side several other open questions are to be solved. Elucidation of definitive genotype-phenotype interactions will become a crucial issue and can only be achieved by well-designed clinical studies. In order to provide evidence that pharmacogenetics improves outcome or save costs, randomized controlled prospective clinical trials represent the most appropriate design for studies that could point the way. Such studies like the PREDICT-1 abacavir study (*see elaborated case study below*), however, are still rare and missing for a lot of promising candidates. Further, the polygenic nature of many drug effects will dictate to move to a genomic rather than genetic strategy for predictive testing. Moreover, a particular gene or set of genes may not always be a rate-limiting determinant of pharmacological response and will explain only a small fraction of drug response variability. In addition, there is much need for studying gene-environment interactions to explain considerable variability in drug response in individuals carrying the same genotype, such as in the *DPYD*

example. Recent advances in the field of “pharmacoeigenetics” underscore the notion that in some cases the accurate prediction of phenotypes will also require the assessment of epigenetic control variables such as gene promoter methylation status [18]. Lastly, a satisfactory level of predictability might even be never achieved in some cases, due to locus heterogeneity, variable penetrance, differential expressivity of alleles, and possibly still unknown other reasons for “Non-Mendelian” inheritance. This implies that a “purely” genomic approach to variability in drug response will probably fail and that a more holistic approach that incorporates non-genetic data, such as serum analytes, physiologic or metabolic measurements, could be more effective.

Case Study: The abacavir example of successful clinical incorporation of genetic testing

Hypersensitivity to abacavir, an anti-HIV reverse-transcriptase inhibitor, constitutes a prominent example for an adverse drug reaction related to genetics with a well-established clinical [27, 28, 61]. Hypersensitivity to abacavir is as a potentially life-threatening idiosyncratic adverse drug reaction affecting ~4% of patients treated. By whole-genome SNP-mapping, a SNP pattern within 3 HLA genes was identified to be highly associated with hypersensitivity to abacavir [20, 47]. There is now evidence from basic science that hypersensitivity is specifically restricted by HLA-B*5701, driven by drug-specific activation of cytokine-producing, cytotoxic CD8⁺ T lymphocytes [6, 61]. Clinical studies revealed that with-holding abacavir in individuals carrying the risk genotypes *HLA-B*5701*, *HLA-DR7*, and *HLA-DQ3* reduced the prevalence of hypersensitivity from 9 to 2.5%. A more recently published double-blind, prospective, randomized clinical study (PREDICT-1), which involved nearly 2000 patients with HIV-1 infection, the prospective genetic screening for the *HLA-B*5701* variant allele proved to significantly lower the incidence of clinically diagnosed hypersensitivity to abacavir (3.4 vs. 7.8% in the control group consisting of patients without prospective screening) [48]. In addition, screening completely eliminated immunologically confirmed hypersensitivity, underlining that a pharmacogenetic test can be used to prevent a specific toxic effect of an antiretroviral therapeutic drug. However, as ~94% of the population are not carriers of the *HLA-B*5701* allele, they are at low risk for hypersensitivity reaction to abacavir. Thus, the cost-effectiveness of *HLA-B*5701* testing is not obvious, mandating the need for inexpensive assays that can be used for genotype screening. Information about

genetic testing is part of the drug label for abacavir, since the FDA supported a recommendation for genetic screening prior to therapy in 2008 [13]. Screening for *HLA-B*5701* is now mandatory according to guidelines of the European AIDS Clinical Society (EACS).

References

1. Aithal GP, Day CP, Kesteven PJ, Daly AK (1999) Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. *Lancet* 353: 717–719
2. Alving AS, Carson PE, Flanagan CL, Ickes CE (1956) Enzymatic deficiency in primaquine-sensitive erythrocytes. *Science* 124: 484–485
3. Bueno-de-Mesquita JM, van Harten WH, Retel VP, et al. (2007) Use of 70-gene signature to predict prognosis of patients with node-negative breast cancer: a prospective community-based feasibility study (RASTER). *Lancet Oncol* 8: 1079–1087
4. Caldwell MD, Awad T, Johnson JA, et al. (2008) CYP4F2 genetic variant alters required warfarin dose. *Blood* 111: 4106–4112
5. Chasman DI, Posada D, Subrahmanyam L, et al. (2004) Pharmacogenetic study of statin therapy and cholesterol reduction. *JAMA* 291: 2821–2827
6. Chessman D, Kostenko L, Lethborg T, et al. (2008) Human leukocyte antigen class I-restricted activation of CD8+ T cells provides the immunogenetic basis of a systemic drug hypersensitivity. *Immunity* 28: 822–832
7. Dave SS, Fu K, Wright GW, et al. (2006) Molecular diagnosis of Burkitt's lymphoma. *N Engl J Med* 354: 2431–2442
8. Desmedt C, Ruiz-Garcia E, Andre F (2008) Gene expression predictors in breast cancer: current status, limitations and perspectives. *Eur J Cancer* 44: 2714–2720
9. Donnelly LA, Doney AS, Dannfald J, et al. (2008) A paucimorphic variant in the HMG-CoA reductase gene is associated with lipid-lowering response to statin treatment in diabetes: a GoDARTS study. *Pharmacogenet Genomics* 18: 1021–1026
10. Dumaual C, Miao X, Daly TM, et al. (2007) Comprehensive assessment of metabolic enzyme and transporter genes using the Affymetrix Targeted Genotyping System. *Pharmacogenomics* 8: 293–305
11. Eichelbaum M, Ingelman-Sundberg M, Evans WE (2006) Pharmacogenomics and individualized drug therapy. *Annu Rev Med* 57: 119–137
12. Evans WE, Hon YY, Bomgaars L, et al. (2001) Preponderance of thiopurine S-methyltransferase deficiency and heterozygosity among patients intolerant to mercaptopurine or azathioprine. *J Clin Oncol* 19: 2293–2301
13. FDA (2008) FDA notifications. Abacavir package insert changes approved. Hypersensitivity, other issues addressed. *AIDS Alert* 23: 102–104
14. Frazer KA, Ballinger DG, Cox DR, et al. (2007) A second generation human haplotype map of over 3.1 million SNPs. *Nature* 449: 851–861
15. Frueh FW, Salerno RA, Lesko LJ, Hockett RD (2009) 4th US FDA-Drug Information Association Pharmacogenomics Workshop, held 10–12 December, 2007. *Pharmacogenomics* 10: 111–115
16. Gasche Y, Daali Y, Fathi M, et al. (2004) Codeine intoxication associated with ultrarapid CYP2D6 metabolism. *N Engl J Med* 351: 2827–2831
17. Ginsburg GS, Willard HF (2009) Genomic and personalized medicine: foundations and applications. *Transl Res* 154: 277–287

18. Gomez A, Ingelman-Sundberg M (2009) Pharmacoeigenetics: its role in interindividual differences in drug response. *Clin Pharmacol Ther* 85: 426–430
19. Gonzalez FJ, Skoda RC, Kimura S, et al. (1988) Characterization of the common genetic defect in humans deficient in debrisoquine metabolism. *Nature* 331: 442–446
20. Hetherington S, Hughes AR, Mosteller M, et al. (2002) Genetic variations in HLA-B region and hypersensitivity reactions to abacavir. *Lancet* 359: 1121–1122
21. Hoffman EP (2007) Skipping toward personalized molecular medicine. *N Engl J Med* 357: 2719–2722
22. Hoffmeyer S, Burk O, von Richter O, et al. (2000) Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc Natl Acad Sci USA* 97: 3473–3478
23. Holleman A, Cheok MH, den Boer ML, et al. (2004) Gene-expression patterns in drug-resistant acute lymphoblastic leukemia cells and response to treatment. *N Engl J Med* 351: 533–542
24. Holleman A, den Boer ML, de Menezes RX, et al. (2006) The expression of 70 apoptosis genes in relation to lineage, genetic subtype, cellular drug resistance, and outcome in childhood acute lymphoblastic leukemia. *Blood* 107: 769–776
25. Hopkins AL, Groom CR (2002) The druggable genome. *Nat Rev Drug Discov* 1: 727–730
26. Hudis CA (2007) Trastuzumab – mechanism of action and use in clinical practice. *N Engl J Med* 357: 39–51
27. Hughes AR, Brothers CH, Mosteller M, Spreen WR, Burns DK (2009) Genetic association studies to detect adverse drug reactions: abacavir hypersensitivity as an example. *Pharmacogenomics* 10: 225–233
28. Hughes AR, Spreen WR, Mosteller M, et al. (2008) Pharmacogenetics of hypersensitivity to abacavir: from PGx hypothesis to confirmation to clinical utility. *Pharmacogenomics J* 8: 365–374
29. Iafrate AJ, Feuk L, Rivera MN, et al. (2004) Detection of large-scale variation in the human genome. *Nat Genet* 36: 949–951
30. Ingelman-Sundberg M (2001) Pharmacogenetics: an opportunity for a safer and more efficient pharmacotherapy. *J Intern Med* 250: 186–200
31. Ingelman-Sundberg M (2008) Pharmacogenomic biomarkers for prediction of severe adverse drug reactions. *N Engl J Med* 358: 637–639
32. Ingelman-Sundberg M, Sim SC, Gomez A, Rodriguez-Antona C (2007) Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetic, pharmacoeigenetic and clinical aspects. *Pharmacol Ther* 116: 496–526
33. Innocenti F, Undevia SD, Iyer L, et al. (2004) Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. *J Clin Oncol* 22: 1382–1388
34. Iyer L, Das S, Janisch L, et al. (2002) UGT1A1*28 polymorphism as a determinant of irinotecan disposition and toxicity. *Pharmacogenomics J* 2: 43–47
35. Jonas DE, McLeod HL (2009) Genetic and clinical factors relating to warfarin dosing. *Trends Pharmacol Sci* 30: 375–386
36. Kerem E, Hirawat S, Armoni S, et al. (2008) Effectiveness of PTC124 treatment of cystic fibrosis caused by nonsense mutations: a prospective phase II trial. *Lancet* 372: 719–727
37. Kinali M, Arechavala-Gomez V, Feng L, et al. (2009) Local restoration of dystrophin expression with the morpholino oligomer AVI-4658 in Duchenne muscular dystrophy: a single-blind, placebo-controlled, dose-escalation, proof-of-concept study. *Lancet Neurol* 8: 918–928
38. Kirchheiner J, Brockmoller J (2005) Clinical consequences of cytochrome P450 2C9 polymorphisms. *Clin Pharmacol Ther* 77: 1–16
39. Lander ES, Linton LM, Birren B, et al. (2001) Initial sequencing and analysis of the human genome. *Nature* 409: 860–921
40. Lazarou J, Pomeranz BH, Corey PN (1998) Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies. *JAMA* 279: 1200–1205

41. Lee A, Ezzeldin H, Fourie J, Diasio R (2004) Dihydropyrimidine dehydrogenase deficiency: impact of pharmacogenetics on 5-fluorouracil therapy. *Clin Adv Hematol Oncol* 2: 527–532
42. Lesko LJ, Woodcock J (2004) Translation of pharmacogenomics and pharmacogenetics: a regulatory perspective. *Nat Rev Drug Discov* 3: 763–769
43. Levy S, Sutton G, Ng PC, et al. (2007) The diploid genome sequence of an individual human. *PLoS Biol* 5: e254
44. Liljedahl U, Kahan T, Malmqvist K, et al. (2004) Single nucleotide polymorphisms predict the change in left ventricular mass in response to antihypertensive treatment. *J Hypertens* 22: 2321–2328
45. Liljedahl U, Lind L, Kurland L, et al. (2004) Single nucleotide polymorphisms in the apolipoprotein B and low density lipoprotein receptor genes affect response to antihypertensive treatment. *BMC Cardiovasc Disord* 4: 16
46. Maitland ML, Vasisht K, Ratain MJ (2006) TPMT, UGT1A1 and DPYD: genotyping to ensure safer cancer therapy? *Trends Pharmacol Sci* 27: 432–437
47. Mallal S, Nolan D, Witt C, et al. (2002) Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. *Lancet* 359: 727–732
48. Mallal S, Phillips E, Carosi G, et al. (2008) HLA-B*5701 screening for hypersensitivity to abacavir. *N Engl J Med* 358: 568–579
49. Mamounas EP, Tang G, Fisher B, et al. (2010) Association Between the 21-Gene Recurrence Score Assay and Risk of Locoregional Recurrence in Node-Negative, Estrogen Receptor-Positive Breast Cancer: Results From NSABP B-14 and NSABP B-20. *J Clin Oncol* 28: 1677–1683
50. Mardis ER, Wilson RK (2009) Cancer genome sequencing: a review. *Hum Mol Genet* 18: R163–168
51. Meyer UA (2004) Pharmacogenetics – five decades of therapeutic lessons from genetic diversity. *Nat Rev Genet* 5: 669–676
52. Mishra PJ, Bertino JR (2009) MicroRNA polymorphisms: the future of pharmacogenomics, molecular epidemiology and individualized medicine. *Pharmacogenomics* 10: 399–416
53. Nebert DW, Vesell ES (2004) Advances in pharmacogenomics and individualized drug therapy: exciting challenges that lie ahead. *Eur J Pharmacol* 500: 267–280
54. Nebert DW, Vesell ES (2006) Can personalized drug therapy be achieved? A closer look at pharmaco-metabonomics. *Trends Pharmacol Sci* 27: 580–586
55. Nebert DW, Zhang G, Vesell ES (2008) From human genetics and genomics to pharmacogenetics and pharmacogenomics: past lessons, future directions. *Drug Metab Rev* 40: 187–224
56. Nelson SF, Crosbie RH, Miceli MC, Spencer MJ (2009) Emerging genetic therapies to treat Duchenne muscular dystrophy. *Curr Opin Neurol* 22: 532–538
57. Niemi M (2010) Transporter pharmacogenetics and statin toxicity. *Clin Pharmacol Ther* 87: 130–133
58. O’Kane DJ, Weinshilboum RM, Moyer TP (2003) Pharmacogenomics and reducing the frequency of adverse drug events. *Pharmacogenomics* 4: 1–4
59. Orth AP, Batalov S, Perrone M, Chanda SK (2004) The promise of genomics to identify novel therapeutic targets. *Expert Opin Ther Targets* 8: 587–596
60. Paik S, Shak S, Tang G, et al. (2004) A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med* 351: 2817–2826
61. Phillips E, Mallal S (2009) Successful translation of pharmacogenetics into the clinic: the abacavir example. *Mol Diagn Ther* 13: 1–9
62. Plewczynski D, Rychlewski L (2009) Meta-basic estimates the size of druggable human genome. *J Mol Model* 15: 695–699
63. Potti A, Dressman HK, Bild A, et al. (2006) Genomic signatures to guide the use of chemotherapeutics. *Nat Med* 12: 1294–1300

64. Potti A, Mukherjee S, Petersen R, et al. (2006) A genomic strategy to refine prognosis in early-stage non-small-cell lung cancer. *N Engl J Med* 355: 570–580
65. Roses AD (2004) Pharmacogenetics and drug development: the path to safer and more effective drugs. *Nat Rev Genet* 5: 645–656
66. Roses AD (2008) Pharmacogenetics in drug discovery and development: a translational perspective. *Nat Rev Drug Discov* 7: 807–817
67. Rudez G, Bouman HJ, van Werkum JW, et al. (2009) Common variation in the platelet receptor P2RY12 gene is associated with residual on-clopidogrel platelet reactivity in patients undergoing elective percutaneous coronary interventions. *Circ Cardiovasc Genet* 2: 515–521
68. Russ AP, Lampel S (2005) The druggable genome: an update. *Drug Discov Today* 10: 1607–1610
69. Schmidt WM, Kalipciyan M, Dornstauder E, et al. (2004) Dissecting progressive stages of 5-fluorouracil resistance in vitro using RNA expression profiling. *Int J Cancer* 112: 200–212
70. Schmidt WM, Spiel AO, Jilma B, Wolzt M, Muller M (2008) In-vivo effects of simvastatin and rosuvastatin on global gene expression in peripheral blood leucocytes in a human inflammation model. *Pharmacogenet Genomics* 18: 109–120
71. Schmidt WM, Spiel AO, Jilma B, Wolzt M, Muller M (2009) In vivo profile of the human leukocyte microRNA response to endotoxemia. *Biochem Biophys Res Commun* 380: 437–441
72. Siddiqui A, Kerb R, Weale ME, et al. (2003) Association of multidrug resistance in epilepsy with a polymorphism in the drug-transporter gene ABCB1. *N Engl J Med* 348: 1442–1448
73. Sordella R, Bell DW, Haber DA, Settleman J (2004) Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. *Science* 305: 1163–1167
74. Tanigawara Y, Aoyama N, Kita T, et al. (1999) CYP2C19 genotype-related efficacy of omeprazole for the treatment of infection caused by *Helicobacter pylori*. *Clin Pharmacol Ther* 66: 528–534
75. Trevino LR, Shimasaki N, Yang W, et al. (2009) Germline genetic variation in an organic anion transporter polypeptide associated with methotrexate pharmacokinetics and clinical effects. *J Clin Oncol* 27: 5972–5978
76. Trusheim MR, Berndt ER, Douglas FL (2007) Stratified medicine: strategic and economic implications of combining drugs and clinical biomarkers. *Nat Rev Drug Discov* 6: 287–293
77. van 't Veer LJ, Dai H, van de Vijver MJ, et al. (2002) Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 415: 530–536
78. van de Vijver MJ, He YD, van't Veer LJ, et al. (2002) A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med* 347: 1999–2009
79. van Deutekom JC, Janson AA, Ginjaar IB, et al. (2007) Local dystrophin restoration with antisense oligonucleotide PRO051. *N Engl J Med* 357: 2677–2686
80. Vasilescu C, Rossi S, Shimizu M, et al. (2009) MicroRNA fingerprints identify miR-150 as a plasma prognostic marker in patients with sepsis. *PLoS One* 4: e7405
81. Venter JC, Adams MD, Myers EW, et al. (2001) The sequence of the human genome. *Science* 291: 1304–1351
82. Voora D, Shah SH, Spasojevic I, et al. (2009) The SLCO1B1*5 genetic variant is associated with statin-induced side effects. *J Am Coll Cardiol* 54: 1609–1616
83. Wang J, Wang W, Li R, et al. (2008) The diploid genome sequence of an Asian individual. *Nature* 456: 60–65
84. Weinshilboum R (2003) Inheritance and drug response. *N Engl J Med* 348: 529–537
85. Weinshilboum RM, Wang L (2006) Pharmacogenetics and pharmacogenomics: development, science, and translation. *Annu Rev Genomics Hum Genet* 7: 223–245
86. Wheeler DA, Srinivasan M, Egholm M, et al. (2008) The complete genome of an individual by massively parallel DNA sequencing. *Nature* 452: 872–876
87. Yang L, Chen J, He L (2009) Harvesting candidate genes responsible for serious adverse drug reactions from a chemical-protein interactome. *PLoS Comput Biol* 5: e1000441

CHAPTER 16

Biomarkers

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1 Introduction

Biomarkers are any measurable and quantifiable biological parameters which serve as indices for health- or disease-related processes. The idea of identifying biological markers as indicators for an underlying disease is an elementary concept in medicine. Since the beginning of the medical skill of healing, signs and symptoms have been interpreted by medical doctors and guided diagnosis and treatment of their patients. With the emergence of the bioanalytical laboratory methods in the 50s of the last century a number of novel serum and urine markers became available. These objectively measurable serum markers were initially intended for studying physiological processes in biology. Over time, the value of such serum based biological markers for clinical decision making emerged as they were correlated with the pathology and the clinical course of disease states. This “traditional identification” of biomarkers as an observational site product of clinical practice led to the identification of the majority of clinical biomarkers employed in clinical practice today.

Biomarker can be classified in two broad categories [1]. The first group are so-called “disease related biomarker”. This class of biomarkers is intended to provide information about the individual risk of a patient to suffer from a disease in the future or the natural course of an already existing disease:

- *Antecedent* biomarkers are for identifying the risk of developing an illness in the future leading to preventive interventions for those at sufficient risk. Examples are the cancer susceptibility genes BRCA1 and BRCA2 for breast cancer. This type of biomarker is currently intensively discussed from the background of personal genomic testing and the challenge to interpret the results in terms of clinical consequences.

Keywords: Biomarker, predictive, prognostic, screening, surrogate endpoint, clinical endpoint, cholesterol as biomarker

- *Screening biomarkers* are intended for detecting subclinical disease enabling intervention at an earlier and potentially more curable stage than under usual clinical diagnostic conditions (e.g. PSA screening for prostate cancer).
- *Diagnostic and staging biomarkers* allowing clinicians to recognize overt disease and categorizing disease severity, respectively (e.g. Troponin T for acute coronary syndrome, pBNP for cardiomyopathy).
- *Prognostic biomarkers* for predicting future disease course and outcome of individual patients or groups of patients in terms of a clinical endpoint, including recurrence of disease (e.g. Her2 expression for breast cancer, cholesterol for CVD).

The second category of biomarker comprises the “therapy related biomarkers”. It differs from the first group of biomarker in that it describes the response of the marker to a therapeutic intervention. This class of biomarkers includes:

- *Predictive biomarkers* are intended to predict the efficacy of a therapeutic intervention *before* it is administered. Predictive biomarkers are context sensitive linking a specific treatment with a specific clinical endpoint and may not be used by implication for similar treatments or clinical endpoints, respectively. In clinical practice, predictive biomarker are employed for identifying patients most likely responding (or not responding!) to a certain drug (e.g. Bcr–Abl mutation in CML for imatinib treatment; HER2 expression in breast cancer for trastuzumab; Ras mutation for not responding to panitumumab in colorectal cancer). Predictive biomarkers are also useful to identify patients least likely to suffer an adverse event when treated with a drug.

Unfortunately, there exists a somewhat confusing mix-up for the terms prognostic and predictive biomarker in the literature. Both, prognostic and predictive biomarker are used upfront to a therapeutic intervention. Whereas the prognostic biomarker allows an estimate about the course of the disease and the outcome, the predictive biomarker enables the clinician to make prediction whether the disease will respond to a specific treatment. The same biomarker may be employed as prognostic and as predictive biomarker. For example, overexpression of the oncogene Her2/neu in breast cancer is a well-established prognostic biomarker for worse prognosis compared to Her2/neu negative tumours [2]. Concurrently, Her2/neu is a positive predictive biomarker for response to treatment with the Her2 targeting monoclonal antibody trastuzumab in breast cancer patients. Thus, Her2 qualifies as both prognostic and predictive biomarker in this setting.

- *Monitoring biomarkers* provide information about the therapeutic response *after* the therapy is administered. Monitoring biomarker allow to adjust the level of intervention (e.g. dose) on a dynamic and personal basis. Examples for monitoring

biomarker are blood glucose or HbA1c level in diabetes patients or tumour markers in oncology.

2 Why do molecular targeting drugs fail in current clinical drug development?

The interest in employing biomarker in clinical pharmacology and drug developments increased tremendously over the last decade. The progress in molecular biology and the deciphering of the human genome enabled us to develop drugs targeting the molecular level of disease. At the same time, the attrition rate of new drug candidates in late stage clinical testing raised. While the number of drug candidate failing due to poor bioavailability could be successfully reduced in the past by implementing intensified PK analyses in clinical trials, there was virtually no improvement for the two leading causes of drug failure in late stage drug development by the end of the last century, namely poor efficacy and unexpected high toxicity in Phase III clinical trial.

This fact inspired a process of reconsidering the drug development strategies applied so far. By standard, drug approval was (and is still) based on evidence from adequate powered and well-controlled clinical trials with clinical meaningful endpoints. However, following this well-established traditional approach it is foreseeable that we will not be able to accomplish all future tasks in drug development. The challenges we are facing can be summarized in the sentence: “It is necessary to do more and faster with less while retaining quality output”. Whereas in the past the number of drug candidates available for clinical testing was the rate limiting step in drug development, there are awaiting currently more candidate compounds clinical testing than financial-, time- and patient resources are available in a reasonable horizon of time. Undeniably, it is not an acceptable alternative to lower the standards in clinical trials compromising patient safety to accomplish these tasks. Thus, there is a clear need to streamline the strategies of the drug approval process. Beside these novel challenges, the traditional approach by large randomized controlled trials reveals obvious shortcomings with respect to patient safety. About a quarter of all finally FDA approved drugs require relabelling (i.e. dose reduction) due to safety concerns [3].

Dose finding for many of the drugs currently on the market was performed according the concept of the “maximum tolerated dose” (MTD). By this approach, a novel investigational drug will be tested for the primary objective of safety and tolerability when administered for the first time in humans (“first in men” study). Beginning with a starting dose, cohorts of patients will be treated with the investigational drug and, if tolerated, dose will be escalated in a next cohort of patients. Dose escalation will be continued up to a dose level where patients experience “dose limiting

toxicities” (DLT) as outlined in the study protocol. The dose level below will be defined as the “maximum tolerated dose” (MTD) and will be carried forward for further testing in Phase II clinical trials as “recommended phase two dose” (RPTD). In the field of oncology the underlying paradigm for this approach is that, dose-related toxicities are regarded as a surrogate for the activity of a drug. Whereas this concept has been employed successfully for drugs with non-specific mechanism of action in the past, it does not seem to be appropriate for the novel generation of molecular targeting therapeutics entering the clinic today. Many of these novel drug candidates are designed to mediate their therapeutic effect by a specific target modulation, while their side effects are not necessarily dependent on this target modulation. Toxicities might be caused by mechanism independent of the therapeutic activity of these compounds. Thus, it has to be questioned whether the concept of “maximum tolerated dose” represent a suitable strategy for future dose finding studies with molecular targeting therapeutics.

To further illustrate this thread, hypothetical dose-response scenarios for molecular targeting compounds are depicted in Fig. 1. In a dose escalation study, increasing the dose of an investigational compound might result in a saturable plateau phase for the therapeutic target activity, whereas toxicity steadily increases as a function of dose

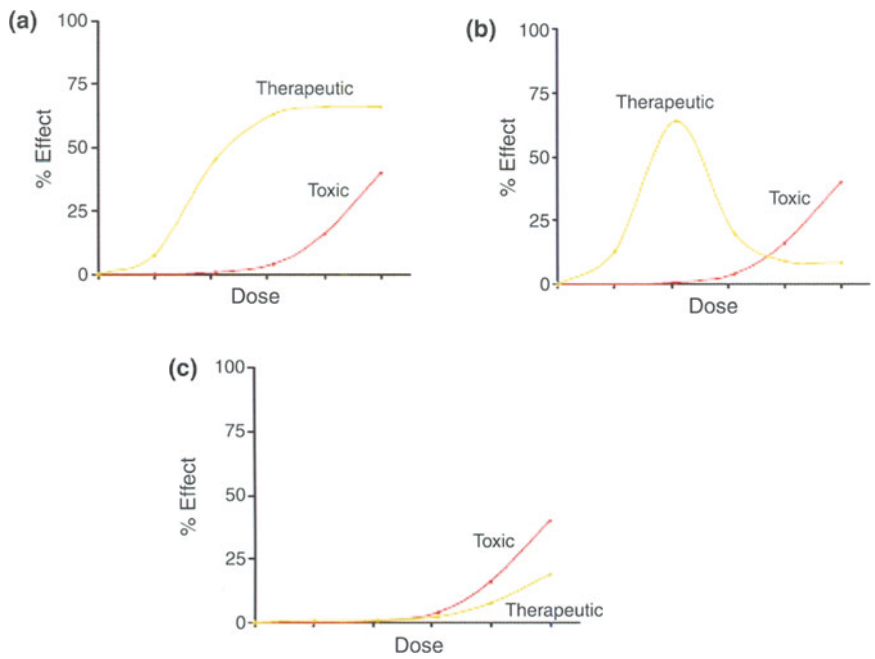


Fig. 1 Hypothetical dose-effect relationship scenarios (from Ref. [4])

(Fig. 1a). In this setting, the same maximum therapeutic target activity of the compound would have been already obtained at a dose considerably lower than the MTD with fewer side effects. This would translate for the patient in an improved risk/benefit ratio with less side effects and the same therapeutic activity. Even worse is the scenario in Fig. 1b, where the dose effect relationship has the shape of an inverse U-curve. After initial increase in activity further dose escalation results in a loss of activity (e.g. due to unspecific receptor binding leading to paradox pharmacological effects). In this setting, the concept of dose escalation to MTD will fail to define a therapeutic active dose and lead to an inappropriate RPTD. And finally, there might be drug candidates which despite promising preclinical data fail to induce any relevant target modulation in humans. Still, it is possible to define a MTD by dose escalation for such compounds, but the concept of MTD will here fail again to define a dose with therapeutic target activity (Fig. 1c).

After dose finding studies are completed, an investigational drug will be assessed for its clinical activity in further clinical trials. In oncology, the endpoints chosen for these clinical trials in the past were optimized for drugs with a non-specific mechanism of action, such as tumour response for cytotoxic chemotherapeutics in oncology. For novel molecular targeting therapeutics such endpoints appear to be only of limited use for evaluating their therapeutic activity. For instance, a clinical benefit of a targeting drug may be expected even without tumour shrinkage (e.g. anti-metastatic activity). What's more, given the complex molecular background of diseases like cancer, neurodegenerative- or autoimmune diseases, it is important to identify potential subgroups of patients most likely to respond to a targeted intervention. Despite a very similar phenotype patients with the aforementioned diseases might differ substantially in their molecular expression pattern making them variable susceptible to a molecular targeting therapeutic. Clinical trials at this stage need to evaluate whether target expression itself or expression of further susceptibility markers (e.g. synthetic lethality) critically influence the activity of a drug candidate.

3 Biomarker in clinical drug development

Biomarkers have the potential to contribute to a solution of all the issues raised above. As a result of a critical appraisal process novel strategies have been proposed promoting implementation of biomarkers as from the beginning of the drug development process. The leading thought of these concepts is to characterize in much more detail the patient disease status and the effect of a given investigational drug in the individual patient with the ultimate aim to provide “the right drug for the right patient at the right dose at the right time”. A biomarker approach should result in better patient outcomes and less side effects relative to a non-biomarker based strategy. Besides optimizing the risk/benefit ratio of the patient's treatment,

insights gained into the molecular mechanism of response should foster the mechanism of action of novel targeting compounds and allow an informed decision-making.

To take advantage of the vast interest and promote the utility of biomarkers in the assessment of clinical research, a consensus on terminology among the various specialties was initiated. In 2001, The National Institutes of Health (NIH) Biomarkers Definition Working Group proposed the following conceptual framework [1, 5]:

1. Biomarker: a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological response to a therapeutic intervention.
 - Type 0 biomarker: a marker of the natural history of a disease that correlates longitudinally with known clinical indices.
 - Type I biomarker: a marker that captures the effects of a therapeutic intervention in accordance with its mechanism of action.Examples for biomarker are, for instance, serum transaminases for liver functions, HbA1c for diabetes or CT scans for tumour response.
2. Clinical endpoint: a characteristic or a variable that reflects how a patient feels, functions, or survives. Clinical endpoints may be further sub-divided in intermediate-and ultimate clinical endpoints.
 - Intermediate (non-ultimate) end point: A true clinical end point (a symptom or measure of function, such as symptoms of angina frequency, exercise tolerance or change in quality-of-life), but not the ultimate end point of the disease.
 - Ultimate end point: survival or the rate of other serious and irreversible morbidity events.
3. Surrogate endpoint: a biomarker that is intended to substitute for a clinical endpoint. A surrogate end point is expected to predict clinical benefit (or harm or lack of benefit or harm) on the basis of epidemiological, therapeutic, pathophysiological, or other scientific evidence. Surrogate endpoints are sometimes also referred to as Type II biomarkers.

The term surrogate endpoint links the biomarker and the clinical endpoint. In the context of a clinical study, a biomarker will be investigated which is deemed to be on the causal pathway of the disease studied. This biomarker and any change of this biomarker will be measured as a substitute for predicting the outcome of the true clinical endpoint. If an intervention is administered (i.e. investigational product) its effect on the biomarker will be measured (Fig. 2). Only if the biomarker is able to predict the true clinical endpoint this biomarker qualifies as a surrogate endpoint and may be measured as a substitute for the ultimate clinical outcome of this disease.

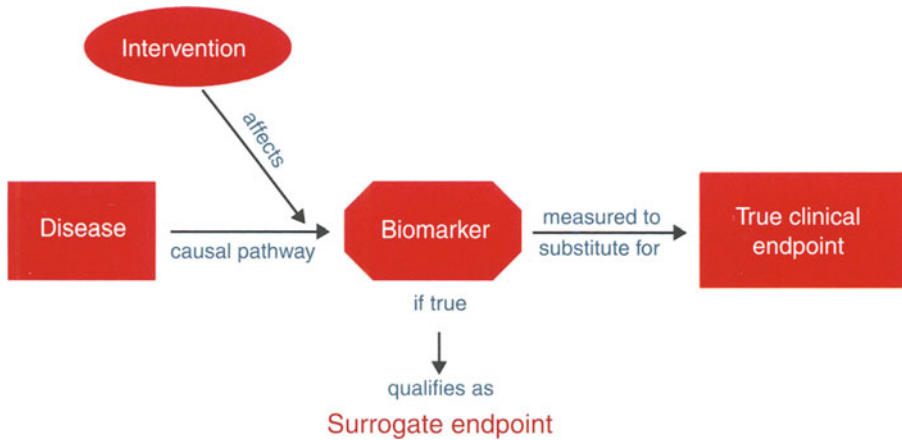


Fig. 2 Schematic framework for the biomarker-surrogate endpoint concept

There has been a lot of criticism about the concept of biomarkers and surrogate endpoints. At least in part, this misconception stems from an improper use of the terms biomarker and surrogate endpoints. It's important to notice that surrogate endpoint biomarkers are used for different purposes in clinical trial development.

4 How biomarkers may improve clinical drug development

Biomarkers have their predominant role in the early phase of drug development. If a novel molecular targeting compound will be tested for the first time in humans it is of critical importance to study whether it acts by the intended mechanism of action. To this end, pharmacodynamic biomarkers may be assessed before and after administration of a investigational drug in “proof of concept” studies. Pharmacodynamic biomarkers are molecular or imaging markers of drug response that can be measured in patients receiving the drug. The marker should be a direct- (e.g. mRNA or protein target downregulation following antisense oligonucleotides therapy[4]) or indirect measure of modulation of the drug target (e.g. increase of angiotensin/renin and decrease of aldosterone following administration of an angiotensin receptor II blocker [6]). Moreover, it should reflect the quantitative changes in response to the dose administered. In case of multiple derivatives of a mother compound, pharmacodynamic biomarker might be of value to select the lead compounds by identifying the drug candidate with the most prominent effect on the pharmacodynamic marker.

Besides proof of concept and lead selection, pharmacodynamic biomarkers provide an elegant tool to overcome the shortcomings of the MTD concept in dose escalation studies. Following the idea that the therapeutic activity of a molecular targeting compound is best if there is a maximum effect on the molecular target, alternative objectives for clinical Phase I have been suggested. Accordingly, while it is definitely still mandatory to establish a safe and tolerable dose, dose escalation for molecular targeting drugs might be guided by the effect on pharmacodynamic biomarkers with the objective to determine an “optimal biological dose” (OBD) rather than the MTD. In this context the “optimal biological dose” is defined as the lowest dose having a maximum effect on the biological target. By describing the dose/target effect relationship the pharmacodynamic marker will be assessed as a surrogate for the activity of the investigational product. Dose escalation will be halted if either the investigational drug display unacceptable toxicities or further dose escalation does not result in an increase of pharmacodynamic biomarker regulation.

To take advantage of this concept it is essential that the biomarker employed meets a couple of stipulations. First, the biomarker must be determinable with a validated assay. Assay validation is key in biomarker research since any biomarker can be only as good as the assay it is measured by. Assays employed have to be tested for accuracy and precision. The process of assay validation is a time and work intensive effort which ideally should start in parallel with pre-clinical testing of a drug candidate for being already established at the beginning of the clinical trial programme. Only if there is solid evidence for a linear correlation between the effect on the biomarker and the molecular target, the biomarker may be of help in clinical development. Furthermore, the biomarker must be measurable in a way which is tolerable for the patient (i.e. non- or minimal invasive). In contrast to the biomarkers mentioned in the introduction, pharmacodynamic biomarkers provide a tool for drug development and are not necessarily suited as monitoring- or predictive biomarker. Likewise, they are not primarily intended to serve as a surrogate endpoint of any regulatory approval process.

An example of a successful implementation of pharmacodynamic biomarker for guiding dose selection is the dose finding of the NK1 receptor antagonist aprepitant [7]. In PET studies the extent of NK1 receptor occupancy in the brain by aprepitant correlated significantly with its antiemetic clinical effects.

Despite the appealing theoretical concept, biomarker are still used only rarely as primary endpoint in Phase I clinical trials for selecting the RPTD. In a review of dose finding strategies employed for molecular targeting therapeutics in the field of oncology only about 5% of all trials relied on a pharmacodynamic biomarker as primary source for defining RPTD. At least approx. 50% of all studies employed pharmacodynamic biomarkers for supporting the RPTD [8]. The reluctance towards the use of biomarkers for dose finding may be argued by the higher investment of time necessary to implement biomarkers in the clinical trial development process and a still existing

shortage of expertise in this research area. However, more recent data indicate that the number of trials using biomarker is steadily increasing.

In this context it is encouraging, that close to 50% of all oncology drugs approved in the last four years by the FDA have in their labelling at least for defining their target population make use of a biomarker. Oncology has become by far the most active field of predictive biomarker development. The stratification of cancer patients according a molecular biomarker led to a number of successful drug approvals in a field where in the past only about 25–80% of all patients were estimated to receive effective treatments [9]. The success story of imatinib targeting the Bcr–Abl translocation in CML, where Bcr–Abl is a diagnostic and monitoring biomarker, raised further enthusiasm to intensify research efforts in this arena. More and more predictive biomarkers for molecular targeting compounds have been identified in parallel with the development of the respective oncological drug. This concept of co-development of a biomarker and a drug, also referred to as companion-development, greatly improves the likelihood of a successful drug development by defining a particular responsive subpopulation. Along these lines, Ras mutations in colorectal cancer patients have been shown to limit the activity of the EGFR targeting antibody panitumumab. Genetic testing of colorectal cancer for ras mutations has become a standard of care to spare these patients from a poorly effective, expensive and side effect intensive therapy. This highlights that only biomarkers addressing an unmet medical need and affecting disease management have a chance to be accepted and become common practice.

Biomarkers provide also a strategy to “rescue” a molecular targeting compound which has initially failed to demonstrate clinical benefit. The EGFR receptor inhibitor gefitinib was studied after successful Phase II clinical data in two large Phase III trials in non-small cell lung cancer (NSCLC) patients in combinations with chemotherapy. The combination with gefitinib did not result in any significant clinical benefit relative to standard of care chemotherapy and gefitinib was withdrawn from the market. However, by careful retrospective molecular analysis subset of patients could be identified showing major response to this therapy. By selecting only NSCLC patients positive for EGFR mutations gefitinib has demonstrated in the meantime superior efficacy, better tolerability and improved quality of life relative to standard of care chemotherapy (i.e. carboplatin/paclitaxel) as first line therapy in NSCLC patients carrying a EGFR mutation [10]. Based on these data gefitinib has been “re-approved” in Europe only for the subgroup of NSCLC patients carrying the predictive biomarker EGFR mutation.

Biomarkers are not only attractive for proof of concept, lead identification, dose guiding, and patient stratification but notwithstanding also for economic reasons. Instead of learning as late as in Phase III clinical trials after spending \$600–800 million that a novel promising drug candidate is not effective, informed go/no-go decisions can be made much earlier by pharmaceutical companies based on the results of “proof of

concept” studies. Also for the community and the health care systems the increased efficiency of drug therapy by selecting responsive subpopulation based on predictive biomarkers bears a clear potential for optimizing resources.

5 Biomarker as surrogate endpoints

Biomarker can be studied beyond their primary role as indicator for a disease or a therapeutic intervention as endpoints in clinical trial. If so, biomarkers are referred to as surrogate endpoints. Surrogate endpoint should be employed with caution. For clinical research questions where it is feasible to set up clinical trials assessing a clinical meaningful this should be preferred. Only if there is an unmet medical need and it is for logistical reasons hardly feasible to study a clinical meaningful endpoint the concept of a surrogate endpoint study should be considered. It is not per se the idea of surrogate endpoints to replace clinical endpoints. There is no doubt that the most reliable way to assess the clinical efficacy of an investigational product is by its impact on well-defined clinical endpoints. However, there are circumstances in clinical research where it appears to be justified in the best interest of patients to assess surrogate endpoints instead of clinical endpoints. In the setting of life-threatening disease and no available alternative standard treatment options clinical trials on surrogate endpoints appear to be warranted to promote development of more effective treatments (e.g. advanced stage disease in oncology). Likewise, clinical trials on surrogate endpoints outperform in conditions where the clinical endpoint will occur delayed and/or rarely. For example, prevention studies require long observation periods and large study cohorts since clinical meaningful endpoints are expected only in few patients in years from now. The acceptance of a surrogate endpoint as outcome parameter of a clinical trial is higher if already a large safety database exists for the investigational product derived from its use for an alternative indication. The validity of surrogate endpoints may be further supported by data showing that the biomarker has already predicted clinical outcome for compounds of the same pharmacological class (or even better several classes in the same indication).

For the use of biomarkers as surrogate endpoints substantial body of evidence has to be available linking the biomarker with a clinical endpoint based on the pathophysiology and epidemiology of the disease. Only if treatment comparisons based on the surrogate endpoint provide a faithful reflection of the true clinical endpoint the reliance on surrogate outcomes is justifiable. In order to guide this selection a widely accepted framework of qualification criteria has been proposed to support the use of a biomarker as surrogate endpoint based on three provisions [11, 12]:

- Efficiency: “although the surrogate endpoint should be easier to assess than the corresponding clinical endpoint, the most important characteristic is its frequency,

i.e. it should occur more often than the corresponding clinical endpoint”. Assessing surrogate endpoints preceding the disease specific morbidity or incidence outcomes increase the feasibility of e.g. prevention studies. Moreover, biomarker measurements should be easy to perform with an acceptable risk/benefit ratio for the patient (e.g. non- or only minimal invasive assessments instead of biopsies). As a result, clinical trials with a surrogate endpoint may be conducted in a shorter timeframe, will require fewer resources and minimize the patient burden.

- Linkage: “the relationship between surrogate and clinical endpoint must be well established, both quantitatively and qualitatively”. There should be a thorough understanding of the pathogenesis of the disease and the mode of action of the investigational drug. In preclinical animal models, target modulation by the investigational drug or by experimental means should be reflected by the intended biological effect in terms of nature and extent. From the point of view of molecular biology, it is preferable to pick a biomarker relatively late on the signalling pathway of a disease as the likelihood of confounding effects downstream of the marker will be reduced. Epidemiologic evidence for the link between the biomarker and the disease should be extensive and consistent to foster the selection of the biomarker as surrogate endpoint. As a whole, data should evidence proximity, causal relationship and specificity of the biomarker and the clinical endpoint.
- Congruency: “an estimate of the expected clinical benefit should be derivable from the estimate of the reduction of the surrogate endpoint”. Any change of the biomarker should be reflected in the change of the clinical outcome allowing a quantitative and sensitive estimate of the clinical effect. The validity of a surrogate endpoint may be conditionally independent of the investigated drug. Confounding factors contributing to the ultimate clinical outcome may not be fully accounted for by the surrogate endpoint assessed. Moreover, depending on the stage of disease the molecular signalling might be alternating so that the surrogate endpoint is affected aberrantly. For practical use it has been suggested that a surrogate endpoint is regarded as useful if it accounts for at least 50% of the effect of an intervention on the outcome of interest [13].

It is obvious that only a subset of biomarkers will have the potential to achieve the status of a surrogate endpoint. Only biomarkers fulfilling the aforementioned criteria are deemed to be “reasonably likely” to serve as a qualified surrogate endpoint in clinical trials. In many therapeutic areas there are currently only “provisional” surrogate endpoints available. In this regard it has to be pointed out that qualification of biomarkers is a “learning by doing” process: biomarkers can only be qualified as surrogate endpoints by conducting interventional clinical studies but at the time these studies are being conducted it is still a non-proven hypothesis whether at the end the biomarker assessed will qualify as a true surrogate endpoint. Even biomarker suitable according to all the above considerations still may not be eligible as surrogate

endpoints if there is no validated assay system available to quantify the biomarker (“*conditio sine qua non*”).

As a prime example for taking advantage of the surrogate endpoint concept in clinical studies is considered the evaluation of the Cox-2 inhibitor celecoxib for preventing familial adenomatosis polyposis (FAP). FAP is a genetic disease characterized by the development of adenomatous polyps in the colon with a near to 100% lifetime risk to suffer from colorectal cancer. Based on observational studies it was known that NSAID may reduce the number of polyps in the colon. In a small clinical trial celecoxib was shown to reduce the adenoma burden by 28% within 6 months of treatment in FAP patients as evidenced by colonoscopy [14]. This effect on the surrogate endpoint “number of adenomatous polyps” led to the approval of celecoxib in patients with FAP. In this setting, the prevention of a life threatening disease coupled with evidence from preclinical and clinical observational studies and a large safety database (i.e. celecoxib was already approved for treating rheumatoid arthritis) was deemed by the regulatory authorities to justify approval on a surrogate endpoint.

6 Outlook

Biomarkers have already changed the drug development process and will continue to change it. By defining subpopulations most likely to benefit from a specific therapy they will help us to get closer to the paradigm of “personalized medicine”. Tandem development of a drug and the corresponding biomarker will become a standard for many therapeutic areas. The active search for novel biomarker enabled by the advent of the – omic technologies in molecular biology is regarded as a key element for more efficient therapies in the future. This new research area of “biomarker discovery” is also expected to overcome some shortcomings which have been inherently present in the biomarker field due to technical limitations in the past. Complex diseases like cancer, CVD or autoimmune diseases arise in the predominant number of cases from a series of molecular perturbations resulting in a diversity of different molecular disease traits. From this background measuring only a single biomarker for predicting the outcome of such complex diseases or their response to a therapeutic intervention appears to be an undue oversimplification. In the future, molecular signature patterns consisting of multiple biomarkers will yield much higher predictive values. In a steep learning curve industry and regulatory authorities have started to team up for establishing biomarkers for new and more efficient ways to test novel drugs. For instance, the Predictive Safety Testing Consortium (PSTC) was formed to establish toxicology biomarker allowing diagnosing organ specific toxicities much earlier and more sensitive than with any other currently available methodology (for more info see <http://www.c-path.org/pstc.cfm>). These joined efforts continue and are expected to make the drug development with biomarkers on the long run more efficient for all parties involved.

Case Study: Cholesterol as biomarker and surrogate endpoint for cardiovascular disease

An example for use of biomarkers and surrogate endpoints in clinical trials is cholesterol in the context of cardiovascular disease (CVD). The development of cardiovascular disease is a complex and long-lasting multistep process. Years before the appearance of any clinical endpoints (e.g. angina pectoris, myocardial infarction) pathogenicity factors like endothelial dysfunction, hyperlipidemia, migration of inflammatory cells into the arterial wall and plaque formation occur which are considered to be on the causal pathway of this disease. Among these factors, the most amenable for surrogate testing in clinical practice is hypercholesterolemia by blood assessment with validated laboratory assays. There is broad and consistent evidence from epidemiological studies supporting the role of serum total cholesterol (TC) and LDL-c levels as a risk factor for development of cardiovascular disease. Moreover, in patients with familial hypercholesterolemia mutations in the gene encoding for the LDL receptor lead to a reduced clearance of LDL-c from the serum. These patients show significantly increased LDL-c serum level and develop premature CVD. In a meta-analysis of 58 trials including 148,321 patients lowering of LDL-c by 1.0 mmol/L reduces overall mortality, coronary mortality and myocardial infarction by 12, 19 and 23%, respectively [15]. This body of evidence from different sources supports the role of LDL-c as a prognostic biomarker for CVD and rendered LDL-c an attractive target for cardiovascular drug development. Consequently, lowering cholesterol level as primary or secondary endpoint for preventing CVD has been accepted by the regulatory agencies as a reliable surrogate endpoint for granting drug approval.

But the therapeutic efficacy of HMG-CoA reductase inhibitors cannot exclusively be attributed to their lipid-lowering effects. Simvastatin, for instance, has shown in clinical trials (e.g. 4S study) that lowering LDL-c results in an about 30% reduction of myocardial infarctions. But this clinical benefit appears to be mediated in part by “pleiotropic” effects of statins. Statins display amongst others anti-inflammatory activity contributing independently to their therapeutic activity. In the JUPITER trial apparently healthy persons without hyperlipidemia but with elevated CRP showed significant reduction in the incidence of major cardiovascular events if treated with rosuvastatin [16]. With respect to the role of LDL-c as a surrogate endpoint it has to be questioned whether LDL-c lowering as a biomarker faithfully reflect the entire therapeutic activity of statins.

Further concerns for the role of LDL-c as a surrogate endpoint derived from the recently published ENHANCE trial. In this study the efficacy of ezetimibe, a selective inhibitor of cholesterol absorption from the intestine, was evaluated in

combination with simvastatin versus simvastatin alone in patients with familiar hypercholesterinemia [17]. Ezetimibe resulted in an additional 16% reduction of LDL-c but no difference between the two treatment groups was noted in the primary endpoint carotid-artery intima-media thickness (CIMT), a commonly used biomarker in CVD prevention studies. Interestingly, CIMT was highly correlated with LDL-c lowering in previous statin trials [18]. The results of this trial put into question whether lowering of LDL-c serum levels as surrogate endpoint may be transferred from one class of drugs (i.e. statins) to other lipid lowering therapeutic strategies (i.e. ezetimibe). Rather it seems that LDL-c is a context-sensitive surrogate endpoint.

To this end, the SEAS trial further substantiated the concern that LDL-c is a valid surrogate endpoint for lipid lowering other than statins (also in combination with statins). In this trial 1873 patients were treated with simvastatin and ezetimibe or placebo and followed up for 52 months [19]. Despite a 61% reduction in LDL-c the combination treatment did not reduce the composite outcome of combined aortic-valve events and ischemic events as primary endpoint of the study. Even so a statistically significant reduction in the incidence of cardiac events in favour for simvastatin and ezetimibe was observed, this 21% reduction was not congruent with the 61% reduction in LDL-c.

Despite careful consideration of surrogate endpoints for clinical trials one always has to bear in mind the risk that an effect on the surrogate endpoint is not necessarily of any value for the patient. LDL-c is unequivocally a prognostic biomarker for CVD but it has to be questioned whether it's widespread acceptance as surrogate endpoint for lipid lowering therapy (i.e. statins) will be sustained in the future. As outlined, recent trial data revealed considerable concerns about the sensitivity and specificity of LDL-c as surrogate endpoint for CVD prevention. The regulatory authorities already reacted and are now reluctant to approve novel lipid lowering drugs with different mode of action on the surrogate endpoint LDL-c. For example, the antisense oligonucleotide mipomersen targeting apoB-100 mRNA outperformed in clinical trials standard lipid-lowering drugs as it concerns LDL-c lowering. However, the FDA advised the sponsor to provide also clinical endpoints for getting marketing authorization other than for familiar hypercholesterinemia [18].

References

1. Biomarkers Definitions Working Group (2001) Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther* 69(3): 89–95
2. Oldenhuis CN, Oosting SF, Gietema JA, de Vries EG (2008) Prognostic versus predictive value of biomarkers in oncology. *Eur J Cancer* 44(7): 946–953

3. Gobburu JV, Marroum PJ (2001) Utilisation of pharmacokinetic-pharmacodynamic modelling and simulation in regulatory decision-making. *Clin Pharmacokinet* 40(12): 883–892
4. Wacheck V (2004) Strategies for designing clinical trials for oligonucleotide therapeutics. *Drug Discov Today* 9(21): 918–923
5. Vasani RS (2006) Biomarkers of cardiovascular disease: molecular basis and practical considerations. *Circulation* 113(19): 2335–2362
6. Lee JW, Hulse JD, Colburn WA (1995) Surrogate biochemical markers: precise measurement for strategic drug and biologics development. *J Clin Pharmacol* 35(5): 464–470
7. Hargreaves R (2002) Imaging substance P receptors (NK1) in the living human brain using positron emission tomography. *J Clin Psychiatry* 63 (Suppl 11): 18–24
8. Parulekar WR, Eisenhauer EA (2004) Phase I trial design for solid tumor studies of targeted, non-cytotoxic agents: theory and practice. *J Natl Cancer Inst* 96(13): 990–997
9. Spear BB, Heath-Chiozzi M, Huff J (2001) Clinical application of pharmacogenetics. *Trends Mol Med* 7(5): 201–204
10. Mok TS, Wu YL, Thongprasert S, et al. (2009) Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* 361(10): 947–957
11. Boissel JP, Collet JP, Moleur P, Haugh M (1992) Surrogate endpoints: a basis for a rational approach. *Eur J Clin Pharmacol* 43(3): 235–244
12. Espeland MA, O'leary DH, Terry JG, Morgan T, Evans G, Mudra H (2005) Carotid intimal-media thickness as a surrogate for cardiovascular disease events in trials of HMG-CoA reductase inhibitors. *Curr Control Trials Cardiovasc Med* 6(1): 3
13. Prentice RL (1989) Surrogate endpoints in clinical trials: definition and operational criteria. *Stat Med* 8(4): 431–440
14. Arber N, Levin B (2008) Chemoprevention of colorectal neoplasia: the potential for personalized medicine. *Gastroenterology* 134(4): 1224–1237
15. Law MR, Wald NJ, Rudnicka AR (2003) Quantifying effect of statins on low density lipoprotein cholesterol, ischaemic heart disease, and stroke: systematic review and meta-analysis. *BMJ* 326(7404): 1423
16. Ridker PM, Danielson E, Fonseca FA, et al. (2008) Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med* 359(21): 2195–2207
17. Kastelein JJ, Akdim F, Stroes ES, et al. (2008) Simvastatin with or without ezetimibe in familial hypercholesterolemia. *N Engl J Med* 358(14): 1431–1443
18. Duivenvoorden R, de GE, Stroes ES, Kastelein JJ (2009) Surrogate markers in clinical trials – challenges and opportunities. *Atherosclerosis* 206(1): 8–16
19. Rossebø AB, Pedersen TR, Boman K, et al. (2008) Intensive lipid lowering with simvastatin and ezetimibe in aortic stenosis. *N Engl J Med* 359(13): 1343–1356

CHAPTER 17

Molecular tools in drug research – translational medicine

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1 Introduction

The advent of high-throughput and increasingly sensitive research techniques in molecular biology in the last 20 years has altered fundamentally our understanding of molecular biology. The unraveling of the human genome has provided unprecedented insights into the molecular pathophysiology of diseases. DNA sequencing techniques allowed collecting huge amounts of genetic information, revealing genetic variation and variable expression of genes pinpointing to the molecular level of diseases. In parallel, analytical methods for evaluating large sets of proteins become available facilitating studies on the functional relevance of transient or stable expression of these molecules for a disease phenotype. These novel molecular techniques are nowadays at the heart of modern drug research as they allow identifying and validating novel molecular targets of disease, drug screening as well as the discovery of biomarkers for predicting and monitoring response to drug therapy.

To take advantage of this gain of information and to bring it into clinical practice “translational medicine” emerged as an “interface” research discipline. Translational medicine is deemed to link preclinical (= basic) science and clinical science. It is a research field, in which many science disciplines are integrated. Physical-, chemical-, biological-, biochemical- and immunology knowledge as well as a solid understanding of clinical medicine is required to get involved in the research about the molecular basis of pathologies and the development of concepts to alter the aberrant molecular signalling by therapeutic interventions. Following the “bench-to-bedside” concept, translational medicine starts with basic laboratory research. Physician-scientists work-

Keywords: Translational medicine, target identification, target validation, target deconvolution, SAGE (serial analysis of gene expression), cloning, sequencing, phage display, transfection, yeast two hybrid, mass spectrometry, knock-out animal, Cre/lox system, FISH, PCR, FACS, Blotting, Western/Northern, ACE2

ing at the interface between the research laboratory and patient care or a team of basic and clinical science investigators identify and explore the potential of novel molecular targets for a specific disease by the tools of molecular biology *in vitro*. After faithful validation of the biological relevance of the target, drug candidates are tested in cell culture based *in vitro* assays and *in vivo* animal models for their potential to modulate the identified pathological signalling pathway and their therapeutic potential. Given positive results, translational medicine transfers the basic laboratory discoveries into clinical “proof-of-concept” trials for improving patient-oriented treatment or prevention of a specific disease.

It is important to emphasize that translational medicine is not a “one-way” directed process rather than a perpetually stimulating circulatory effort. Besides “bench-to-bedside” the inverse “bedside-to-bench” research activities are a tremendous fruitful source of novel information of the underlying molecular pathophysiology of a disease. Fluids (e.g. blood, urine or cerebrospinal fluid) or tissue samples collected from patients are analysed by the tools of molecular biology in more and more detail for biomarker discovery. The availability of the novel – omics technologies in combination with biobanks, that is large collections of samples from patients with well-characterized clinical history, allow linking molecular expression patterns (“molecular fingerprint”) with a disease state. Lessons learnt from these re-translational efforts provide the origin for novel research hypotheses to be tested then in preclinical. This illustrates that a close collaboration between clinical and pre clinical science, such as biochemistry, physics, chemistry, molecular biology and medicine is key for successful translational medicine.

The molecular tools available today in concert with translational medicine pave the way for unprecedented opportunities for drug research. These research activities will have great impact for understanding and combating diseases such as malignancy and infectious and autoimmune disease, as well as cardiovascular, metabolic and neurological diseases. In the following we will highlight exemplarily molecular tools and techniques employed in drug research for target identification, target validation, drug discovery and monitoring pharmacodynamic biomarkers in translational clinical trials.

2 Molecular tools for drug target identification

The identification of novel molecular targets in drug research may derive from different conceptual approaches. With the advances in molecular biology and biochemistry novel techniques emerged allowing whole-genome wide comparative analysis of patient and samples from healthy volunteers. Samples collected (e.g. tissue, serum and urine) are analysed for differential expression of genes and proteins. Differentially expressed genes (up- or downregulated) will then further characterized for their biological function and

their functional relevance. This “target screening” approach was clearly expedited by the availability of various molecular techniques in the last decade.

Alternatively, there are strategies starting from an observed phenotype induced by a drug candidate, which then will be further characterized to identify the target modulated. These so called “target deconvolution” strategies start from a given compound library. The compounds in such libraries are studied typically in mammalian cell culture high-throughput screening (HTS) assay systems for a phenotype of interest caused by the test compound. Depending on the drug effect desired, such phenotype might be morphological changes of the cell (e.g. cell shape, neurite outgrowth) or specific cellular effects, which will be measured by biochemical assays. For instance, cell viability measured by specific dyes relying on the metabolic ability of cells to reduce formazan salt, are employed to screen the cytotoxic activity of potential novel anti-cancer drugs. Reporter gene assays are employed to detect the activation of a particular signalling pathway of interest. For this purpose an easily detectable reporter gene (e.g. luciferase) is fused to the promoter sequence of a downstream target gene of the studied pathway. In case a compound screened interacts with the specific signalling pathway, the expression of the report gene will be activated or inhibited, which would become detectable by a change of luminescence. In the following, molecular tools employed for “target screening” and “target deconvolution” will be presented.

2.1 At the nucleotide level

As far as it concerns gene expression, the *DNA micro-array* technology represents a convenient, versatile and (in the meantime) affordable tool to assess the mRNA expression in samples. Gene expression profiles derived from mRNA micro-array may identify genomic signatures pinpointing to signalling pathway altered in a specific disease state and lead to the identification of new molecular targets. Moreover, micro-array may be used to link gene expression signatures from small molecules. By so-called “connectivity maps” gene expression profiles from human cell cultures treated with small molecules were linked successfully to identify molecules with a common mechanism of action. The micro-array technology is described into detail in Chapter 15. Briefly, by hybridization of the fluorescently-labelled “target” mRNA (sample) to the oligonucleotide probe sequences spotted on the array, the mRNA in a sample can be quantified by means of fluorescence intensity, which is proportional to the abundance of the target sequence in the sample. A limitation of the micro-array technology is the fact that it is by nature a biased approach. Since only these mRNA transcripts can be detected, which have complementary oligonucleotide probe sequences on the array, any further transcripts will not be detected. This might limit the use of micro-arrays for finding novel drug targets.

As an alternative, the *serial analysis of gene expression* (SAGE) provide an unbiased approach to measure the number of mRNA transcripts, since it is inde-

pendent on prior knowledge of what transcripts to study. SAGE is an effective method for analysing mRNA gene expression, which makes use of short cDNA fragments, so called sequence tags. These small sequences of nucleotides can effectively use to identify the original mRNA transcript. The gene tags are linked together, sequenced

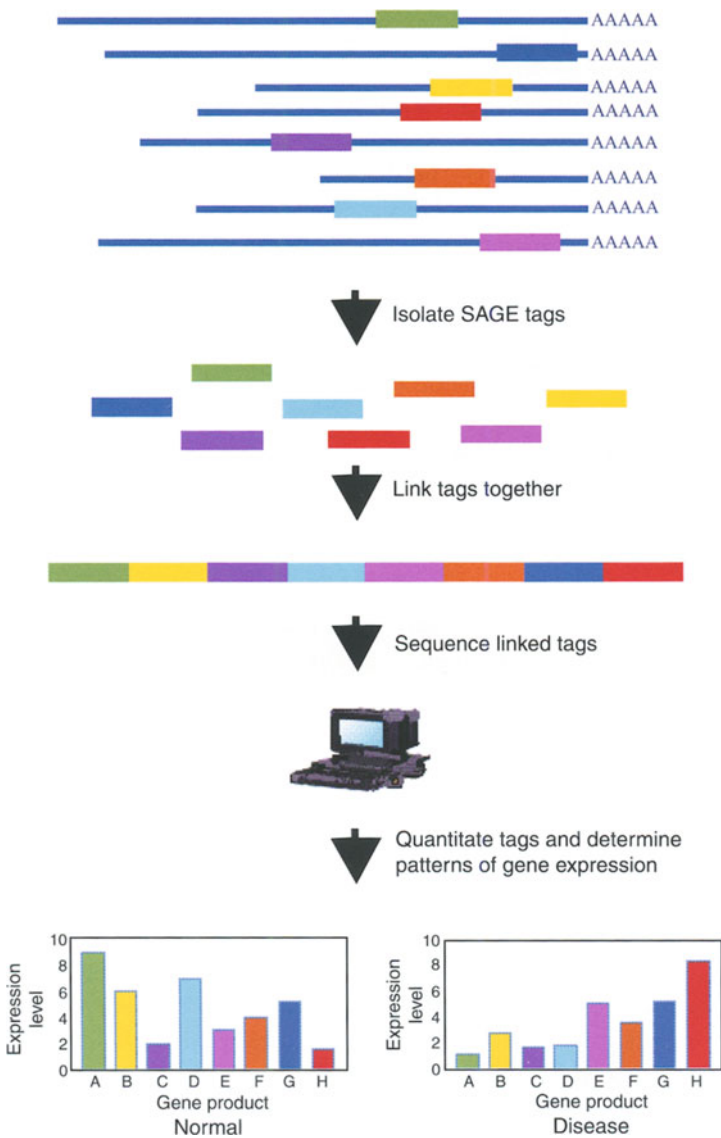


Fig. 1 Scheme of serial expression of gene expression (from <http://www.sagenet.org/findings/index.html>)

and numbered to deduce the quantitative expression of the original mRNA expression in a sample.

The first step of SAGE is extraction of the mRNA from a sample to be studied for its mRNA transcription levels (Fig. 1). The mRNA will be transcribed by the enzyme reverse transcriptase into cDNA (copyDNA), which is much more stable than mRNA. The total of the transcribed mRNA of a sample (e.g. tumour biopsy) is referred to as cDNA library as it comprises all transcribed sequences of a sample. Next, short sequence fragments (“tags”) of 26–28 basepairs are cleaved from the cDNA strands by restriction endonuclease enzymes cutting the cDNA at defined nucleotide motifs. The cut cDNA fragments are amplified by PCR and subsequently linked to each other to form one long string of “tags” (called “concatemer”). This chain of tags is then introduced into a vector to be cloned.

Cloning is the process of producing multiple copies of a defined DNA sequence. It allows amplification of DNA fragments. Basically, the isolated DNA fragment to be amplified (= “insert”; in case of SAGE the concatemer) will be ligated enzymatically into a plasmid, which is an extra chromosomal DNA molecule capable to replicate independently from the chromosomal DNA (Fig. 2). Next, the plasmid with the inserted cDNA will be introduced into bacteria (for example *E. coli*) for propagation. Since this process of transformation yields typically rather low efficiency, plasmids contain a selection marker conferring antibiotic resistance. Only bacteria successfully transformed with the plasmid will grow on media containing this antibiotic. After forming single colonies, bacteria will be picked and further cultured before finally the amplified DNA fragment will be extracted from the bacteria.

To identify and quantify the “tags” in the cloned concatemer it will be finally sequenced. *Sequencing* is a method to determine the order of nucleotides bases in a molecule of DNA. The first techniques for gene sequencing were introduced in the 1970s by 2D-chromatography or the wandering-spot method. Later, the chain-termination method developed by Sanger (who received the Nobel Price for its development) became the method of choice. The Sanger method is based on the use of dideoxynucleotide triphosphates (ddNTPs) as DNA chain terminators. The DNA sample to be sequenced is analysed in four separate sequencing reactions. For each reaction all four of the standard deoxynucleotides (dATP, dGTP, dCTP and dTTP) are added but only one of the four dideoxynucleotides (ddATP, ddGTP, ddCTP or ddTTP), which are the chain-terminating nucleotides. By addition of DNA polymerase DNA strand extension starts until incorporation of a modified nucleotide terminates DNA strand elongation resulting in DNA fragments of varying length. By gel electrophoresis running each of the four reactions in a separate lane the DNA bands can be visualized and the relative position of the DNA bands translates into the DNA sequence. In extension of the Sanger method, dye-terminator sequencing employs four different fluorescent dyes, which permits sequencing in a single reaction instead of running four reactions in parallel. The fluorescently labelled ddNTPs and

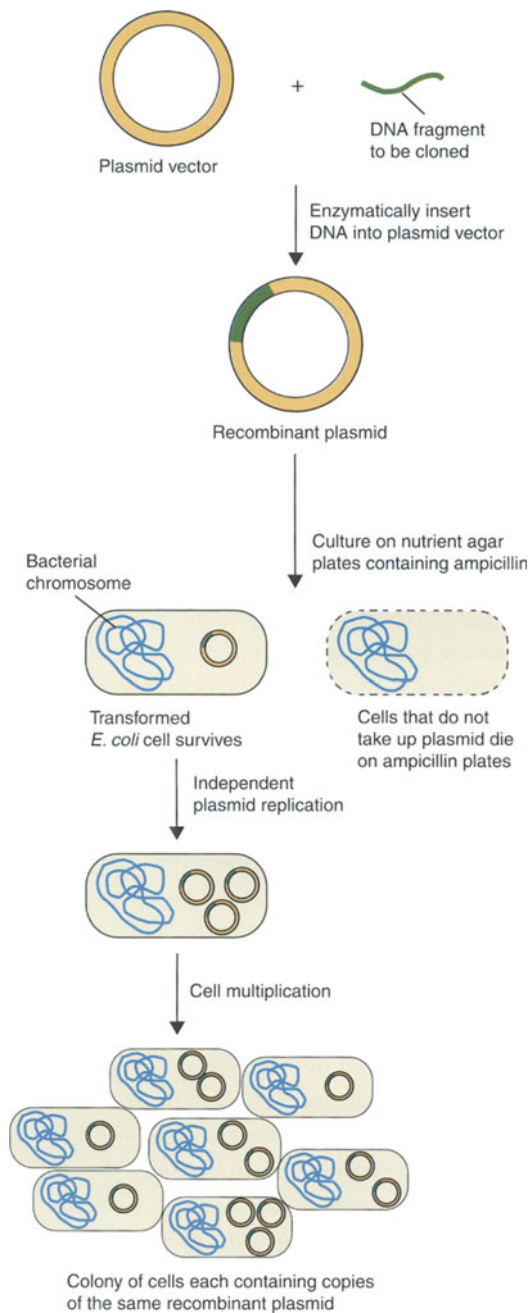


Fig. 2 Cloning of a DNA fragment into a plasmids (from <http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=mcb&part=A1582&rendertype=figure&id=A1590>)

primers paved the way for automated, high-throughput DNA sequencing device employed today.

After sequencing, the tags within the concatemer can be identified by their sequence and attributed quantitatively to their original gene. The sequence of the tags will be correlated by means of a “sequence-similarity search” to the original mRNA and gene, respectively. Due to the fact that SAGE is a sequence-based technique, it is more accurate for quantifying gene expression compared to the hybridization based DNA micro-arrays. At the downside, SAGE experiments are much more work-intensive than micro-array studies and therefore the latter are preferred for large scale screening studies.

2.2 At the protein level

Proteomic is the analysis of proteins starting from the systematic separation and identification of all proteins within a cell, tissue, or other biological sample. For drug research, proteomic techniques are employed for “target screening” as well as for “target deconvolution”.

In analogy to the DNA micro-arrays, *protein-arrays* have become available. Protein-arrays (also termed protein-chips) allow detecting antibody–antigen, protein–protein, protein–DNA or protein–small molecule interaction. There are different types of protein arrays. For “target screening” approaches protein lysates from a sample of interest (e.g. tumour biopsy) are putted on an “antibody micro-array” with a library of specific capture antibodies immobilized on its surface. Proteins in the probe will be captured by the spotted antibodies and subsequently labelled by a second fluorescence antibody for detection. Alternatively, the lysate probe itself can be spotted on the array and antibodies will be added. The binding reaction in these “reverse phase protein micro-arrays” is again detected by a fluorescently labelled second-step antibody. The beauty of the “reverse phase protein micro-arrays” is that they allow to test simultaneously many different lysates on a single chip. For target deconvolution, “antibody-arrays” performed from cells exposed to a drug candidate permit identification of protein signature profiles pinpointing to the potential mechanism of action in short time. Moreover, “reverse transfected cell micro-arrays” enable to evaluate in a high-throughput manner the interaction of proteins and drug candidates. Such micro-arrays are spotted with cDNAs from a library in expression vectors and overlaid with a transfection reagent and mammalian cells to generate a “living” micro-array. Cells are transfected with the spotted cDNA vectors and start expressing the respective proteins. Incubation with a labelled small molecule permits detection of cells interacting with the small molecule due to expression of the transfected protein. The beauty of the concept is that “Reverse transfected cell micro-arrays” allow screening proteins derived from any cDNA library without necessitating production of individual purified proteins.

A further method to identify proteins, their posttranslational modifications and interaction with other molecules is given by *mass-spectrometry*. Mass-spectrometry is an analytical technique based on measuring the mass-to-charge ratio of molecules. Biological samples often comprise a complex mixture of proteins requiring fractionation before analysis by mass spectroscopy. For this purpose, proteins are separated by two-dimensional gel electrophoresis (2DE) or by high performance liquid chromatography (HPLC) after enzymatic digestion. Such fractionated peptides are then assessed by mass-spectrometry such as MALDI-TOF (matrix-assisted laser desorption–ionization–time of flight mass spectrometry). A laser beam passes through the sample to be analysed and causes vaporization and ionization of the sample. Ionized molecules fly upward into a tube. “Time of flight” through the tube correlates directly to mass: with lighter molecules having a shorter time of flight than heavier ones. Mass-spectrometry is used for qualitative and quantitative analysis of proteins.

In order to study physical drug–target interaction expression-cloning-based techniques are of particular interest. Recombinant proteins are expressed from cDNA libraries and exposed to the drug candidate to identify proteins interfering with the drug candidate.

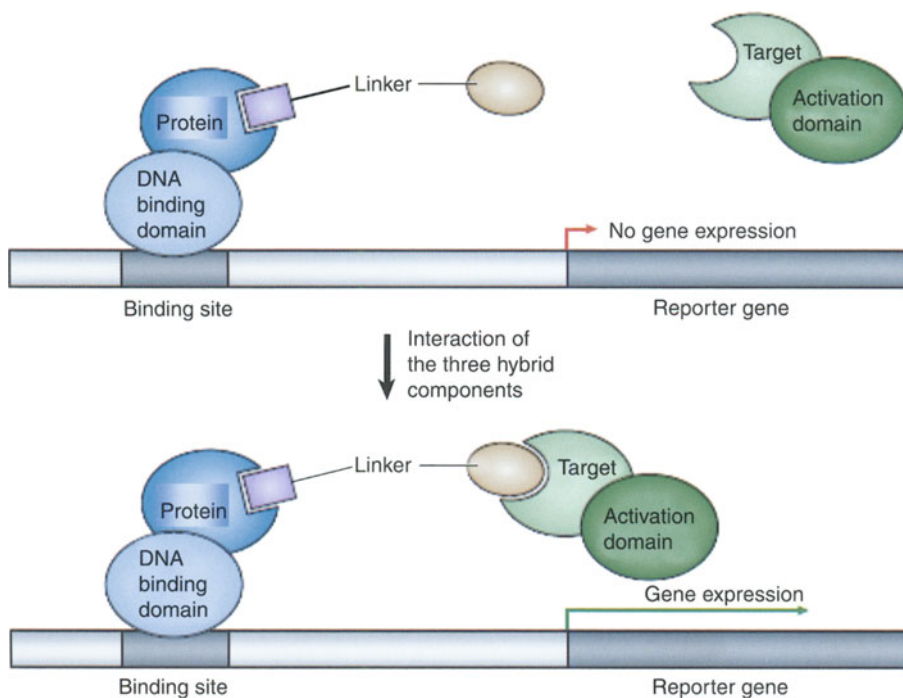


Fig. 3 Yeast three hybrid system (adapted from Ref. [1])

The *yeast two-hybrid* (Y2H) (see Fig. 3) is a molecular tool in biology to assess protein–protein or protein–DNA interactions. This technique was first designed to study protein–protein interaction in yeast (therefore “yeast” two-hybrid). In essence, it is based on the activation of a downstream reporter gene upon binding of a transcription factor to an upstream activating sequence. By splitting the transcription factor into a DNA binding domain and an activating domain, the interaction of a protein coupled to the activating domain (“prey”) and a protein coupled to the DNA binding domain (“bait”) brings the two fragments of the transcription factor in close proximity resulting in transcriptional activation of the reporter gene. For target deconvolution of drug candidates, the Y2H technique was adapted to a “yeast three-hybrid” technique by putting in between the two interacting proteins the drug candidate (“third hybrid”), which is coupled by a linker to the “bait” protein. Only if the target protein binds to the drug candidate reporter gene expression is activated.

Based on the reporter gene chosen a change in cellular phenotype is observed, allowing selection of cells expressing the protein interacting with the drug candidate. For generation of target “prey” proteins, random libraries or cDNA libraries (e.g. from a patient sample) are employed, which are ligated into plasmids to express the respective fusion protein in yeast. Y3H systems have successfully employed to deconvolute the targets of small molecule kinase inhibitors. However, Y2H (as well as Y3H) systems are prone to false positive results. Among other causes, this is due to overexpression of the fusion protein (= non-specific interactions) and the use of yeast as primitive organisms. To overcome, a mammalian cell based derivative was only recently introduced (MASPIT).

Phage display is another molecular biology technique to screen protein interactions drug discovery makes use of. Basically, cDNAs from a library are cloned into bacteriophages (“bacteria infecting viruses”) to produce fusion proteins of the bacteriophage coat protein and the proteins corresponding to the respective cDNAs. This phage display library is then exposed to the target molecules immobilized on a surface. Only phages (short for bacteriophages) expressing proteins interacting with the target molecules will be eluted by washing steps and transfected in bacteria for amplification. The cDNA of the enriched phage population will be isolated and sequenced to identify the target protein. Phage display have been primarily employed for antibody lead selection but are also particularly well suited to identify new ligands (e.g. receptor blocker or agonists, antibodies) for low-abundance proteins and targets of low-affinity ligands.

3 Molecular tools for target validation

Following target identification by “target screening” and “target deconvolution” it is essential for drug development of molecular targeting drugs to confirm that the target

identified is of functional relevance for the phenotype of the disease of interest. Despite thoughtful target identification strategies applied, it might turn out that the target identified is a false positive one, in that the target indeed is expressed in a given disease state but its modulation does not result in any change of phenotype (“epiphenomenon”).

To accomplish this task there are several molecular tools and model systems available. In principle, the identified molecular target will be either (over)expressed to promote the phenotype or down-regulated to suppress the phenotype of a disease with the aim to foster the causal relationship between the molecular target and the disease.

3.1 Target (over)expression

The idea of *transfection* studies is to transform a well characterized mammalian cell line to express the gene of interest. The phenotype of the generated new cell clone will be evaluated in functional assays in comparison to the original cell line and any change in phenotype will be attributed to expression the gene of interest. For successful transfection experiments it is essential to bring as much genetic copies of the recombinant DNA in as many cells as possible in a way that preferably many cells “survive” the transfection procedures.

To introduce a transgene into cells typically plasmids will be employed as vectors. As described above, plasmids are extra-chromosomal DNA molecules, which are separated from the chromosomal DNA and can therefore replicate autonomously. The gene of interest will be inserted into the plasmid at the so called “Multiple Cloning Site”, which consists of restriction sites allowing enzymatic cleavage of the plasmid, insertion of the transgene and re-ligation of the plasmid with the inserted cDNA encoding for the gene of interest. For replication, vectors have an “origin of replication” (= sequence of DNA capable of directing the propagation of itself and any linked sequence) for initiating semi-independent replication of the plasmid in host cells.

Mammalian cells are protected by their cell membrane from potential environmental risks such as foreign DNA. In order to surmount the lipid bilayer and facilitating the uptake of DNA into the host cell a number of different transfection methods are available in molecular biology. The “classical” approach of transfection is based on a transient increase in the permeability of the cell membrane. Calcium phosphate forms precipitates with DNA, which are then taken-up by cells. Cationic lipids and polymers bind the negatively charged DNA and cellular uptake is mediated by fusion of the complex with the cell membrane or endocytosis, respectively.

The most efficient and reliable approach for transfection of mammalian cells is by the use viral vectors. Formally, the transfer of DNA into mammalian cells by use of viral vectors is referred to as “transduction” in contrast to the chemical based methods mentioned above. Viral mediated transduction strategies consist of a recombinant viral

vector/plasmid with the inserted gene of interest, which is transduced into a complementing packaging cell line to produce infectious virions (= extracellular virus particle). The virions are harvested and employed to transduce the target cells. The resembling of the naturally way of virus infection leads to high yields with near to 100% of transduced target cells. Whereas adenovirus based system result in transient transduction of cells, retro- and lentiviral systems are integrated into the DNA of the target cells leading to stable expression of the insert in dividing mammalian target cells. For transduction of non-dividing and other cells not easily to transfect, lentiviral systems are considered to be the most promising vectors for. To avoid any uncontrolled virus spread viral transduction systems typically employ only replication incompetent viruses lacking essential genes necessary for generation of structural proteins. Still, the conduct of viral transfection experiments requires biosafety level II laboratory facilities. Given the considerable advantages of viral transfection systems they have become the standard for target validation studies *in vitro*.

3.2 Target downregulation

A point of criticism inherent to any target overexpression strategy for validation experiments arise about the question whether a target gene overexpressed in a mammalian cell line properly reflects the biological function of the endogenously expressed gene. Overexpression of a transgene might overwrite the fine-tuned balance of intracellular signalling leading to a phenotype aberrant from the one caused by expression of the endogenous target gene. Not only, but also for this reason the reverse strategy of target downregulation is often employed for target validation. By targeted suppression of the gene of interest the resulting phenotype can be attributed to the gene's biological function.

For *in vitro* experiments, the most versatile tool for target silencing is given by *RNA interference*. RNA interference is a naturally existing cellular mechanism of post transcriptional gene silencing. Mello and Fire published in 1998 that gene specific silencing may be achieved by double-stranded RNA complementary to the sequence of the target gene leading to degradation of the target mRNA by induction of an endogenous RNA induced silencing complex (RISC; for details see Chapter 20). Degradation of the target mRNA deprives the cell of the template for its protein translation machinery. Thus, RNA interference allows gene specific silencing of virtually any gene of interest. For target validation *in vitro*, the RNA interference mechanism may be induced by two different technical approaches.

siRNA (= small or short interfering RNA) are short double-stranded RNA fragments, which are introduced intracellular by transfection. Transfection of a cell with siRNA leads to activation of the RISC and subsequent target-mRNA degradation. siRNA experiments are easy to perform straight forward experiments for target validation, but they result only in transient downregulation of target mRNA.

For long-term target silencing RNA interference may be induced by introducing a vector into cells encoding a “*small hairpin RNA*” (sh-RNA). sh-RNA is a RNA sequence of about 70 nucleotides consisting of a sense – and a complementary antisense strand, which are connected by a short sequence of nucleotides. Since the sense- and the antisense strand tend to form a double strand by complementary base pairing with a loop at the top formed by the nucleotide sequence connecting the both strands the construct resembles the shape of a hairpin. The transcribed sh-RNA is exported from the nucleus to the cytoplasm where it is sliced by an enzyme called dicer into siRNA fragments, which then in turn activate the RISC for target mRNA degradation. RNA interference by siRNA is currently mainly limited to *in vitro* experiments since the delivery of siRNA molecules *in vivo* has not yet been adequately addressed to obtain reliable target silencing *in vivo* (despite in the liver).

An alternative strategy for studying the biological relevance of a target gene is the use of *knock-out* animals. Knock-out animals (typically mice) are genetically modified

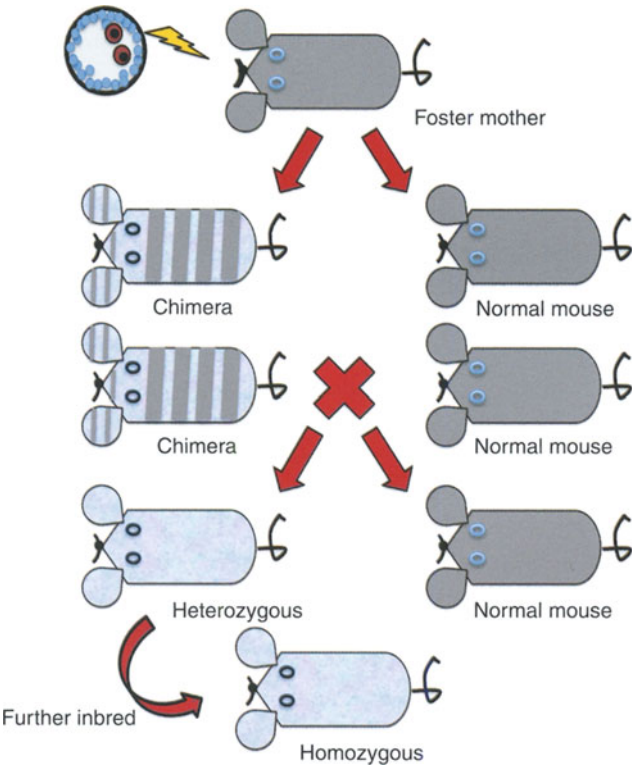


Fig. 4 Generation of knock-out mice

animals (GMA) in which one or multiple genes are deactivated by genetic manipulation of the animal's germ line. Embryonic stem cells are isolated from a mouse-blastocyst and propagated *in vitro*. By microinjection with a tiny glass pipette a DNA sequence is introduced into the nucleus of an embryonic stem cell. The introduced DNA sequence is highly similar (homologous) to the target gene sequence, except that by mutations the sequence is coding for a non-functional copy of the target gene. In some embryonic stem cells this nucleotide sequence is incorporated into the DNA by replacing the original copy of the gene (= homologous recombination). Following selection the successfully transformed embryonic stem cells are inserted into a mouse blastocyst and implanted into a uterus of a female mouse (see Fig. 4). The offspring of this foster mouse have a "mixed" genotype (= chimera) since they carry both, the original "wild-type" gene from the stem cell of the foster mouse and the "knock-out" gene from the engineered stem cells. By back-crossing these chimeric mice with wild-type mice heterozygous animals are obtained (= two different alleles of the gene of interest). Further inbred of these heterozygous mice results in homozygous knock-out mice with no copy of the functional gene of interest.

Since the first knock-out mouse models created in 1989, there are in the meantime several thousand strains of knock-out mice available for studies of diseases such as cancer, metabolic- or neurologic disorders (see <http://www.knockoutmouse.org>). Knock-out mice have become a valuable and reliable tool for validating the functional relevance of a gene of interest. Even simultaneous knock-out of two (= double knock-out) or three (= triple knock-out) genes and allele-specific knock-out to create homo- and heterozygous mice is feasible for studying the interaction and functional dependence of genes.

In comparison to gene silencing by sh-RNA, knock-out animals are in general more appropriate to study the phenotype of a complete suppression of a target gene ("knock-out"). In contrast, animals with gradual expression of sh-RNA constructs allow studying quantitative target-effect relationship for drawing conclusions about the degree of inhibition necessary for influencing the disease phenotype. Despite the attractiveness of knock-out models, it needs to be emphasized that like overexpression also gene silencing/knock-out provides only an approximation to the human situation. There are examples in the literature where the phenotype of a knock-out mouse displayed different characteristics relative to the loss of function of the same gene in humans (e.g. p53 knock-out mice), underlining the genetic inter-species differences between mouse and men. A further limitation of knock-out models stems from the fact that a substantial number of genes are of critical importance during embryonic development. In about 15% of all cases gene knock-out causes embryonic lethality of the transformed embryo. Since genes may serve different functions during embryogenesis and in adult animals, such gene might still be relevant for the phenotype of a human disease in adult mice but knock-out models are under such circumstances no way to validate this gene as drug target.

3.3 Conditional target regulation

In order to circumvent the limitation of constitutive knock-out animals with permanent gene knock-out animal model with conditional target regulation have been developed. Conditional target regulation facilitates expression or knock-down of a target gene limited to restricted tissues or only during a specific timeframe by employing tissue specific promoter and inducible systems, respectively. According to the course of a disease, a gene of interest can be switched off and on any time allowing dynamic studies for the biological relevance of the target over the course of disease.

In this context, the *Cre/lox system* is widely used. The Cre-lox system is a site specific recombination system for enzyme mediated cleavage and ligation at defined DNA sequences. It consists of the Cre (which stands for “Cyclization Recombination”) recombinase protein and the loxP (“locus of X-over P1”) recombination sites containing a binding site for Cre. To knock-out a gene of interest, loxP sites are inserted in the DNA sequence before and after the sequence encoding the gene of interest (also referred to as “to flox a gene”). The Cre recombinase binds to the inserted loxP sites, cuts out the double strand between the two loxP sites and recombines the DNA ends at the loxP sites. This will result in a deletion of the gene of interest flanked by the loxP sites. For animal models, Cre protein and loxP sites are introduced separately as transgenes in mice. The offspring of these mice express then a complete functional Cre/lox system.

For conditional target regulation the expression of the Cre protein can be set under control of a tissue specific promoter. A tissue specific promoter expresses the genes under its control only in defined tissues. For instance, Cre protein under control of liver specific promoter allows the knock-down of a floxed target gene only in the liver of an animal. Likewise, tissue specific promoters are also employed to express a gene in a tissue restricted fashion to evaluate the “overexpression” phenotype of a gene of interest.

For time restricted expression of a gene of interest the Cre/lox systems has been adapted. The Cre protein was fused with a modified ligand binding domains of the estrogen receptor, which is no more activated by endogenous estrogen but by the selective estrogen modulator tamoxifen. By feeding animals with tamoxifen, tamoxifen binds to the modified estrogen receptor and induces Cre protein expression leading to deletion of the “floxed” target gene. Upon stop of tamoxifen administration the knock-out phenotype can be reverted.

Another approach for conditional target expression is given by the *tetracycline controlled transcriptional* (Tet) activation system. Expression of the gene of interest is controlled by tetracycline dependent promoters, which upon presence of externally administered tetracycline (or doxycycline as the more stable tetracycline analogue) either induce target gene expression (Tet-On) or indirectly repress target gene expression (Tet-Off). Relative to (inducible) Cre/lox systems, the Tet system enables

tighter control of target gene expression but requires more time before change in target expression due to induction/set-off becomes effective.

4 Molecular tools for monitoring pharmacodynamics in translational studies

After successful target identification/deconvolution and validation, a drug candidate undergoes GMP production and toxicology testing before it is allowed to be studied finally in first in men clinical trials. Given a molecule with a defined mechanism of action it is of outstanding importance in these early clinical trials to learn, beyond clinical tolerability, whether the postulated mechanism of action “translates” into the human setting (“bench-to-bedside”). In such “proof-of-concept” studies the changes induced by the drug candidate on the molecular level of the disease needs to be closely monitored by pharmacodynamic analyses and correlated with the pharmacokinetics of the drug candidate (PK/PD studies). Pharmacodynamic “biomarker” will be measured in samples from the tissue involved in the pathogenic process or from a surrogate matrix (e.g. normal skin, blood, urine) before and after treatment. Target modulation as an effect of the drug candidate administered will be assessed by molecular analysis. For monitoring pharmacodynamic biomarkers, a wide range of molecular tools for mRNA-, protein-analyses, and functional assays is available. Depending on the molecular target addressed and the accessibility of the target tissue either the direct – (e.g. down-regulation or phosphorylation of the target) or an indirect effect on the molecular target (“downstream” effect) will be measured.

The molecular tools applied for monitoring pharmacodynamics in translational studies are greatly overlapping with the ones employed for target identification and validation. However, in contrast to the – omic approaches used for target identification/deconvolution the techniques applied for biomarker assessment in clinical trials are more focused (i.e. target-orientated), not at least due to the demanding standards required for biomarker assay validation.

At the nucleotide level, *fluorescence in-situ-hybridization* (FISH) is a standard molecular tool to detect DNA or mRNA in cells or tissue samples. FISH is based on binding of a labelled DNA oligonucleotide (“probe”) complementary to the DNA or mRNA sequence of interest. Following denaturation of the DNA in the sample by heating the “probe” binds to the target DNA and is detected by its fluorescent label with fluorescence microscopy.

While FISH allows qualitative analysis, quantitative analysis of mRNA expression may be accomplished by *Northern blotting*. The technique of Northern blotting was first described in 1977 as a derivative of southern blotting used for DNA detection. Following extraction of mRNA from a sample, RNA's are separated by size via gel electrophoresis. Next, RNA samples are transferred to a nylon membrane (“blotting”) and fixed on the

surface of the membrane by heat or UV light. For detection of specific mRNA, a labelled probe (radiolabelled or chemoluminescence) with an oligonucleotide sequence complementary to the mRNA of interest will be added for hybridization. The binding of the labelled probe is visualized on a X-ray film and can be quantified by densitometry. Northern blotting is characterized by a high specificity but a low sensitivity and a somewhat poor performance for quantitative analysis of mRNA.

In contrast, the *polymerase chain reaction* (PCR) is a highly sensitive molecular method for detection of mRNA in a patient sample. PCR multiplies the mRNA of interest by repeated amplification cycles allowing also detection of low abundant mRNA. Basically, mRNA of a biological sample is first reversely transcribed in more stable cDNA. Short sequences of DNA (so called “primers”) hybridize to defined sequences of the cDNA of interest and initiate with an added polymerase selective extension and amplification of the cDNA of interest in cycles of repeated heating and cooling (“chain reaction”). For quantification of cDNA copies generated by PCR, real-time PCR (RT-PCR) intercalates fluorescent dyes in the PCR products so that the intensity of fluorescence detected directly correlates with the relative number of cDNA copies generated. RT-PCR (also coined q(uantitative)PCR) has become the most efficient tool for mRNA quantification in translational research studies.

While the transcriptome is by nature always only the “intention of protein expression”, measuring target modulation at the protein level is considered to provide more reliable data in “proof-of-concept” studies. For assessment of protein based biomarkers there are several molecular methods available.

To analyse target protein expression in patient tissue samples, immunohistochemistry (IHC) is a well-established technique. Tissue slices are exposed to antibodies specific for the target of interest. Binding of the primary antibody to the antigen of interest is detected by a labelled secondary antibody, which reacts with the primary antibody. The secondary antibody is coupled with an enzyme catalyzing the reaction with a chromogenic substrate for visualization. The convenience of IHC for translational studies is given by the fact that it allows protein biomarker assessment of cells within their histological context. For multiplex histological analysis, “tissue microarray” (TMA) have been introduced only recently, allowing to assess simultaneously up to 1000 small tissue sample cores embedded in a single paraffin block instead of staining all samples on separate slides. At the downside, quantification of IHC is observer dependent, requires skilled pathologists and is complex to standardize. Typically, results are presented as a mixed score consisting of the percentage of cells positive and gradual staining intensity for the biomarker in a limited number of regions of the sample. To overcome the observer and selection bias, digital tissue analysis system have been developed enabling automated acquisition and subsequent quantification of IHC stained sections (“tissue FACS”).

For quantitative analysis of protein expression, *Western blotting* has been the molecular standard tool for many years. In contrast to Northern blotting, proteins

(not mRNA) are separated for size and charge by gel electrophoresis. Following transfer to a membrane (“blotting”) the protein of interest are detected by specific antibodies. Binding of the specific antibody (“primary antibody”) is visualized by a second step antibody binding to the primary antibody and catalyzing a “reporter reaction” (e.g. chemiluminescence or colorimetric reaction). This reporter reaction is detected as bands on an X-ray film whereby the intensity of the band correlates with the amount of protein in the sample. Western blotting as a standard laboratory tool is characterized by a satisfying specificity but it is considered as a semi-quantitative technique only and therefore only rarely employed as a biomarker assay for drug development.

Fluorescence-activated cell sorting (FACS) or flow cytometry enables quantitative detection of multiple proteins of individual cells in a standardized manner. In principle, while cells pass through a laser beam in a stream of fluid, the cells and their capacity to bind fluorescently labelled antibodies specific for the proteins of interest is quantified by detecting the scattering of light and the fluorescence excited by the light of the laser beam. Flow-cytometry is suited for all type of samples where cellular protein expression in cell suspensions is of interest (e.g. peripheral blood mononuclear cells). On a cautionary note, the specificity of FACS is purely dependent on the specificity of the antibodies employed, which needs to be validated carefully before a FACS assay may be employed as biomarker for decision making in translational studies.

To measure non-cellular protein expression in body fluids *Enzyme-linked Immunosorbent Assay* (ELISA) is employed. ELISA rest upon detection of antigens present in fluids such as serum, urine or cerebrospinal fluid by binding to specific antibodies. In a standard “sandwich” ELISA, specific antibodies immobilized on a surface of a microtiter plate capture the protein of interest in the sample fluid. After washing the plate for removing unbound proteins, a second antibody specific for the protein of interest is added, which is linked to an enzyme reacting with a subsequently added substrate for detection (e.g. horseradish peroxidase or alkaline phosphatase). ELISA might also reversely be designed to detect antibodies fluids by exposing the corresponding antigen in the assay or to detect secretory products of activated immune cells cultured in microtiter plates (i.e. ELISPOT). The most recent progress in ELISA development allows simultaneous detection of multiple antigen within one sample (up to 100 antigens) in so called Multiplex ELISA (= Luminex). These assays make use of colour coded beads (“microspheres”) for labelling the individual reactions taking place simultaneously in the same well.

In addition to this general set of molecular tools outlined here, a multitude of techniques fitting the needs of particular research fields are currently emerging, which have the potential to impact substantially future drug research (for instance “circulating tumour cells” as “liquid biopsy” in oncology [2] or “circulating endothelial progenitor cells” for angiogenesis research [3]).

Case Study: Re-translational studies – the case of ACE2

As mentioned in the introduction, translational research may not be seen exclusively as a “one-way process” bringing basic research success stories into clinical trials but also as the inverse process by inspiring basic research with novel clinical relevant information for hypothesis forming and testing. A recent example illustrating this fruitful circle of “bedside-to-bench-to-bedside” in translational research is the development of the Angiotensin converting enzyme 2 (ACE2) recombinant protein currently in clinical testing.

The angiotensin converting enzyme (ACE) is part of the Renin-Angiotensin-Aldosterone-System (RAAS). It converts angiotensin I to angiotensin II and plays an important role in regulation of the cardiovascular system including blood pressure, blood volume and serum electrolytes. After its first description in 1956 [4] and the deciphering of its functional role in the 70s of the last century [5], ACE inhibitors blocking the enzymatic activity of ACE are in daily clinical use since 1981.

In 2000 it was published that there is a second ACE gene (ACE2) [6]. This human homologue was identified as a result of a re-translational research effort. From the explant of a female patient undergoing heart transplantation due to dilated cardiomyopathy tissue samples were collected. mRNA was extracted from the explanted cardiac left ventricle and reversely transcribed in cDNA. From this cDNA, a human cardiac left ventricle cDNA library was prepared by using standard cloning techniques. A total of 19,000 different clones were generated and sequenced by high throughput methods. The nucleotide sequences obtained were checked for homology with any already known gene sequence by means of a “sequence-similarity search”. Among the 19,000 clones, a so far unknown gene sequence was identified showing 42% identity with the amino domain of the known ACE gene. In order to study the tissue distribution of this novel ACE2 gene, 23 different human tissue types were evaluated by northern blotting with ACE2 specific probes. ACE2 mRNA tissue expression differed from ACE expression with preferential expression in heart, kidney, testis and at a lower level in the colon and the lung. To define cellular and subcellular localization of ACE2 expression, immunohistochemical examination of human ventricular myocardium with an ACE2 specific antibody was performed. ACE2 protein expression was found to be localized to the endothelium of most intramyocardial vessels including capillaries and venules. In order to study the functional role of ACE2 expression, mammalian cells were transfected transiently with a vector encoding the human ACE2 gene. There was no apparent difference in phenotype of transfected and non-

transfected control cells. However, mass-spectrometry revealed that only in the supernatant of cells transfected with ACE2 Angiotensin I was converted into Angiotensin 1–9 and Angiotensin II into Angiotensin 1–7. This effect could not be inhibited by addition of the ACE inhibitor lisinopril. Thus, ACE2 differed in its enzymatic activity from ACE.

However, at this point in time the biological role of ACE2 was still unknown. To gain insight into the biological relevance of ACE2 a knock-out mouse was created [7]. Disruption of the murine ACE2 gene resulted in increased levels of Angiotensin II and progressive worsening of cardiac contractility with age. It turned out that ACE2 apparently counterbalance the function of ACE within the local RAAS. While ACE increases Angiotensin II level promoting diseases such as cardiomyopathy, ACE2 degrades Angiotensin II to Angiotensin 1–7 protecting from cardiomyopathy. The counterbalancing effect of ACE2 is also observed in other organs like the kidney. Deletion of ACE2 in Akita mice, a mouse model with a mutation causing a diabetic phenotype, leads to spontaneous onset of nephrotic glomerulonephritis and diabetic kidney injury [8].

A crucial role of ACE2 in the respiratory system emerged by the discovery that the SARS virus is able to bind to ACE2. In an elegant study it was shown that ACE2 is essential for SARS infections *in vivo*. ACE2 knock-out mice infected with SARS were protected from SARS virus replication in the lung [9]. At the same time, SARS virus replicating in lungs of normal wild-type mice leads to down-regulation of ACE2 protein expression in the lungs.

The SARS virus is one of many potential inducers of the most serious form of acute lung injury, the acute respiratory distress syndrome (ARDS). ACE2 knock-out mice suffering from acute lung injury induced by different stimuli show more severe symptoms in form of enhanced vascular permeability, increased lung edema, neutrophil accumulation and worsened lung function [10]. Of note, ACE2 is able to counteract these effects by playing a protective role in acute lung injury with therapeutic potential. In mice with acute lung injury, treatment with recombinant ACE2 protein improved the symptoms of acute lung injury [10]. This was observed in wild-type as well as in ACE2 knock-out mice.

These multiple lines of evidence foster the validity of ACE2 as a promising molecular target for treating acute lung injury by recombinant ACE2 and provided the rationale for translating the concept into clinical testing. Only recently, a Phase I study in healthy volunteers was announced to be completed for clinical developing of recombinant human ACE2 as an enzyme biotherapeutic in patients with ARDS (<http://www.apeiron-biologics.com>).

References

1. Terstappen GC, Schlupen C, Raggiaschi R, Gaviraghi G (2007) Target deconvolution strategies in drug discovery. *Nat Rev Drug Discov* 6: 891–903
2. Pantel K, Alix-Panabieres C, Riethdorf S (2009) Cancer micrometastases. *Nat Rev Clin Oncol* 6: 339–351
3. Bertolini F, Mancuso P, Shaked Y, Kerbel RS (2007) Molecular and cellular biomarkers for angiogenesis in clinical oncology. *Drug Discov Today* 12: 806–812
4. Skeggs LT Jr, Kahn JR, Shumway NP (1956) The preparation and function of the hypertensin-converting enzyme. *J Exp Med* 103: 295–299
5. Ferreira SH, Greene LH, Alabaster VA, Bakhle YS, Vane JR (1970) Activity of various fractions of bradykinin potentiating factor against angiotensin I converting enzyme. *Nature* 225: 379–380
6. Donoghue M, Hsieh F, Baronas E, et al. (2000) A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1–9. *Circ Res* 87: E1–E9
7. Crackower MA, Sarao R, Oudit GY, et al. (2002) Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature* 417: 822–828
8. Wong DW, Oudit GY, Reich H, et al. (2007) Loss of angiotensin-converting enzyme-2 (ACE2) accelerates diabetic kidney injury. *Am J Pathol* 171: 438–451
9. Kuba K, Imai Y, Rao S, et al. (2005) A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat Med* 11: 875–879
10. Imai Y, Kuba K, Rao S, et al. (2005) Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* 436: 112–116

SECTION 4

Topics in Clinical Pharmacology

CHAPTER 18

Pharmaceutical drug safety

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Summary

The occurrence of sometimes life-threatening adverse drug reactions (ADRs) jeopardizes patients' health during drug treatment and additionally imposes an increased financial burden on the healthcare system. The withdrawal of already marketed drugs because of ADRs furthermore erodes public confidence in the way drugs are approved. In this chapter the development and current status of drug monitoring systems will be discussed and the future of pharmacovigilance, the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other medicine-related problem will be outlined.

1 Introduction

We are fortunate to live in an era in which diverse diseases can be treated and cured with an increasing armamentarium of therapies, including drug treatment. The downside of the development and availability of new medicines, however, is the risk of experiencing adverse reactions to those drugs. Adverse drug reactions (ADR) are a common, though preventable cause of illness and are defined as “a response to a medicine which is noxious and unintended, and which occurs at doses normally used in man” (Table 1) [1]. In most instances ADRs are of relatively mild intensity, and disappear when the drug is discontinued or the dose is changed. In ~5% of therapeutic drug courses, however, ADRs complicate medical treatment and require admission to a hospital [2]. In a meta-analysis of 39 prospective studies from hospitals in the United States it was even estimated that more than 100,000 deaths can be attributed annually to serious ADRs and it

Keywords: Adverse drug reaction (ADR), pharmacovigilance, drug safety, spontaneous reporting systems, risk management, periodic safety update report (PSUR), drug withdrawal, causality assessment, cerivastatin, micafungin

Table 1 Glossary of adverse drug reaction terms (modified from Refs. [1, 21])

| | |
|--|---|
| Adverse drug reaction (ADR), suspected adverse (drug) reaction | A response to a medicinal product which is noxious and unintended and which occurs at doses normally used in man for the prophylaxis, diagnosis or therapy of disease or for the restoration, correction or modification of physiological function. Response means that a casual relationship between a medicinal product and an adverse event is at least a reasonable possibility. ADR also includes adverse clinical consequences associated with use of the product outside the terms of the Summary of Product Characteristics or other conditions laid down for the marketing and use of the product (including prescribed doses higher than those recommended, overdose or abuse). |
| Serious adverse reaction | ADR which results in death, is life-threatening, requires in-patient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability or incapacity, or is a congenital anomaly/birth defect. |
| Unexpected adverse reaction | ADR, the nature, severity or outcome of which is not consistent with domestic labelling or market authorization, or expected from characteristics of the drug. |
| Adverse event or adverse experience | Any untoward medical occurrence that may present during treatment with a medicine but which does not necessarily have a causal relationship with this treatment. The basic point here is the coincidence in time without any suspicion of a causal relationship. |
| Side-effect | Any unintended effect of a pharmaceutical product occurring at doses normally used by a patient which is related to the pharmacological properties of the drug. |
| Signal | Reported information on a possible causal relation between an adverse event and a drug, the relation being previously unknown or incompletely documented. Usually more than a single report is required to generate a signal, depending on the seriousness of the event and the quality of the information. |

was concluded that ADRs rank from the fourth to sixth leading cause of death [3]. A more recent study in England ascertained the current burden of ADRs through a prospective analysis of all hospital admissions [4]. It could be shown that at any time the equivalent of up to seven 800 bed hospitals may be occupied by patients admitted with ADRs [4]. Besides the impact on the individual's health status, ADRs thus impose a high financial burden on the healthcare system. Some countries spend up to 15–20% of their hospital budget dealing with drug complications [5] with high costs also in the ambulatory setting [6]. These direct costs should be added to the indirect costs such as loss of productivity.

No drug is completely safe and it is recognized that most of the deleterious effects of a drug remain unknown until the product is marketed. However, this fact is not intuitively comprehensible to the patients and healthcare practitioners who demand safe and effective drugs. They also expect that correct prescription and directed use of medications result in beneficial effects without significant harm.

In the middle of the 20th century information about drug related problems was often only available from publications in the medical literature. Experiencing the thalidomide disaster – as exemplified in detail below – was necessary to trigger the development of today's drug monitoring systems to capture drug effects, both intended and unwanted, so that good evidence is available upon which an assessment of risk versus effectiveness or benefit can be made and unexpected adverse reactions and their risk factors can be early identified. Since the early 1990s – pharmacovigilance, an umbrella term used to describe the processes for monitoring and evaluating ADRs – has become a key component of effective drug regulation systems, clinical practice and public health programmes [7]. The World Health Organization (WHO) defines pharmacovigilance as the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other medicine-related problem [7].

2 Thalidomide – a disaster as starting point for the methodical assessment of drug safety

Thalidomide became known to a wide public in the context of one of the biggest drug disasters in recent history. Thalidomide, also known as Contergan[®], was introduced to the market in 1957. It was marketed as a “nontoxic” hypnotic and antiemetic for morning sickness during pregnancy. By the end of the 1950s thalidomide was widely prescribed under at least 37 names worldwide [8]. In 1961, reports were published suggesting that thalidomide was responsible for a dramatic increase in the incidence of a rare birth defect called phocomelia, a condition involving shortening or complete absence of limbs. Epidemiological studies provided strong evidence for the association of this birth defect with thalidomide use by women during the first trimester of pregnancy, whereby as little as a single dose of thalidomide was sufficient for the teratogenic effect. Consequently, the drug was withdrawn from the market in 1961. It is estimated that about 10,000 infants worldwide, approximately half of them in Germany, were affected, from which approximately half survived severely disabled. In the United States, the Food and Drug Administration (FDA) did not approve the drug due to safety concerns. Today, despite its disastrous toxicity in pregnancy, thalidomide is regarded as a relatively safe drug for humans other than the fetus and it is now allowed by the FDA for limited use as a treatment for cancer and inflammatory diseases. Its history serves as a lesson in drug development that underscores the need to understand a compound's activity as well as its toxicity [8].

There are several reasons, why such tragic events could occur. In the 1950s in Germany, there were no guidelines and legal foundations regulating development, production and marketing of medicinal products. Therefore it was possible to register thalidomide without any governmental review of the existing documentation. Furthermore, testing for harmful teratogenic effects was not standard practice at that time and

also seemed not indicated as pharmacological and toxicological investigations carried out in rodents – only rats were tested – revealed no sign of any risk [9].

As a consequence of the thalidomide disaster, it was widely acknowledged that the basis for the authorization of new medicines was insufficient. First measures to ensure risk minimization in connection with the licensing of newly developed pharmaceuticals were the development of spontaneous reporting systems and legislation in Europe (EC Directive 65/65), such as the UK's "Yellow Card" system. In the United States, the FDA initiated important reforms, including giving it the power to require that a manufacturer demonstrates efficacy before a new drug can be marketed [10].

3 During drug development only frequent ADRs can be detected

In order to be authorized to market a new drug, a company has to apply to a regulatory authority. In Europe, this is the European Medicines Agency (EMA); the equivalent in the United States is the FDA. For an informed decision, the agencies rely on the collection of data from preclinical studies and clinical studies, which are performed in 3 study phases. Altogether less than 5000 subjects will be exposed to the new drug, most of them not reflecting the population in which the drug will be marketed after approval. Two or more confirmatory trials are required that demonstrate before marketing that a drug is effective and reasonably safe for its recommended use. With this system, ADRs occurring with a frequency of >1% are usually captured and described before marketing, whereas rarer ADRs might fail to be detected. In the recent Institute of Medicine report it was noted that "... a drug's risk-benefit profile necessarily evolves over the drug's life cycle" [11].

In their publication "Safety of Medicines – A guide to detecting and reporting adverse drug reactions", the WHO summarizes the main issues that complicate the detection of less common, but sometimes very serious ADRs during drug development [1]:

- Tests in animals are insufficient to predict human safety;
- Patients used in clinical trials are selected and limited in number, the conditions of use differ from those in clinical practice and the duration of trials is limited;
- By the time of licensing exposure of less than 5000 human subjects to a drug allows only the more common ADR to be detected;
- At least 30,000 people need to be treated with a drug to be sure that you do not miss at least one patient with an ADR which has an incidence of 1 in 10,000 exposed individuals;
- Information about rare but serious adverse reactions, chronic toxicity, use in special groups (such as children, the elderly or pregnant women) or drug interactions is often incomplete or not available.

4 ADR reporting and worldwide pharmacovigilance

As exemplified by the WHO, pre-marketing trials do not usually allow identifying ADRs with a low frequency due to the low number of participating subjects. Furthermore, the comparably short duration of clinical trials makes it difficult to detect ADRs with a long latency and the characteristics of study populations do not readily correspond to the characteristics of the patients, who will receive the drug after approval. Consequently, it is essential that new and medically still evolving treatments are monitored for efficacy and safety under real life conditions especially in combination with other drugs post-marketing (i.e. in phase 4 of drug development).

In the aftermaths of the thaloidomide disaster the first systems for reporting ADRs had been created and introduced. One example, the Yellow Card Scheme in the UK, was established in 1964 and permits any suspected ADRs to be reported to the UK Medicines and Healthcare products Regulatory Agency (MHRA). These reports are then stored in the MHRA sentinel database [12]. The FDA operates a similar scheme, in which reports are stored in the Adverse Event Reporting System (AERS) database or the Vaccine Adverse Events Reporting System (VAERS). In these systems, healthcare professionals and patients are asked to report ADRs to regulatory authorities, and the pharmaceutical industry is obliged to submit reports of clinically serious reactions [13].

International collaboration is the basis for the WHO International Drug Monitoring Programme, which was established in 1968 and provides a forum for WHO member states to collaborate in the monitoring of drug safety. Within the Programme, individual case reports of suspected adverse drug reactions are collected and stored in a common database, presently containing over 4.7 million case reports. In each of the countries participating in the Programme, the government has designated a National Centre for pharmacovigilance. The WHO Programme consists of a network of the National Centres, WHO Headquarters, Geneva and the WHO Collaborating Centre for International Drug Monitoring, the Uppsala Monitoring Centre, in Uppsala, Sweden. As of September 2009, 96 countries had joined the WHO Drug Monitoring Programme, and in addition, 30 “associate members” were awaiting compatibility between the national and international reporting formats [14].

For Europe, pharmacovigilance is coordinated by the EMA and conducted by the National Competent Authorities (NCA). Since 2001, EudraVigilance, a data processing network and management system for reporting and evaluating suspected adverse reactions during the development and following the marketing authorization of medicinal products in the European Economic Area (EEA) is in operation [15].

In the United States the main pillars of pharmacovigilance are the FDA through MedWatch, the FDA safety information and adverse event reporting programme [16], the pharmaceutical industry and academic non-profit organizations, such as RADAR (Research on Adverse Drug events And Reports) [17, 18].

5 Spontaneous reporting systems

Spontaneous reporting systems have become the primary method of collecting post-marketing information on drug safety. Spontaneous reporting is defined as “a system whereby case reports of adverse drug events are voluntarily submitted by health professionals and pharmaceutical companies to the national pharmacovigilance centre” [14]. Spontaneous reporting systems have the strength to early identify signals of new, rare and serious ADRs [19]. Further advantages are that they can be used throughout the life cycle of a drug by all stakeholders at relatively low costs [13]. Spontaneous reporting is also used by the pharmaceutical industry to collect information about their drugs. One signal successfully detected by spontaneous reporting is cardiac valvular disease caused by fenfluramine. This ADR was discovered after 24 years of marketing, mainly as a result of a sudden increase in its use as an anorectic agent [12]. One further example of an ADR identified through spontaneous reporting is QT prolongation caused by cisapride, which led to its withdrawal from the US market in 2000.

During the clinical phases of drug development, all ADRs must be reported. After approval and during marketing, surveillance, evaluation, and reporting must continue for any ADRs, which are related to use of the drug including overdose, accident, failure of expected action, events occurring from drug withdrawal, and unexpected events not listed in labelling. Events that are both serious and unexpected must be reported to the regulatory agencies within 15 days (Table 1) [20, 21]. ADRs may act through the same physiological and pathological pathways as different diseases and thus, they are difficult and sometimes impossible to distinguish. The WHO therefore suggests a step-wise approach that may be helpful in assessing possible drug-related ADRs [1]:

1. Ensure that the medicine ordered is the medicine received and actually taken by the patient at the dose advised;
2. Verify that the onset of the suspected ADR was after the drug was taken, not before and discuss carefully the observation made by the patient;
3. Determine the time interval between the beginning of drug treatment and the onset of the event;
4. Evaluate the suspected ADR after discontinuing the drugs or reducing the dose and monitor the patient's status. If appropriate, restart the drug treatment and monitor recurrence of any adverse events;
5. Analyze the alternative causes (other than the drug) that could on their own have caused the reaction;
6. Use relevant up-to-date literature and personal experience as a health professional on drugs and their ADRs and verify if there are previous conclusive reports on this reaction. The National Pharmacovigilance Centre and Drug Information Centres

are very important resources for obtaining information on ADR. The manufacturer of the drug can also be a resource to consult;

- 7. Report any suspected ADR to the person nominated for ADR reporting in the hospital or directly to the National ADR Centre.

Once a suspected ADR has been identified, causality has to be assessed to determine whether the observed reaction is drug related, as issues like concomitant medication, underlying diseases or treatments not mentioned by the patient might complicate a definite assignment. The WHO has developed a system for the causality assessment of suspected adverse reactions that is available on their web-page [14] and that is depicted in Table 2.

Spontaneous reports of suspected ADRs can only be regarded as signals of a potential hazard in the use of a drug and are a hypothesis generating instrument and cannot readily be used as a reliable measure for a definite risk–benefit assessment. Signal detection has for long been based on a case-by-case analysis of reports. Recently, new

Table 2 Causality assessment of suspected adverse reactions (modified from Ref. [14])

| Causality term | Assessment criteria |
|---------------------------------|---|
| Certain | A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to drug administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be definitive pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary. |
| Probable/likely | A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition. |
| Possible | A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear. |
| Unlikely | A clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations. |
| Conditional/ unclassified | A clinical event, including laboratory test abnormality, reported as an adverse reaction, about which more data is essential for a proper assessment or the additional data are under examination. |
| Unassessable/ unclassifiable | A report suggesting an adverse reaction which cannot be judged because information is insufficient or contradictory, and which cannot be supplemented or verified. |

statistical data mining techniques have gained importance, which have improved the analysis of large databases of adverse event reports, thereby permitting more rapid, robust and comprehensive detection of signals that indicate the possibility of safety issues [13, 19]. After signal identification, these signals require evaluation to see if they are false-positive indications or reflect a true problem, including evaluation of frequency, causality mechanisms and preventability to possibly identify risk or protective factors to better inform prescribers and patients. This is usually done by pharmaco-epidemiological studies, designed to confirm or refute the findings by hypothesis-testing techniques, such as case-control or cohort studies or randomized controlled clinical trials [13].

A special form of active, intensified surveillance, prescription event monitoring (PEM), was developed in the early 1980s [22]. This system uses prescription data to identify users of a certain drug. The prescriber of the drug is asked about any adverse event occurring during the use of drug. Data are collected and analyzed for new signals [19]. PEM is non-interventional and observational, provides real world clinical data, is capable of identifying signals for events that were not necessarily suspected as being ADRs of the studied drugs and also enable the incidence of ADR to be estimated, thus enabling quantification of the risk of certain ADRs [19]. Limitations stem from the lack of control group which does not allow estimating the true background incidence of events. Furthermore, the percentage of unreported ADRs is unknown and data on smoking status, concomitant medication, or body weight are not routinely recorded. Several ways in which the clinical information for active surveillance can be collected include patient registries, studies using databases of medical records and clinical trials [13].

Although spontaneous reporting systems remain the primary and best method for identifying ADRs to newly marketed drugs [23], they have also been criticized as being fundamentally a 1950s-era approach [23] with inherent disadvantages such as poor quality of submitted reports, often with inadequate documentation and details, the potential of selective reporting and underreporting [19]. A systematic review with the aim to describe the extent of under-reporting of ADRs to spontaneous reporting systems found that a median of 94% of ADRs are not reported at all [24]. As a result, false conclusions about the safety profile of a drug might be drawn, which leads to either overlooking a true risk or erroneously ascribing an adverse event to a drug. Reporting is furthermore more complete for newer and more recently marketed drugs than older drugs and external influences can easily modify reporting rates [23].

Despite all post-marketing efforts, in recent years, a number of drugs had to be withdrawn from the market after authorization due to safety problems and serious ADRs (Table 3). A comprehensive up-to-date list of marketing authorization withdrawals and suspensions of medicinal products for human use in the European Union can be found on the EMA homepage [25]. In-depth information on drug safety for the

Table 3 Recent drug withdrawals due to adverse drug reactions (ADRs)

| Year of withdrawal | Substance | Approved for (year of approval) | Reason for withdrawal |
|--------------------|--------------|---|--|
| 1998 | Mibefradil | Treatment of angina and hypertension (1997) | Serious drug interactions and risk of QT prolongation and Torsades de Pointes |
| 2001 | Cerivastatin | Treatment of hyperlipidemia (1999) | Increased risk of rhabdomyolysis |
| 2004 | Rofecoxib | Treatment for osteoarthritis, rheumatoid arthritis and higher dose-strengths are indicated for short-term relief of acute pain (1999) | Increased risk of confirmed serious thrombotic events (including myocardial infarction and stroke) compared to placebo, following long-term use (over 18 months) |
| 2004 | Parecoxib | Prevention of venous thromboembolic events in patients undergoing elective hip- or knee-replacement surgery (2002) | Cardiovascular and serious skin adverse events |
| 2005 | Valdecoxib | Treatment of symptomatic relief in the treatment of osteoarthritis or rheumatoid arthritis and the treatment of primary dysmenorrhoea (2003) | Cardiovascular and serious skin adverse events |
| 2006 | Ximelagatran | Prevention of venous thromboembolic events in patients undergoing elective hip- or knee-replacement surgery (2005) | Severe liver injury during longer-term treatment |
| 2007 | Tegaserod | Treatment of irritable bowel syndrome with constipation and chronic idiopathic constipation in women younger than 55 | Higher chance of heart attack, stroke and unstable angina |
| 2007 | Aprotinin | Symptomatic relief in the treatment of osteoarthritis of the hip and knee (systemic medicines containing aprotinin have been available since 1974) | Increased mortality for patients receiving aprotinin |
| 2007 | Lumiracoxib | Symptomatic relief in the treatment of osteoarthritis of the hip and knee (2005) | Risk of serious side-effects affecting the liver |
| 2008 | Rimonabant | Adjunct to diet and exercise for the treatment of obese patients (BMI $\geq 30 \text{ kg/m}^2$), or overweight patients (BMI $> 27 \text{ kg/m}^2$) with associated risk factor(s), such as type 2 diabetes or dyslipidaemia (2006) | Concerns over suicidality, depression, and other related side-effects |

US market is summarized on the FDA homepage [26], One of the drugs withdrawn due to safety reasons was the statin cerivastatin. Example 1 describes the events around the withdrawal in detail and also discusses the role of drug safety systems.

6 Drug safety issues after Lipobay and Vioxx

When cerivastatin and later rofecoxib (Vioxx[®]) [27] had to be withdrawn from the market due to safety problems, the Thalidomide tragedy seemed almost forgotten. In the United States it had been proposed that the FDA should approve beneficial new drugs more quickly and concurrently develop a better system of monitoring for adverse events once the drugs were in routine use. The 1992 Prescription Drug User Fee Act allowed pharmaceutical companies to pay the FDA to cover the costs of additional agency staff required to review new drug applications rapidly. The time required for approval dropped sharply, but post-marketing studies were not financed. The FDA was accused of having become too industry friendly and to demand only surrogate rather than hard clinical outcomes for drug approval studies. Fast approvals also came along with an increase of drug recalls. A further fundamental problem of this system was that it had to increasingly rely on the pharmaceutical industry to conduct its own post-marketing safety evaluation. This raised the concern that a pharmaceutical company's appraisal of suspected ADRs may be influenced by economic considerations. It could be shown that fewer than half of the post-marketing studies that were agreed upon as a condition of drug approval, has been completed or even initiated [33] and that the rate of those studies declined between the 1970s and the 1990s [19]. The FDA also had no authority to legally force companies to fulfill their post-marketing commitments such as a change in labels to reflect new safety concerns, creation of a patient registry, conduction of patient or physician education, or restricted advertising. Mechanisms to alert physicians to new safety information, such as "black-box" warnings or letters to physicians, were criticized as being not sufficient to induce increased safety awareness.

When it was revealed that some FDA experts had direct financial interest in the drug or topic they were evaluating [28], the whole discussion culminated in 2004 when FDA scientist David Graham even accused the agency of not being capable of protecting the American people from unsafe drugs [10]. A recent study aimed to determine whether the deadlines imposed by the Prescription Drug User Fee Act for the completion of drug reviews by the FDA were truly associated with post-marketing safety problems [29]. The authors conclude that the approval decisions of the FDA have been affected and that once medications are in clinical use, the discovery of safety problems is more likely for drugs approved immediately before a deadline than for those approved at other times [29].

7 Recent and future developments in pharmacovigilance

Following the events around recent drug withdrawals, efforts have been undertaken to develop solutions to enhance drug safety, including the introduction of legislation that expands the power of drug regulatory agencies, new data transparency standards and

increased requirements for funding of post-marketing surveillance [30]. In the European Union, for example, a proactive risk management strategy has been introduced in 2005, which gives the regulatory agencies the power to demand a risk management plan that describes commitments for post-marketing pharmacovigilance in detail. This plan has to be submitted already with the application for marketing authorization [31]. Furthermore, drug companies are obliged to provide periodic safety update reports (PSUR) on the new drug after its approval. Recently, the EMA has introduced conditional marketing authorizations that are valid for a limited time and require further studies to be renewed. The advantage of this approach is earlier access to a potentially highly beneficial drug, which is desirable from a public health perspective, although there is not a complete dataset on the risk–benefit ratio at the time of approval. At the same time, the need to continuously provide new data, addresses the issue of inadequate follow-up by post-marketing studies and allows for prompt regulatory actions, as soon as safety problems become evident. Example 2 briefly describes how the risk management strategy has been imposed on a drug company by the EMA.

Besides the legal tools for regulatory actions, regulators in the future will also need improved strategies for collection, integration and analysis of data related to post-marketing safety [13]. These strategies include larger clinical trials, use of meta-analysis of trials of individual drugs or drug classes and results from observational studies, involving electronic records that link drug-use data to health outcomes for a large number of patients [31]. Furthermore, there will be more emphasis on the adoption of tools for active drug surveillance to systematically collect clinical information, such as databases of medical records or patient registers [13]. One example for a patient register, which is used to collect data on a defined patient population over a defined period of time, is a register involving natalizumab, an antibody for the treatment of severe relapsing multiple sclerosis [13]. Clinical trials and post-marketing data had identified a safety signal – natalizumab was associated with an increased risk of progressive multifocal leukoencephalopathy (PML) – that subsequently led to the voluntary withdrawal of the drug in 2005. After regulatory review of safety and efficacy data, natalizumab was reintroduced into the market in 2006 under a risk minimization programme, in which patients receiving the drug are registered and monitored [13].

Additionally, pharmacovigilance may increasingly rely on the use of personalized medicine. Use of data on the genetic background and variability of drug response and ADRs has moved from the experimental stage to the clinics, although not in the pre-intended proportion. To date, there are only a few examples of pharmacogenetic tests that are routinely employed to identify genetic variants that confer risk to ADRs. Testing for the human leukocyte antigen (HLA)-B*5701 allele has been shown to predict the risk of hypersensitivity in patients with HIV scheduled to receive therapy with abacavir [32]. In 2007, warfarin received a new label with advice on the altered metabolism that is seen in patients with particular variants in the cytochrome P450 2C9 or vitamin K epoxide reductase complex subunit 1 (VKORC1) genes [32]. Whereas submission of genetic

data is currently performed on a voluntary basis, the future might see an increasing number of such submissions as a further means for risk stratification.

Although at the moment pharmacovigilance is still used mainly as a tool to detect unknown ADRs and might lead to regulatory actions such as changing the summary of product characteristics or withdrawing the drug from the market, in the future this data will need to be translated into information that can assist a healthcare professional or patient in the decision-making process of whether or not to use a drug in a timely matter [19].

8 Conclusions

With regard to public perception, there is a long term need to broaden awareness that no drug is completely safe or always effective and that, despite the best efforts, some safety issues may not be identified before a drug reaches the market. Withdrawal of approved and widely used drugs because of serious life threatening adverse events, however, has eroded public confidence in the medical care system. Pharmacovigilance of tomorrow must be able to identify new safety issues without delay. To successfully achieve this goal and to implement changes in the way drug safety is assessed during drug development and post-marketing, collaboration between industry, academia and government is essential, as future strategies rely on the involvement of all stakeholders. Success in this area is needed to increase the patients' confidence in drug therapy.

Case Study 1: Cerivastatin – the withdrawal of a blockbuster drug is needed to expose problems with drug safety systems

In 1987 lovastatin was the first member of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, also known as statins, to be commercially marketed for the management of dyslipidemia. In August 2001, cerivastatin (marketed as Lipobay® in Europe and Baycol® in the US), the 6th member of the statin family which was approved in 1997, was voluntarily withdrawn from the US market and Europe and subsequently in Japan by the Bayer Company, cerivastatin's manufacturer, because of an increasing number of reports concerning fatal rhabdomyolysis. Among patients on cerivastatin, 52 deaths were attributed to drug-related rhabdomyolysis, which led to kidney failure. Thirty-one fatalities were reported in the United States, a further 21 deaths worldwide. In addition there were 385 nonfatal cases reported among the estimated 700,000 users in the United States,

most of whom required hospitalization. In many of the fatal cases, patients had received the full dose of cerivastatin (0.8 mg/day) or were using gemfibrozil concomitantly [33]. Bayer faced 7800 claims for compensation in the USA and about 500 in Germany [34]. In a US court case, Bayer was forced to release confidential company documents revealing just how much the company knew about the problems with the drug before withdrawing it in 2001 [35]. At the time of drug withdrawal, dose dependent myopathy and rhabdomyolysis, were known serious adverse events for patients taking statins and it was also recognized that concomitant use of drugs that increase blood levels of statins and combination with fibrates, such as gemfibrozil, potentially increase myopathy. A review of reports of the Adverse Event Reporting System of the FDA showed that fatal rhabdomyolysis is a rare event among statin users. For cerivastatin, however, the rate of fatal rhabdomyolysis was 16–80 times as high as the rates for any other statin [36].

The withdrawal of cerivastatin was intensely discussed by experts and the public alike and raised questions concerning the responsibilities in this case in particular about the contributions of the pharmaceutical industry, the regulatory agencies and the status of the drug approval process and post-marketing surveillance system in general that had failed to prevent serious ADRs despite the knowledge about their potential occurrence. It was argued that that Bayer was aware of problems associated with cerivastatin since its approval by the FDA in 1997 and it was suspected that not all adverse events were reported to the FDA. Bayer insisted that it acted properly and in a timely manner when informing regulatory authorities about myopathy with cerivastatin and accused doctors still prescribing cerivastatin along with gemfibrozil, despite changes in labelling and a warning from the company that this could result in adverse reactions [34].

The approval history of cerivastatin has been summarized and quoted as an example how the approval system had failed to properly react when rare and potentially serious adverse events were emerging [37]: In June 1997, cerivastatin was launched at low doses of 0.2 and 0.3 mg. The risk of rhabdomyolysis was added as a warning to the approved label in July. In August 1998 a supplemental New Drug Application (NDA) was submitted requesting approval of a 0.4-mg dose and soon after the first case of a cerivastatin and gemfibrozil interaction associated with rhabdomyolysis was published. A change was made to the 0.4 mg dose NDA in May 1999, adding a warning regarding concomitant use with gemfibrozil. The NDA for the 0.8-mg dose was submitted in September 1999, followed by a letter to practitioners in December warning of the contraindication for using gemfibrozil with cerivastatin. In July 2000 the FDA approved the dose increase, because of a lack of efficacy at the lower dose. By the spring of 2001 the FDA noted a sudden increase in reports of adverse reactions with the drug. This

prompted discussions with Bayer and resulted in the company's decision to withdraw cerivastatin from the market in August 2001 [35]. Of note, an increased risk of myopathy in thin, elderly women given the 0.8 mg dose had already been recognized and reported by an FDA medical reviewer but, in the final analysis, this was not considered significant enough to prevent approval [37]. In the end, the safety problems can in many cases be explained by a combination of the authorities' failure to react properly on known safety signals, combined with the clinician's enthusiasm to prescribe a new substance that was heavily marketed by the manufacturing company.

Cerivastatin received initial approval based on surrogate criteria, i.e. on its effects on serum lipoproteins. At the time of withdrawal, documentation for long-term efficacy and safety was weak or non-existent. As a consequence, the approval of the next statin, rosuvastatin, resulted in the generation of a database containing 4 times the number of patients of that of any previously approved statin [37].

Case Study 2: Micafungin – safety issues prompt EMA to demand submission of a risk management strategy as a condition of market authorization in the European Union (if not otherwise specified, the EMA public assessment report, EPAR [38] was quoted)

In 2006, Astellas Pharma GmbH submitted an application for Marketing Authorization to the EMA for Mycamine[®]. Mycamine[®], with the active compound micafungin, belongs to the echinocandin lipopeptides, a new class of antifungal agents [39]. Micafungin was the third echinocandin after caspofungin and anidulafungin to apply for marketing authorization in the European Union. At the time of application, Mycamine[®] had already been approved in several countries including Japan and the USA. At the time of release of the EPAR in April 2008, post-marketing experience was available from approximately 220,000 patients worldwide. The reported adverse events (AEs) were in line with the known safety profile of micafungin, and in particular underlined its hepatotoxic potential. Reports of hepatic AEs had accounted for approximately 25% of all adverse events, including 20 fatal cases considered at least as possibly causal related to micafungin (1/3 of all fatal related AEs).

In its review of pre-clinical and clinical data, The Committee for Medicinal Products for Human Use (CHMP), which is responsible for preparing the EMA's opinions on all questions concerning medicinal products for human use, had stressed out that already during the toxicological development programme liver

toxicity had been an issue, as micafungin induced irreversible foci of altered hepatocytes (FAH) and hepatocellular tumours in rats after treatment for 3 months and longer. The mechanisms for FAH and tumour development have not been elucidated so far. However, the assumed threshold for tumour development in rats had been approximately in the range of clinical exposure. At this threshold, the AUC in female rats was in the range of human AUCs at therapeutic doses, i.e. there were no safety margins at least for the high therapeutic doses. The CHMP stated that the relevance of this finding for the therapeutic use in patients cannot be excluded. At the same time the CHMP, however, acknowledged that there is need for new antifungal agents, because of the development of fungal resistance as well as emerging fungal pathogens. Therefore, micafungin would be approvable as a first-line therapeutic option, if the risk for hepatocarcinogenicity could be excluded. As this risk cannot be excluded for the time being, the benefit/risk ratio of all other antifungals was considered “superior” in “uncomplicated” situations. In other cases micafungin might be an adequate treatment option in life threatening situations despite this potential risk.

In 2008, the CHMP issued a positive opinion for granting a Market Authorization to Mycamine[®] as a treatment option only when the use of other antifungals is not appropriate. The applicant was furthermore obliged to fulfill a number of measures to evaluate the potential risk for the development of liver tumours in patients and the applicant submitted a risk management plan. After its review, the CHMP was of the opinion that pharmacovigilance activities in addition to the use of routine pharmacovigilance were needed to investigate further some of the safety concerns. These activities include the conduction of observational studies in different patient populations, a pharmacokinetic study in patients with severe hepatic dysfunction, close monitoring and specific standardized follow-up questionnaires. Furthermore, additional risk minimization activities were defined, such as warnings and the listing of additional ADRs in the Summary of Product Characteristics, prescriber check-lists and a nurse administration and monitoring guide. The applicant has agreed to fulfill the follow-up measures within agreed time limits.

References

1. http://whqlibdoc.who.int/hq/2002/WHO_EDM_QSM_2002.2.pdf (last accessed January 15th 2010)
2. Kongkaew C, Noyce PR, Ashcroft DM (2008) Hospital admissions associated with adverse drug reactions: a systematic review of prospective observational studies. *Ann Pharmacother* 42: 1017–1025

3. Lazarou J, Pomeranz BH, Corey PN (1998) Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies, *JAMA* 279: 1200–1205
4. Pirmohamed M, James S, Meakin S, et al. (2004) Adverse drug reactions as cause of admission to hospital: prospective analysis of 18,820 patients. *BMJ* 329: 15–19
5. White TJ, Arakelian A, Rho JP (1999) Counting the costs of drug-related adverse events. *Pharmacoeconomics* 15: 445–458
6. Field TS, Gilman BH, Subramanian S, et al. (2005) The costs associated with adverse drug events among older adults in the ambulatory setting. *Med Care* 43: 1171–1176
7. http://whqlibdoc.who.int/hq/2004/WHO_EDM_2004.8.pdf (last accessed January 17, 2010)
8. Franks ME, Macpherson GR, Figg WD (2004) Thalidomide. *Lancet* 363: 1802–1811
9. http://www.contergan.grunenthal.info/ctg/en_EN/pdf/ctg_en_en_ctg_brosch.pdf (last accessed January 17th 2010)
10. Avorn J (2006) Evaluating drug effects in the post-Vioxx world: there must be a better way. *Circulation* 113: 2173–2176
11. Stevens JL, Baker TK (2009) The future of drug safety testing: expanding the view and narrowing the focus. *Drug Discov Today* 14: 162–167
12. Atuah KN, Hughes D, Pirmohamed M (2004) Clinical pharmacology: special safety considerations in drug development and pharmacovigilance. *Drug Safety* 278: 535–554
13. Wise L, Parkinson J, Raine J, Breckenridge A (2009) New approaches to drug safety: a pharmacovigilance tool kit. *Nat Rev Drug Discov* 8: 779–782
14. <http://www.who-umc.org/> (last accessed January 15, 2010)
15. <http://eudravigilance.emea.europa.eu/highres.htm> (last accessed January 15, 2010)
16. <http://www.fda.gov/Safety/MedWatch/default.htm> (last accessed January 15, 2010)
17. <http://cancer.northwestern.edu/radar/> (last accessed January 15 2010)
18. Carson KR, Focosi D, Major EO, et al. (2009) Monoclonal antibody-associated progressive multifocal leucoencephalopathy in patients treated with rituximab, natalizumab, and efalizumab: A Review from the Research on Adverse Drug Events and Reports (RADAR) Project. *Lancet Oncol* 10: 816–824
19. Härmark L, van Grootheest AC (2008) Pharmacovigilance: methods, recent developments and future perspectives. *Eur J Clin Pharmacol* 64: 743–752
20. FDA Guidance for Industry. Postmarketing Safety Reporting for Human Drug and Biological Products Including Vaccines. <http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm092257.pdf> (last accessed January 15, 2010)
21. EMEA Guidelines on Pharmacovigilance for Medicinal Products for Human Use. http://ec.europa.eu/enterprise/sectors/pharmaceuticals/files/eudralex/vol-9/pdf/vol9a_09-2008_en.pdf (last accessed January 15, 2010)
22. Mann RD (1998) Prescription-event monitoring – recent progress and future horizons. *Br J Clin Pharmacol* 46: 195–201
23. Strom BL (2004) Potential for conflict of interest in the evaluation of suspected adverse drug reactions: a counterpoint. *JAMA* 292: 2643–2646
24. Hazell L, Shakir SA (2006) Under-reporting of adverse drug reactions : a systematic review. *Drug Safety* 29: 385–396
25. Marketing Authorisation (MA) Withdrawals and Suspensions – Medicinal Products for Human Use. <http://www.ema.europa.eu/htms/human/withdraw/withdraw.htm> (last accessed January 15, 2010)
26. <http://www.fda.gov/Safety/default.htm> (last accessed January 15, 2010)
27. Krumholz HM, Ross JS, Presler AH, Egilman DS (2007) What have we learnt from Vioxx? *BMJ* 334: 120–123
28. Fontanarosa PB, Rennie D, DeAngelis CD (2004) Postmarketing surveillance – lack of vigilance, lack of trust. *JAMA* 292: 2647–2650

29. Carpenter D, Zucker EJ, Avorn J (2008) Drug-review deadlines and safety problems. *N Engl J Med* 358: 1354–1361
30. Ray A (2009) Beyond debacle and debate: developing solutions in drug safety. *Nat Rev Drug Discov* 8: 775–779
31. Eichler HG, Abadie E, Raine JM, Salmonson T (2009) Safe drugs and the cost of good intentions. *N Engl J Med* 360: 1378–1380
32. Mallal S, Phillips E, Carosi G, et al. (2008) HLA-B*5701 screening for hypersensitivity to abacavir. *N Engl J Med* 358: 568–579
33. Furberg CD, Pitt B (2001) Withdrawal of cerivastatin from the world market. *Curr Control Trials Cardiovasc Med* 2: 205–207
34. How a statin might destroy a drug company (2003) *Lancet* 361: 793.
35. Marwick C (2003) Bayer is forced to release documents over withdrawal of cerivastatin. *BMJ* 326: 518
36. Staffa JA, Chang J, Green L (2002) Cerivastatin and reports of fatal rhabdomyolysis. *N Engl J Med* 346: 539–540
37. Jacobson TA (2006) Statin safety: lessons from new drug applications for marketed statins. *Am J Cardiol* 97: 44C–51C
38. EMEA Assessment Report for Mycamine, International Nonproprietary Name: mycافunjin, Procedure No. EMEA/H/C/000734. <http://www.ema.europa.eu/humandocs/Humans/EPAR/mycamine/mycamine.htm> (last accessed January 19, 2010)
39. Bal AM (2010) The echinocandins: three useful choices or three too many? *Int J Antimicrob Agents* 35: 13–18

CHAPTER 19

Drug interactions

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1 Definition

A drug interaction is a situation in which a drug, food or other extrinsic and intrinsic factors affect the activity of a medication, i.e. the effects of the medication are increased or decreased, or the combination of substances produces a new effect that neither of them produces on its own. Thereby often the efficacy or toxicity of a medication is changed.

2 Relevance of interactions

Adverse drug reactions cause more than 100,000 deaths each year in US and are responsible for approximately 7% of all hospital administrations in Europe [1, 2]. Drug interactions, in turn, are the leading cause of adverse drug reactions. However, the true incidence of overall drug interactions as well as their clinical significance can only be estimated.

The importance of interactions is highlighted by the continuous increase of drug interaction studies over previous decades [3]. This observation is in line with regulatory requirements of FDA and EMEA for preclinical and clinical studies on drug–drug interactions before a new compound may enter the market [4, 5]. Nevertheless, often the interaction profile of a new drug is not fully understood until several years after it was introduced into the market.

3 Categories of interaction

When talking about drug interactions most commonly interactions based on the Cytochrome (CYP) P450 system come to mind. Indeed interactions based on this

Keywords: Absorption, ADME, adverse drug reaction, antibiotics, Cytochrome P 450 (CYP) family, distribution, elimination, metabolism, pharmacodynamics, pharmacokinetics, polypharmacy, P-glycoprotein (P-gp), statins

class of isoenzymes are considered most important; however drug interactions include a much wider field and can be distinguished by a range of different categories.

- (a) *Mechanism of interaction – pharmacokinetic or pharmacodynamic interaction.* Pharmacodynamic interaction: the activity of a substance is modified without changes in the drug concentration vs. time profile, usually when two drugs are competitors for binding to the same receptor. Drugs may act additively, synergistically or antagonistic.

Pharmacokinetic interaction: the drug concentration vs. time curve in the human body is modified. Pharmacokinetic interactions can be based on all aspects of pharmacokinetics of a drug, i.e. absorption, distribution, metabolism and elimination (ADME).

- (b) *Type of interacting factor.* Typically drug interactions with other drugs come to mind (drug–drug interaction, classified by the class of drugs involved). However, interactions may also exist with food (drug–food interactions), as well as with herbal medications and nutritional supplements (drug–herb interactions) or with intrinsic factors like enzymes or plasma binding proteins.
- (c) *Role of the drug in the interaction.* The drug can be substrate (the activity of the drug itself is modified), inducer or inhibitor (other drugs are modified). Inducers or inhibitors can act competitively (the drug itself is substrate or competitive ligand of a receptor) or independently by other mechanisms.
- (d) *Result of the interaction.* The activity and/or the toxicity are enhanced or reduced.
- (e) *Relevance or interaction.* Drug interactions are regarded clinically meaningful if they reduce therapeutic efficacy or produce toxicity to an extent that the dose of the drug has to be modified in order to retain activity or avoid side effects. Thereby, interactions can be classified as minor (no clinical relevance, no change of therapy), moderate (require adjustment of dose and frequent monitoring of drug levels, therapeutic effect and toxicity, but do not preclude concomitant use of the drugs) or severe (drugs should not be used in combinations, if known usually labeled as contraindication in the SPC).

4 Factors promoting interactions and their clinical relevance

In many respects drug interactions might be compared to road traffic. As long as a car drives on a lonely highway, collision with other traffic members is unlikely, independent on the type of vehicle one drives. As soon as traffic becomes dense, the risk for car accidents increases tremendously. Indeed, regarding drug interactions the number of used drugs in one patient might often be considered more important than the characteristics of the drug itself.

A strong relationship between the number of dispensed drugs and potential drug interactions has been described, especially for potentially serious drug interactions

[6, 7]. A US study found that the risk of non intended drug interactions increased from 13% for patients taking two medications to 82% for patients taking seven or more medications [8]. According to a survey in developed countries average patients take seven different generic substances at the time of admission to a hospital; with other words they have at least 82% chance of occurrence of drug interactions.

However, the extent of drug interaction varies markedly among individuals; i.e. it is dependent on inter-individual differences in CYP3A4 tissue content, pre-existing medical conditions and most importantly age [6–9]. Elderly patients have a much higher probability of drug interactions than younger subjects [6, 10]. Thereby the elderly suffer from risk of drug interactions both due to changes in metabolism and renal excretion as well as their frequent polypharmacy. If possible, minimizing the number of drugs prescribed to the elderly is of outstanding importance to avoid drug interactions. Use of over-the-counter (OTC) medication and herbal supplements for self-treatment can contribute to polypharmacy in chronically ill patients and is often unknown to the health care team [11]. Lifestyle factors like chronic alcoholism or smoking may impact drug metabolism and thereby the probability for drug interactions. Alcohol induced hepatic dysfunction may reduce the ability to metabolize drugs [12].

Out of 540 drug–drug interaction studies performed between 1992 and 1997, 80 (15%) resulted in clinically significant labelling statements. New molecular entities with highest probability of drug interactions were neuropharmacology, cardiorenal, anti-viral, and anti-infective drugs, while drug classes such as oncology drug products and radioimaging products were least likely to include drug–drug interaction studies in their submissions [3].

An interaction is “clinically relevant” when

- (a) the therapeutic activity and/or toxicity of a drug is changed to such extent that a dosage adjustment of the medication or medical intervention might be required and
- (b) the concomitant use of two interacting drugs can occur when both are used as therapeutically recommended [4].

Again, the clinical importance of any drug interaction depends on factors that are drug-, patient- and administration-related. Generally, a doubling or higher increase of plasma drug concentrations has the potential for enhanced adverse or beneficial drug effects. Less pronounced pharmacokinetic interactions may still be clinically important for drugs with a steep concentration-response relationship or narrow therapeutic index. The relevance of an interaction is mainly driven by the therapeutic index, i.e. the ratio of the concentration resulting in 50% of lethal toxicity to the concentration necessary for reaching 50% of the maximum effect of the respective drug (Fig. 1).

Drug interactions may be most apparent when patients are stabilized on the affected drug and the interacting agent is then added to the regimen. Temporal relationship

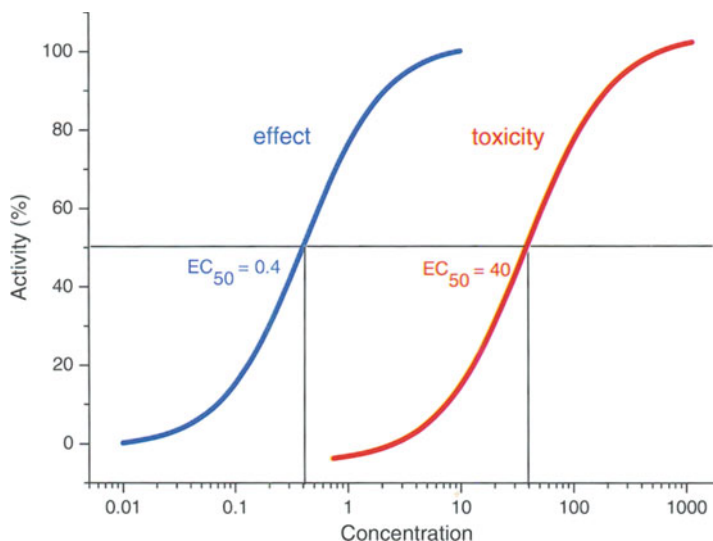


Fig. 1 The therapeutic index is the ratio of the concentration resulting in 50% of lethal toxicity to the concentration necessary for 50% effect. For optimal drugs 90% of activity can be achieved at concentrations at which no or very little toxicity is expected

between the administration of a new drug and occurrence of interaction further helps to reveal its extent [9].

Most severe interactions occur if both mechanisms of interactions, pharmacokinetic and pharmacodynamic, are combined. A famous example are the described lethal cases of rhabdomyolysis associated with co-administration of cerivastatin and fibrates, where gemfibrozil increased plasma levels of cerivastatin by inhibition of its metabolism but the substance gemfibrozil also independently had toxic effect on muscle tissue [13].

5 Most important mechanisms of interactions according to the ADME schemata

5.1 ADME – interactions based on drug absorption

Drug absorption can be modulated by factors which influence the amount of drug available for absorption (chelation or conformational changes due to high or low pH), by modifying the speed of gastrointestinal passage or by directly modifying the penetration of a substance from the gastrointestinal tract into the blood (activity of transport proteins or intestinal CYP450 metabolism). Some drugs impact absorption *via* more than one mode of action. Antacids, for example may adsorb drugs in the

gastrointestinal drug, may change solubility of a drug due to the increase of gastric pH and may speed up gastric emptying [14]. In addition, antacids may alkalinize urine, thereby modifying excretion of pH – sensitive drugs. Thus, generally antacids should not be given together with other drugs at the same time.

Both the rate (speed) and/or the extent (percentage) drug absorbed might be affected. Affecting the rate of absorption is most important for analgetics or all drugs used in emergency indications where the onset of effect is crucial and delay of action might cause danger or discomfort to the patient. Interactions based on drug absorption might even occur for drugs which are not given orally. The cholesterol lowering ion-exchanger cholestyramine may not only interact with orally administered drugs, but may also impact parenterally administered drugs like digoxin that undergo enterohepatic circulation [15].

Interactions based on modifications of drug absorption might also occur outside the gastrointestinal tract and might not necessarily be based on drug–drug interactions but intrinsic factors. Bioavailability of intranasally applied agents for example might be modified by nasal pathologies [16]. Uptake of depot insulin is highly dependent on the site of application, the perfusion and the constitution of the subcutaneous adipose tissue in individual patients [17].

Perhaps the most frequent condition that might interact with drug absorption is the concomitant consumption of food and fluids. Food intake exerts a complex influence on the bioavailability of drugs and might both increase and decrease bioavailability of drugs for several hours. It may interfere not only with tablet disintegration, drug dissolution and drug transit through the gastrointestinal tract, but may also affect the metabolic transformation of drugs in the gastrointestinal wall and in the liver [18]. Food may interact in unpredictable ways, even with drugs that are chemically related, therefore, the net effect of food on drug bioavailability can be assessed only by direct clinical studies of the drug in question. Many substances, especially antibiotics (isoniazid, rifampicin, tetracycline, penicillin and ampicillin) are better absorbed by an empty stomach [18]. While for those drugs food will lower or at least delay absorption, food might also strongly increase systemic availability. For example bioavailability of beta blockers (propranolol and metoprolol) and the antiepileptics phenytoin and carbamazepine is significantly increased and exposure to cyclosporine is doubled when given together with meals [18–20]. Repeated intake of protein-rich meals enhances, while repeated intake of carbohydrate-rich meals reduces the rate of oxidation of antipyrine and theophylline. Thus, food and its components and contaminants may have both short and long-term effects on both the absorption and biotransformation processes influencing systemic availability of drugs. Besides affecting bioavailability food may also interact with local action of a drug thereby modifying the gastric tolerability, e.g. for nitrofurantoin, doxycycline and lithium the presence of food markedly reduces the incidence of local gastrointestinal side effects [21].

Since food interactions might impact absorption of drugs tremendously regulatory agencies require investigation of food interaction for each novel drug early in drug development [4]. The commonly accepted threshold for clinically relevant change of bioavailability of a drug is 20%. Since food dependency will obviously hamper correct intake of a drug, pharmaceutical companies try to develop drugs and formulations which are less susceptible to interactions based on food intake.

6 ADME – interactions based on drug distribution

Drug distribution might be affected by mechanisms which modulate passive diffusion of substances from the central compartment to peripheral tissues or by interactions based on active drug transport.

Binding to plasma proteins plays a major role in drug therapy as it provides a depot for many compounds, affects pharmacokinetics of drugs, and may influence the metabolic modification of ligands [22]. Only the protein unbound fraction of a drug in plasma can penetrate into and equilibrate with the extravascular space [23]. This is highly important as the majority of targets are located in the interstitial fluid of tissues rather than in blood [24]. Protein binding also affects drug clearance from the body. As high protein binding keeps the drug in the bloodstream, for drugs that are eliminated by tubular secretion or hepatic metabolism increase of plasma protein binding is associated with lowered drug elimination. Likewise protein binding negatively correlates with glomerular filtration, since only the free drug may be filtered [25].

For half a century it is known that endogenous substances like bilirubin and synthetic compounds like sulphonamides or cephalosporins can compete for binding sites resulting in displacement of drug molecules leading to changes of unbound fractions of the drug [26, 27]. The mechanism may be either competitive, meaning that drugs bind to the same site, or non-competitive, with one drug causing a conformational change in the protein, which, in turn, modifies its binding capacity for another drug [25]. Drugs interacting for binding sites on plasma proteins often additionally interact at the level of metabolism and excretion, resulting in a potentiating effect [25]. Obviously interactions with impact on protein binding are most important for drugs with high protein binding and narrow therapeutic index.

In contrast to plasma protein binding, which prevents a drug from leaving the blood stream, transport proteins may work as an efflux pump on biological barriers like the intestinal wall or the blood brain barrier. Drugs may impact the activity of an efflux pump for a certain medication by competition, induction or inhibition of the transport protein. Although transport proteins may be involved in drug interactions that alter the absorption, distribution, metabolism and elimination of medications, their main importance is within drug distribution.

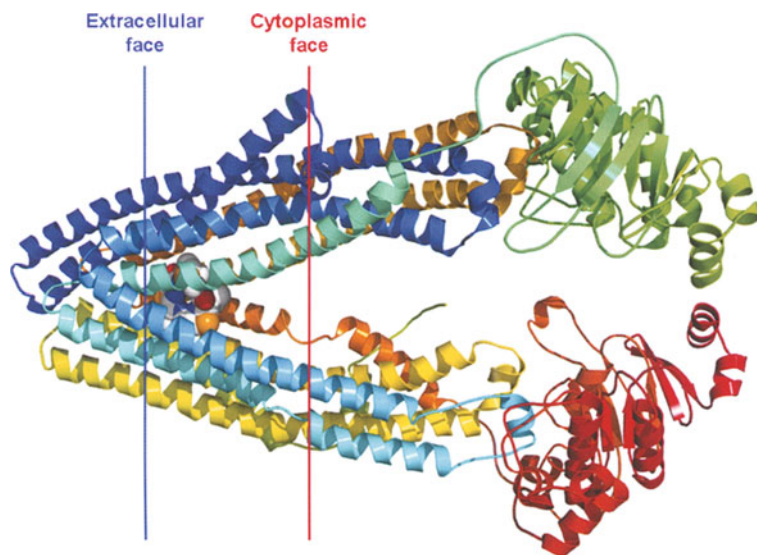


Fig. 2 Structure of P-glycoprotein (obtained from <http://en.wikipedia.org/wiki/P-glycoprotein>). The approximate positioning of the protein in the cell membrane is indicated by the blue (extracellular face) and red (cytoplasmic face) lines

P-glycoprotein (P-gp) which is encoded by the human multidrug resistance (MDR) gene belongs to the family of ATP-binding-cassette (ABC) transporters and is commonly considered to be the most important transporter (Fig. 2). P-gp is located throughout the human body, but is especially expressed at barriers like the blood brain barrier, the blood testis barrier, the placenta, the renal proximal tubuli, hepatic cells and the intestinal epithelium. In general, P-gp thereby aims at limiting exposure of the human body or certain areas towards xenobiotics and toxins, excreting drug into bile at the liver, into the intestinal lumen in the gut, into renal tubules in the kidney or into the blood stream from the brain and other organs or even from cells like lymphocytes. The considerable overlap in drug specificity for P-gp and CYP3A4, the most important liver isoenzyme (see Tables 1 and 2) further underlines the central role of P-gp in the bodies defence against potentially harmful substances.

Modulation of P-gp mediated transport has significant pharmacokinetic implications for the respective substrates. Pharmacokinetic interaction may occur at the systemic (blood concentrations), regional (organ or tissue concentrations) or local (intracellular concentrations) level [28]. P-gp has broad substrate specificity, transporting a large number of endogenous and exogenous substances. Examples of clinically important substrates of P-gp are presented in Table 1.

Many of the initially identified inhibitors of P-gp, like the calcium channel blocker verapamil or the immunosuppressive agent cyclosporin A turned out to be themselves

Table 1 Examples of P-glycoprotein substrates (modified from Ref. [29])

| Antineoplastic agents | Protease inhibitors | Corticoids | Others |
|-----------------------|---------------------|----------------|-------------------------------------|
| Vinblastine | Saquinavir | Dexamethasone | Cimetidine (H2-Receptor antagonist) |
| Vincristine | Ritonavir | Hydrocortisone | Loperamide (Antidiarrheal) |
| Paclitaxel | Nelfinavir | Corticosterone | Ondansetron (Antiemetic) |
| Docetaxel | Indinavir | Triamcinolone | Verapamil (Ca-channel blocker) |
| Mitoxantrone | Lopinavir | | Digoxin (Cardiac glycoside) |
| Etoposide | Amprenavir | | Cyclosporin A (Immunosuppressant) |
| Actinomycin D | | | Erythromycin (Antibiotic) |

substrates of P-gp, suggesting that they act as competitive inhibitors [29]. Clinically significant interactions with inhibitors of P-gp have been described. Gastrointestinal absorption of digoxin was significantly enhanced in presence of rifampin [30]. Loperamid, an opioid without central activity used for treatment of diarrhoea, was shown to cause respiratory depression when co-administred with P-gp inhibitors, which was linked to suppression of the gate keeper effects of P-gp at the blood brain barrier [31].

As previously mentioned, P-gp is also held responsible for the phenomenon of MDR. One example of P-gp induced MDR is failure of chemotherapy with different classes of cytotoxic agents like anthracyclins, vinca alkaloids, taxanes and epipodophylotoxins [32]. Resistance toward the cytotoxic agent is often not a pre-existing ability of the tumour but develops during the treatment. Increased expression of P-gp has been also determined in epileptogenic brain regions of patients with pharmacoresistant epilepsy [33]. Therefore, controlled inhibition of P-gp might yield an important therapeutic target in cancer chemotherapy and other indications [34]. Although P-gp inhibitors were developed as far as phase II none of these substances was approved as therapeutic agent so far [35].

Beside P-gp other transporters such as the multidrug resistance protein 1 (MRP1) and MRP2, the organic anion transport polypeptides (OATPs), organic cation transporters (OCTs) and multidrug resistance related proteins (MRPs) also contribute to drug distribution in the human body, although to a lesser extent than P-gp. Like P-gp, MRP1 has the capacity to mediate transport of many drugs and other compounds but has also a protective role in preventing accumulation of toxic compounds and drugs in epithelial tissue covering the choroid plexus/cerebrospinal fluid compartment, oral epithelium, sertoli cells, interstitial tubules and urinary collecting duct cells. MRP2 primarily transports weakly basic drugs and bilirubin from the liver to bile. Most compounds that efficiently block P-gp have only low affinity for MRP1 and MRP2. Currently there are only few effective and specific MRP inhibitors available, none of them being approved for clinical use in this indication [34].

Table 2 Important substrate, inhibitors and inducers of cytochrome P450 isoenzymes

| Ezyme | Class | Substrate | Inhibitor | Inducer |
|---------|---------------------------------------|--|---|--|
| CYP 3A4 | Analgetics | codeine fentanyl lidocaine methadone paracetamol | | |
| | Antiarrhythmics/ Antihypertensives | amiodarone amlodipine digitoxin diltiazem nifedipine propranolol salmeterol verapamil | amiodarone diltiazem verapamil | |
| | Antibiotics | clarithromycin erythromycin | clarithromycin erythromycin | rifabutin rifampin |
| | Antidepressives/ Antipsychotics | aripiprazole buspirone fluoxetine haloperidol quetiapine risperidone ziprasidone | fluvoxamine | |
| | Antidiabetic | | | pioglitazone troglitazone |
| | Antiepileptics/ Benzodiazepines | alprazolam carbamazepine diazepam midazolam triazolam | | carbamazepine oxcarbazepine phenobarbital phenytoin |
| | Antifungals | | fluconazole itraconazole ketoconazole voriconazole | |
| | Antivirals | indinavir nelfinavir ritonavir saquinavir | indinavir nelfinavir ritonavir saquinavir | efavirenz nevirapine |
| | Immune Modulators/ Zytostatics | cyclosporine docetaxel irinotecan sirolimus tacrolimus tamoxifen vincristine | imatinib | |

(continued)

Table 2 (Continued)

| Ezyme | Class | Substrate | Inhibitor | Inducer |
|--------|---------------------------------------|---|---|---|
| CYP1A2 | Statins | atorvastatin lovastatin simvastatin | | |
| | Steroids/Analogues | dexamethasone hydrocortisone progesterone testosterone | | dexamethasone |
| | Others | caffeine cocaine dextromethorphan ondansetron warfarin | cimetidine grapefruit juice | St. John's wort |
| | Analgetics | naproxen paracetamol ropivacaine | | |
| | Antiarrhythmics/ Antihypertensives | propranolol verapamil | amiodarone | |
| | Antidepressives/ Antipsychotics | amitriptyline clomipramine clozapine fluvoxamine haloperidol imipramine olanzapine cyclobenzaprine | fluvoxamine | |
| | Antiepileptics/ Benzodiazepines | | | |
| | Steroids/Analogues | estradiol | | |
| | Others | caffeine ondansetron theophylline tizanidine warfarin zolmitriptan | interferon | broccoli brussel sprouts grilled meat insulin omeprazole tobacco |
| CYP2C9 | Analgetics | diclofenac ibuprofen lornoxicam meloxicam naproxen piroxicam celecoxib | | |
| | Antiarrhythmics/ Antihypertensives | irbesartan losartan | amiodarone | |
| | Antibiotics | | isoniazid sulfamethoxazole sulfaphenazole | rifampin |
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(continued)

Table 2 (Continued)

| Ezyme | Class | Substrate | Inhibitor | Inducer |
|---------|--|---|---|---------------|
| CYP2C19 | Antidepressives/ Antipsychotics Antidiabetic | amitriptyline fluoxetine glibenclamide glimepiride glipizide glyburide nateglinide rosiglitazone tolbutamide phenytoin | fluvoxamine | secobarbital |
| | Antiepileptics/ Benzodiazepines | | | |
| | Antifungals | | fluconazole | |
| | Immune Modulators/ Zytostatics | tamoxifen | | |
| | Statins | fluvastatin | fluvastatin lovastatin | |
| | Others | warfarin | fenofibrate zafirlukast indomethacin | |
| | Analgetics | | | |
| | Antiarrhythmics/ Antihypertensives | propranolol | | |
| | Antibiotics | proguanil | | rifampin |
| | Antidepressives/ Antipsychotics | amitriptyline citalopram clomipramine hexobarbital imipramine | fluoxetine fluvoxamine | |
| | Antiepileptics/ Benzodiazepines | citalopram diazepam phenobarbital phenytoin | oxcarbazepine topiramate | carbamazepine |
| | Antifungals | | ketoconazole | |
| | Antivirals | nelfinavir | | |
| | Immune Modulators/ Zytostatics | cyclophosphamide | | |
| | Steroids/Analogues | progesterone | | prednisone |
| | Others | lansoprazole omeprazole pantoprazole rabeprazole warferin | cimetidine lansoprazole omeprazole pantoprazole rabeprazole | |
| | | codeine lidocaine oxycodone | celecoxib | |
| CYP2D6 | Analgetics | | | |

(continued)

Table 2 (Continued)

| Ezyme | Class | Substrate | Inhibitor | Inducer |
|-------|--|---|---|---------------|
| | Antiarrhythmics/ Antihypertensives | tramadol carvedilol metoprolol nebivolol propafenone propranolol timolol | amiodarone | |
| | Antibiotics Antidepressives/ Antipsychotics | amitriptyline aripiprazole chlorpromazine clomipramine desipramine duloxetine fluoxetine fluvoxamine haloperidol imipramine nortriptyline paroxetine perphenazine risperidone thioridazine venlafaxine | halofantrine bupropion chlorpromazine citalopram doxepin duloxetine escitalopram fluoxetine paroxetine sertraline | rifampin |
| | Antifungals Antivirals Immune Modulators/ Zytostatics Steroids/Analog Others | tamoxifen amphetamine dextromethorphan metoclopramide nicotine ondansetron | terbinafine ritonavir doxorubicin cimetidine cocaine diphenhydramine methadone metoclopramide ranitidine ticlopidine | dexamethasone |

Modified from University of Indiana [41] and EMEA (1997) “note for guidance on the investigation of drug interactions” [4]

6.1 ADME – interactions based on drug metabolism

The cytochrome P450 isoenzymes (CYPs) represent a superfamily of heamoprotein enzymes localized on the membrane of the endoplasmatic reticulum (Fig. 3). They are

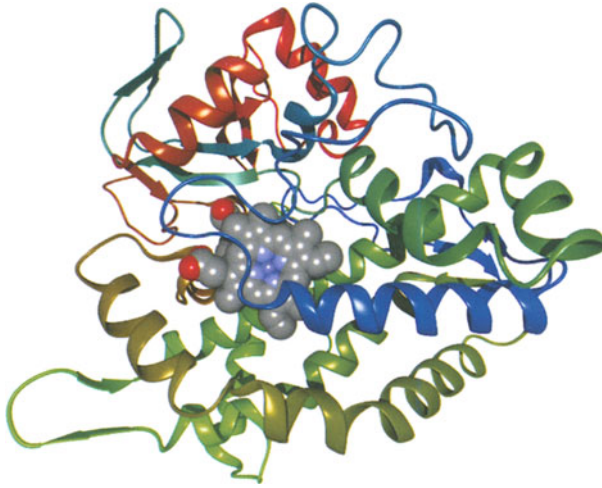


Fig. 3 Three dimensional structure of Cytochrome P450 (obtained from http://de.wikipedia.org/wiki/Cytochrom_P450)

responsible for catalyzing the metabolism of a large number of endogenous and exogenous compounds. CYPs are mainly based in the liver, but can be also found in lung, intestine, kidneys and other organs. The location of CYPs in the small bowel and liver permits an effect on both presystemic and systemic drug disposition.

Cytochrome P450 isoenzymes are identified by a code consisting of two numbers and a letter like CYP3A4, where the first number identifies the enzyme family, the letter the subfamily and the last number the individual genes [36]. While in humans approximately 20 families and subfamilies and 60 genes have been identified, the

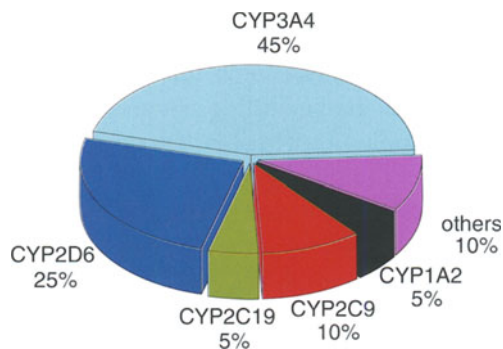


Fig. 4 Percentage of drugs metabolized by individual Cytochrome P450 isoenzymes (based on Kalra BS 2007) [36]

majority of drugs is metabolized by families 1, 2 and 3 (Fig. 4). Obviously drugs for which more than one pathway of metabolism and excretion exist, for example drugs which might be metabolized by different CYPs or may be also excreted as parent compound *via* bile or kidneys, are less susceptible to CYP450 interactions than those with only one possible pathway of elimination.

Inhibition of Cytochrome P450 isoenzymes leads to a decrease in the rate of hepatic biotransformation of the involved drugs causing increased serum concentration and possibly toxicity. Inhibition of CYP enzymes can be further classified into reversible inhibition and irreversible inhibition [37]. Reversible interactions are based on overlapping substrate specificity of CYPs, i.e. two drugs that are metabolized by the same isoenzyme compete for one enzyme binding site, and belong to the most common mechanisms in documented drug–drug interaction cases. The determinant of potency of an inhibitor is the strength of the bond between the binding site of the enzyme and the inhibitor. In contrast, irreversible inhibition is caused by reactive metabolites generated from CYP-catalyzed reactions. The first type of irreversible inhibition involves the formation of metabolic intermediate complexes. Inhibition of CYP3A4 by erythromycin is a well documented example that results from a metabolic intermediate complex [37]. Erythromycin contains a tertiary amine in the amino sugar ring. Transformation reactions, such as N-hydroxylation, N-demethylation and N-oxidation catalyzed by CYP3A, generates a nitroso metabolite that binds tightly to the haem portion of the CYP enzyme to form a stable metabolic intermediate complex, which is pharmacologically inactive.

Enzyme induction on the other hand is mainly based on enhancing the rate of enzyme synthesis by activation of the transcription of genes encoding for metabolic enzymes, probably by ligand activated nuclear receptors [38]. Enzyme induction by reduction of degradation due to protein stabilization of CYPs has been described but seems to be less important [36]. Obviously induction of a certain isoenzyme might lead to increased metabolism and decrease of elimination half life of substrates and thereby may shorten or weaken drug effect (“pharmacokinetic tolerance”).

While interactions based on induction or inhibition of cytochrome P450 isoenzymes start with the first dose of the modulating substance, maximum effect is often not seen before several days or weeks of application. The onset and duration of induction depends both on the kinetics of the drug and on the half life of CYP enzyme, which ranges from 1 to 6 days [39]. Usually it takes 4–14 days for peak induction, which may increase enzyme activity up to 40-fold. After withdrawing the inducer the enzyme activity returns to its original level in 1–3 weeks [40]. Thus, regulatory agencies recommend clinical investigation of interaction after multiple dose rather than single dose [4].

Since most drug–drug interactions involve CYPs it is important to identify substrates as well as inducers and inhibitors of CYPs to allow foreseeing certain drug interactions. Usually these investigations are based on *in vitro* or *in vivo* studies

combining the novel drug with typical probe drugs that act as well known substrates, inhibitors or inducers of a defined CYP isoenzyme.

Table 2 provides an overview of a range of substrates, inducers and inhibitors of the most important cytochrome P450 isoenzymes. Please note that the table should be considered as a list of certain drugs with high interaction potential rather than as an exhaustive list. The coexistence of many drugs both as substrate and as inhibitor of an isoenzyme highlights the prevalence of competitive inhibition.

Sex differences in cytochrome P450 activity have been reported with increased CYP3A4 activity in women compared with men while CYP1A2 activity is lower in females than males [42]. Inter-individual differences in the expression of certain isoenzymes may lead to differences in susceptibility with regard to efficacy and toxicity [43]. Genetic variations can cause a patient to metabolize drugs abnormally fast, abnormally slow, or not at all. Genetic polymorphism is the most common cause of the inter-individual differences in metabolism of CYP2D6 substrates, while CYP2C9 shows high inter-ethnic and intra-ethnic variability.

It has to be pointed out that most Cytochrome P450 mediated interactions do not preclude combination of certain drug classes as such, since metabolic pathways of different members of the same drug class may vary considerably. One example might be given by the frequently concomitantly used class of proton pump inhibitors. While all five proton pump inhibitors omeprazole, esomeprazole, lansoprazole, pantoprazole and rabeprazole are metabolized by CYPs, lansoprazole and pantoprazole are the most potent *in vitro* inhibitors of CYP2C19 and CYP2C9, respectively. On the other hand lansoprazole lacks interaction with CYP3A4, which is a relevant isoenzyme for metabolism of all other proton pump inhibitors [44].

Another class of substances which are typically associated with drug interactions are 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (statins). However, Fig. 5 depicts that individual statins are differently susceptible when exposed to typical CYP3A4 inhibitors [45]. Pravastatin, fluvastatin and cerivastatin (which was withdrawn from the market in 2001) apparently lack interaction with common probe drugs used to detect interactions based on CYP3A4 isoenzymes. On the other hand atorvastatin, lovastatin and simvastatin show up to 20-fold increase of systemic exposure after administration of isoconazole, erythromycin, mibefradil or grapefruit juice.

6.2 Examples for clinically relevant interactions based on CYP3A4

Although not all interactions based on the CYP3A4 isoenzyme that can be detected on a pharmacokinetic base are clinically relevant, many of them have been associated with fatal events. Torsades de pointes, a life-threatening ventricular arrhythmia associated with QT prolongation, can occur when CYP3A4 inhibitors are coadministered with terfenadine, astemizole, cisapride or pimozide [46]. As mentioned above, rhabdomyolysis has been reported after the coadministration of some statins and various CYP3A4

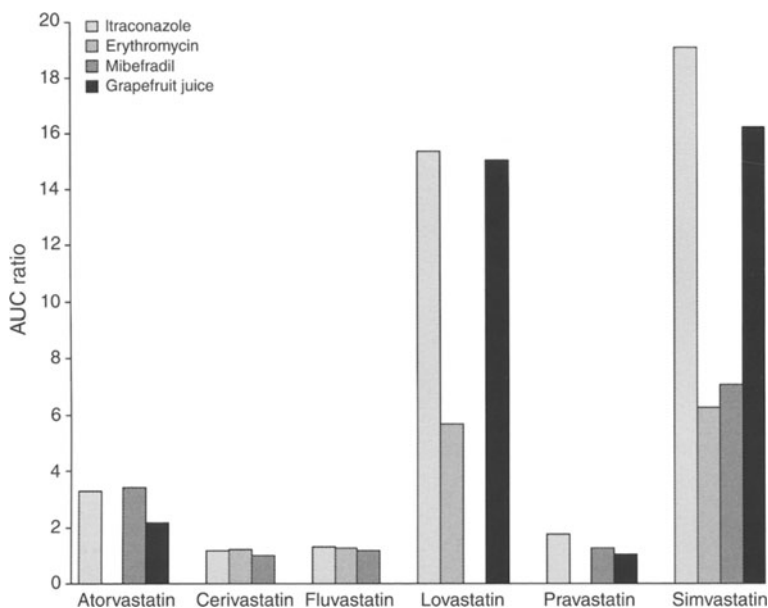


Fig. 5 Interactions of statins and various CYP3A4 inhibitors. “AUC ratio” is the area under the concentration–time curve (AUC) of the statin after combined administration divided by the AUC after administration alone; values close to 1 therefore indicate a lack of interaction [45]. The use of this figure was generously granted by Wolters Kluwer Pharma solutions

inhibitors [45, 47]. Excessive sedation, sometimes together with respiratory depression can result from concomitant administration of benzodiazepines (midazolam, triazolam, alprazolam or diazepam) or non-benzodiazepine (zopiclone and buspirone) hypnotics and CYP3A4 inhibitors [48, 49].

Likewise, induction of the CYP3A4 isoenzyme was associated with life threatening events. CYP3A4 inducers like rifampicin, barbiturates or some antiepileptic drugs may lead to decreased plasma levels of tacrolimus or cyclosporine promoting the risk of acute allograft rejection in transplant patients [50, 51]. Special care should be given to St. John's wort, a widely used over the counter antidepressant agent with significant CYP3A4 inducing activity, which has even been associated with cases of transplant rejection and many more interactions [52, 53]. Although not life threatening, the interaction of CYP3A4 inducers with oral contraceptives should be considered in young female patients under polypharmacy [54].

However, also beneficial drug interactions have been described for CYP3A4. Sometimes interactions can even be deliberately used to improve pharmacokinetics of a medication. One example of a drug interaction used in this context is the co-administration of carbidopa and levodopa for treatment of Parkinson's disease [55]. To

avoid metabolism of levodopa before it reaches the brain, the widely inactive carbidopa is co-administered to inhibit the peripheral metabolism of levodopa. Thereby more levodopa can reach the brain un-metabolized and peripheral side effects that would result from higher dosing of isolated levodopa can be reduced. Likewise administration of a CYP3A4 inhibitor with cyclosporin may allow reduction of the dosage and cost of the immunosuppressant. In HIV treatment the bioavailability of otherwise poorly absorbed protease inhibitors like saquinavir can be profoundly increased by the addition of ritonavir [56]. Beside decreasing costs of treatment this interaction most importantly may increase compliance of HIV patients by lowering their pill burden.

6.3 ADME – interactions based on drug excretion

The kidneys play a major role in the elimination of drugs. However, only for drugs where renal clearance is a major contributor to the total clearance (at least 50%) the potential for clinically significant renal drug–drug interactions is given. Four potential mechanisms exist for drug interactions at the renal level: (1) competition at a tubular secretion site resulting in a decrease in drug excretion; (2) competition at the tubular reabsorption site resulting in an increase in drug excretion; (3) a change in urinary pH and/or flow that may increase or decrease drug excretion depending on the pKa of the drug and (4) inhibition of renal drug metabolism [57]. Additionally also change in renal perfusion and change of the amount available for filtration (see interaction by protein binding above) may influence renal excretion.

The best known renal drug interaction is competitive inhibition of tubular secretion, ultimately leading to an increase in plasma drug concentration. Drugs like probenecid or salicylates may interfere with drugs that undergo active tubular secretion by inhibiting or competing for drug transport in the kidneys. Historically coadministration of probenecid with penicillin has been used to delay renal excretion of penicillin in order to reduce the required amount of the, at this time difficult to manufacture, antibiotic [58]. More recent examples include renal interactions following the coadministration of methotrexate and nonsteroidal antiinflammatory drugs (NSAIDs) [59].

Passive diffusion and reabsorption of drugs into and from urine may be altered by change of pH of urine. Plasma levels of salicylates and phenobarbital have been shown to decrease after administration of antacids or bicarbonate [60, 61]. In addition, change of pH of urine may affect activity of drugs that develop their main action in urine, like antibiotics in case of urinary tract infections [62].

7 Management of potential drug interactions

Often drug interactions can be avoided. Inappropriateness in choice of drug, dosage or administration route was reported in 50% of fatal cases of adverse drug reactions [7].

Being aware of the potential for interactions allows the physician to minimize risk by applying the following principles:

- Correct and up to date patient history.
- Identifying patients at high risk for developing interactions (i.e. elderly patients, preexisting polypharmacy and narrow therapeutic index of the medication).
- Avoiding unnecessary polypharmacy including OTC, food additives and herbs.
- Weighing the risk of the interaction against the benefits of a new medication.
- Determining if the interaction applies to all drugs within the same class or just a subset.
- Selecting an alternative agent with less interaction potential.
- Actively managing potential interactions by modification of administration schedules and dosage adjustments.
- Careful patient monitoring for clinical signs of drug interactions.
- If indicated and technically feasible therapeutic drug monitoring (measurement of blood levels of the drug).

Clinical pharmacologists, pharmaceutical industry and regulatory agencies have to provide clinicians with updated information regarding drug interactions by easy to handle media. Information on drug interaction can be obtained for the SPC of the individual drug as well as various commercially available software programmes. In addition, a range of online sites have recently been established to provide help for assessment of the potential of interaction either by providing updated lists of enzymatic pathways or by online drug interaction programmes. Examples include:

University of Indiana (<http://medicine.iupui.edu/flockhart>)

Drugs.com (http://www.drugs.com/drug_interactions.php)

FDA (<http://www.accessdata.fda.gov/Scripts/cder/DrugsatFDA>)

Medscape (<http://www.medscape.com/druginfo/druginterchecker>)

While these internet platforms have the advantage of continuously providing up to date information, attention should be paid to the source of information behind the page. In situations in which patients take multiple drugs, clinicians should always consider that interaction effects may be additive and should be aware that the extent of drug interactions is difficult to predict based on pharmacokinetic studies only examining two drugs.

Case Study: Interactions based on a drug class: antibiotics

In the western world antibiotics frequently are given as “ad on” to a persisting scheme of polypharmacy to deal with an acute illness, the infection. Many patients

receiving antibiotics are old or suffer from other conditions like malignant disease, chronic obstructive disease or diabetes, which usually are associated with a considerable number of baseline medications [63, 64]. Since the antibiotic usually is given for a very limited period of time, adjustment of the preexisting treatment regime is often considered too extensive.

However, some antibiotics belong to the most potent modulators of the Cytochrome P450 isoenzymes, i.e. active interaction due to antibiotics may impact preexisting medication. Passive modulation of the pharmacokinetics of antibiotic itself on the other hand is problematic for two reasons: first, most antibiotics have a narrow therapeutic index [65]. Second, antibiotics display a unique correlation between their pharmacokinetics and their pharmacodynamic effect. Beside the host defense, the success of an antibacterial treatment is widely driven by well defined correlations between pharmacokinetics of the antibiotic and their pharmacodynamic effect. The pharmacodynamic action of an antimicrobial is commonly described by the minimal inhibitory concentration (MIC), i.e. the concentration of an antimicrobial at which no visible growth of a given bacterial strain can be observed after 24 h. By combining the MIC with pharmacokinetic parameters pharmacokinetic/pharmacodynamic (PK/PD) indices are generated which can be used to predict antimicrobial efficacy of a treatment. C_{\max} (maximum concentration) and AUC_{0-24} (area under the concentration–time curve over 24 h) to MIC ratio (C_{\max}/MIC , AUC_{0-24}/MIC) as well as the time (t) that the concentration of the antibiotic exceeds the MIC ($t > MIC$) are considered as most important PK/PD indices (Fig. 6) [66–70]. The relevance of each of these indices for predicting antimicrobial and clinical outcome varies for different

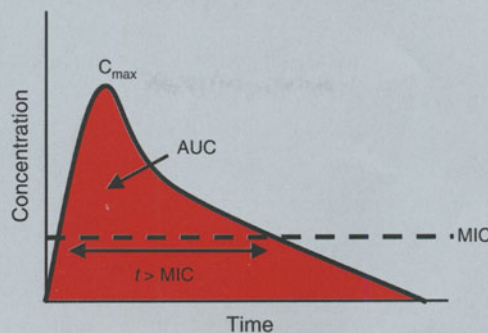


Fig. 6 Area under the concentration–time curve (AUC) and maximal concentration (C_{\max}) to the minimal inhibitory concentration (MIC) as well as the time period during which the concentration of the antibiotic exceeds the MIC ($t > MIC$) of a bacterium are considered the most important PK/PD parameters

antimicrobial classes. Beta-lactam-antibiotics display a “time-dependent” pattern of activity and $t > \text{MIC}$ is considered most predictive for outcome. In contrast, for aminoglycosides, the $C_{\text{max}}/\text{MIC}$ is a good predictive index and determines the antimicrobial efficacy. To achieve fast bacterial eradication aminoglycosides should be given infrequently in high doses as long as this is not precluded by toxicity. Thereby a simple delay of absorption might impact both antimicrobial action and side effect, even if the overall absorbed rate of the drug is not effected. Likewise, increased elimination of β -lactams by the kidney could reduce activity although the maximum concentration may not be impacted.

In the following examples of drug interactions with antibiotics will be given for all aspects of “ADME”.

Absorption

Active action of antibiotic: Erythromycin is a potent stimulator of gastrointestinal motility and can be a useful agent to treat gastrointestinal stasis in patients who are critically ill. However, it is not licensed for this indication and (beside other drug interactions caused by erythromycin, see below) the possible modulation of the uptake of other orally administered drugs should be kept in mind [71].

Alteration of normal gut flora by antibiotics has been proposed as a mechanism to explain alterations in the concentrations of several drugs, including digoxin, oral contraceptives and warfarin. It has been speculated that some cases of digoxin toxicity might be based on killing of the gut commensal *Eubacterium lentum* by macrolides, leading to a decrease in bacterial digoxin metabolism by these bacteria in the intestine and thereby increased systemic exposure [72, 73]. However, this pathway exists only in rare patients who are colonized with *E. lentum*. Carriers often can be identified by digoxin blood concentrations that are much lower than predicted by pharmacokinetic calculations. In this case appropriate therapy should include the selection of an alternative antibiotic without activity against *E. lentum* or, if this is not possible, a temporary reduction of digoxin dosage. Similarly, failure of oral contraceptives has been attributed to a reduction in the drug’s enterohepatic recirculation secondary to loss of hydrolysis of steroid conjugates by gut flora [74]. However, the relevance of this mechanism remains unclear. Other interaction leading to increased or decreased absorption might be based on inhibition (macrolides) or induction (rifampicin) of P-gp transport in the intestines (see below).

Antibiotic passively affected: Acid-peptic diseases belong to the most common illnesses in Western countries. Prevention of the absorption of antibacterials such as tetracycline, azithromycin and quinolones belong to the most important interac-

tions of H2 antagonists, proton pump inhibitors and prokinetic agents [75]. Most important, drug absorption may be limited by the formation of insoluble complexes that may result when drugs are exposed to di- and trivalent cations in the gastrointestinal tract. Quinolone or tetracycline antibiotics chelate with coadministered magnesium, aluminum, calcium or iron-containing products, significantly limiting absorption when coadministered within 2 h [76, 77]. Even if the bioavailability of a drug is not changed, delaying the absorption might reduce the C_{\max} and thereby the efficacy of concentration dependent drugs (see above). Indeed the clinical relevance of impaired absorption was demonstrated in 3134 patients who received a course of oral levofloxacin. Coadministration of divalent or trivalent cation-containing compounds was significantly associated with subsequent identification of a levofloxacin-resistant isolate [78]. Also azole antifungals like itraconazole or ketoconazole require acidic conditions for adequate absorption [79]. Therefore, most antimicrobials should be administered at least two hours before or after antacids and should be given with care when coadministered with proton pump inhibitors, H2 blockers or cation containing supplements. To prevent chelation of intravenous formulations quinolones or tetracyclines should not be given in the same intravenous line with multivalent cations.

Distribution

Antibiotic passively affected: It is well known that only the non protein bound fraction of an antibiotic is microbiologically active and can penetrate into the interstitial space fluid of tissues, where most infections are located [80–82]. Drug–drug interactions can lead to a disproportionate increase in free concentrations of protein-bound drugs [26]. The mechanism may be either competitive, meaning that drugs bind to the same site, or non-competitive, with one drug causing a conformational change in the protein molecule, which, in turn, inhibits

Table 3 Protein binding of selected highly bound antimicrobials (obtained from the approved labels)

| | |
|----------------|---------------------|
| Glycopeptides | Teicoplanin: 90–95% |
| Cephalosporins | Ceftriaxone: 85–95% |
| Carbapenems | Ertapenem: 92–95% |
| Tetracyclines | Doxycycline: 82% |
| | Tigecycline: 71–89% |
| Lipopeptides | Daptomycin: 90% |

the binding of the other drug [25]. Changes in the free fraction might become clinically relevant, when drugs with a narrow-therapeutic-range and a high degree of protein binding are administered or if protein binding interactions due to concomitant administration of other highly-protein-bound drugs are expected [26]. Table 3 provides examples for antibiotics with binding to plasma proteins above 80%.

Metabolism

Active action of antibiotic: Rifampin and its derivates as well as macrolides are most important modulators of the Cytochrome P450 system and P-gp (Table 4).

Rifampin (=Rifampicin) is indicated as component of the standard drug regiment for treatment of tuberculosis and for the prophylaxes of *Neisseria meningitidis* and *Staphylococcus aureus* infections. Among all antibiotics rifampin is the most potent inducer of the Cytochrome P450 isoenzymes and may cause severe drug interactions if this potency is not considered. The three commercially available rifamycin derivatives rifampin, rifabutin and rifapentine have different isoenzyme induction potencies. In vitro data demonstrate that rifampin is the most potent, followed by rifapentine and rifabutin [85]. Rifampin induces the isoenzymes CYP3A4, 2C8, 2C9, 2C19, 2B6 and the transporter P-gp [86]. When co-administered with drugs that are substrates of the same enzymes, their metabolism may be accelerated resulting in lower concentrations and less efficacy. The enzyme induction effect is only gradually reduced over a 1- to 2-week period, and sometimes longer, after rifampin is discontinued. Important CYP3A4 substrates are listed in Table 2. Rifampicin can cause acute transplant rejection in patients treated with immunosuppressive drugs, such as cyclosporin [87]. In addition, ri-

Table 4 Influence of antibiotics on Cytochrome P450 isoenzymes and P-gp [4, 5, 41, 83, 84]

| Drug | Inhibitor | Substrate | Inducer |
|-----------------------------|-----------|-----------|--------------------------------|
| Rifampin/Rifabutin | - | | 3A4, 2C8, 2C9, 2C19, 2B6, P-gp |
| Erythromycin/Clarithromycin | 3A4, P-gp | 3A4 | |
| Ciprofloxacin | 1A2 | | |
| Trimethoprim | 2C8 | | |
| Sulphamethoxazole | 2C9 | | |

fampicin reduces the plasma concentrations of methadone, potentially leading to symptoms of opioid withdrawal [88]. Rifampicin also induces CYP2C8/9/19-mediated metabolism and thus reduces the plasma concentrations of the CYP2C9 substrate warfarin, making frequent monitoring of anticoagulation necessary. In addition, rifampicin can reduce the plasma concentrations of drugs that are not metabolized by inducing drug transporters such as P-glycoprotein (see Table 1). Potential drug interactions should be considered whenever starting but also when discontinuing prolonged rifampicin treatment. It is particularly important to remember that the concentrations of many of the other drugs used by the patient will increase when rifampicin is discontinued after many month of tuberculostatic treatment as the induction starts to wear off [87].

Erythromycin and to a lesser extent clarithromycin and roxithromycin, commonly used macrolides, are known to be both substrates and inhibitors of CYP3A4 and P-gp. Complex interactions with potentially serious toxic consequences have been observed when this group of antibiotics was combined with CYP3A4 substrates. Concomitant use of macrolides with drugs like the benzodiazepine midazolam, which usually has a short half live, have been associated with massively prolonged sedation of patients [89, 90]. Theophylline intoxications have been described when this drug with narrow therapeutic index was coadministered with erythromycin, a common treatment combination for respiratory infect exacerbations [91]. If alternatives are available, erythromycin and clarithromycin should not be prescribed as part of complex drug regimes. Azithromycin is not an inhibitor of CYP3A4 and may be used as substitute if clinically indicated.

The fluoroquinolone ciprofloxacin is an inhibitor of CYP1A2. Even a low dose of ciprofloxacin can lead to a clinical significant increase of serum concentrations of the antiepileptic clozapine and systemical toxicities have been ascribed to concomitant use of ciprofloxacin and ropivacaine, a local anaesthetic drug [92, 93]. Levofloxacin and moxifloxacin are weak or no inhibitors of CYP1A2 and may be used as substitute [94].

Antibiotic passively affected: Macrolides and fluoroquinolones as well as other classes of antimicrobial agents have been associated with prolongation of cardiac repolarization including case reports of torsades de pointes [95]. Since erythromycin is extensively metabolized by CYP3A4 the risk of ventricular arrhythmias and sudden cardiac death was five fold increased by the concurrent use of strong inhibitors of CYP3A4 like antifungal agents, diltiazem or verapamil [96]. The concurrent use of erythromycin and strong inhibitors of CYP3A4 should thus be generally avoided.

Excretion

Active action of antibiotic: Active interactions of antibiotics with other drugs are rather due to pharmacodynamic aspects than to a change of the pharmacokinetics. Due to the nephrotoxic potential of aminoglycosides and vancomycin the concurrent use of other nephrotoxic agents such as amphotericin B, cisplatin or other cytostatic drugs should be avoided because of the potential of additive effects.

Antibiotic passively affected: As previously mentioned probenecid inhibits the renal excretion of beta-lactam antibiotics that are mainly eliminated by renal tubular secretion and its use may result in increased concentrations and prolonged elimination time [97]. Beta-lactams are known to compete also with other drugs for renal tubular secretion mediated by the organic anion transport system; however, this is usually not of major concern, given the wide therapeutic index of these antimicrobials. In contrast, therapeutic failure of beta-lactams might be related to coadministration of drugs which increase renal clearance by means of enhanced cardiac output and/or renal blood flow, such as dopamine, dobutamine and furosemide [98]. Therefore, in settings like intensive care, where concomitant use of such agents with beta-lactams is common, standard dosing regimes should be reconsidered.

In conclusion, in most cases significant interactions with antibiotics occur when antibiotics are used together with complex therapeutic regimes in patients under polypharmacy. Where possible antibiotics like rifampin, erythromycin, clarithromycin and ciprofloxacin should be avoided in those patients or, at least, the patients should be carefully monitored for occurrence of drug interactions.

References

1. Ingelman-Sundberg M (2001) Pharmacogenetics: an opportunity for a safer and more efficient pharmacotherapy. *J Intern Med* 250: 186–200
2. Lazarou J, Pomeranz BH, Corey PN (1998) Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies. *JAMA* 279: 1200–1205
3. Marroum PJ, Uppoor RS, Parmelee T, et al. (2000) In vivo drug–drug interaction studies – a survey of all new molecular entities approved from 1987 to 1997. *Clin Pharmacol Ther* 68: 280–285
4. EMEA (1997) Note for guidance on the investigation of drug interactions (www.emea.europa.eu/pdfs/human/ewp/056095en.pdf)
5. FDA (2004) Drug interaction studies – study design, data analysis, and implications for dosing and labeling. (http://www.fda.gov/ohrms/dockets/ac/04/briefing/2004-4079B1_04_Topic2-TabA.pdf)

6. Johnell K, Klarin I (2007) The relationship between number of drugs and potential drug–drug interactions in the elderly: a study of over 600,000 elderly patients from the Swedish Prescribed Drug Register. *Drug Safety* 30: 911–918
7. Buajordet I, Ebbesen J, Erikssen J, et al. (2001) Fatal adverse drug events: the paradox of drug treatment. *J Intern Med* 250: 327–341
8. Goldberg RM, Mabee J, Chan L, Wong S (1996) Drug–drug and drug–disease interactions in the ED: analysis of a high-risk population. *Am J Emerg Med* 14: 447–450
9. Dresser GK, Spence JD, Bailey DG (2000) Pharmacokinetic-pharmacodynamic consequences and clinical relevance of cytochrome P450 3A4 inhibition. *Clin Pharmacokinet* 38: 41–57
10. Routledge PA, O'Mahony MS, Woodhouse KW (2004) Adverse drug reactions in elderly patients. *Br J Clin Pharmacol* 57: 121–126
11. Beijnen JH, Schellens JH (2004) Drug interactions in oncology. *Lancet Oncol* 5: 489–496
12. Smith RG (2009) An appraisal of potential drug interactions in cigarette smokers and alcohol drinkers. *J Am Podiatr Med Assoc* 99: 81–88
13. Staffa JA, Chang J, Green L (2002) Cerivastatin and reports of fatal rhabdomyolysis. *N Engl J Med* 346: 539–540
14. Gugler R, Allgayer H (1990) Effects of antacids on the clinical pharmacokinetics of drugs. An update. *Clin Pharmacokinet* 18: 210–219
15. Rawashdeh NM, al-Hadidi HF, Irshaid YM, Battah AK (1993) Gastrointestinal dialysis of digoxin using cholestyramine. *Pharmacol Toxicol* 72: 245–248
16. Jones NS, Quraishi S, Mason JD (1997) The nasal delivery of systemic drugs. *Int J Clin Pract* 51: 308–311
17. Vora JP, Burch A, Peters JR, Owens DR (1993) Absorption of radiolabelled soluble insulin in type 1 (insulin-dependent) diabetes: influence of subcutaneous blood flow and anthropometry. *Diabet Med* 10: 736–743
18. Melander A (1978) Influence of food on the bioavailability of drugs. *Clin Pharmacokinet* 3: 337–351
19. Ptachcinski RJ, Venkataramanan R, Rosenthal JT, et al. (1985) The effect of food on cyclosporine absorption. *Transplantation* 40: 174–176
20. Liedholm H, Melander A (1986) Concomitant food intake can increase the bioavailability of propranolol by transient inhibition of its presystemic primary conjugation. *Clin Pharmacol Ther* 40: 29–36
21. Welling PG, Kendall MJ, Dean S, et al. (1980) Effect of food on the bioavailability of alafosfalin, a new antibacterial agent. *J Antimicrob Chemother* 6: 373–379
22. Fasano M, Curry S, Terreno E, et al. (2005) The extraordinary ligand binding properties of human serum albumin. *IUBMB Life* 57: 787–796
23. Bergogne-Berezin E (2002) Clinical role of protein binding of quinolones. *Clin Pharmacokinet* 41: 741–750
24. Wise R (1983) Protein binding of beta-lactams: the effects on activity and pharmacology particularly tissue penetration. II. Studies in man. *J Antimicrob Chemother* 12: 105–118
25. Lindup WE, Orme MC (1981) Clinical pharmacology: plasma protein binding of drugs. *Br Med J (Clin Res Ed)* 282: 212–214
26. Dasgupta A (2002) Clinical utility of free drug monitoring. *Clin Chem Lab Med* 40: 986–993
27. Odell GB (1959) The dissociation of bilirubin from albumin and its clinical implications. *J Pediatr* 55: 268–279
28. Padowski JM, Pollack GM (2010) Pharmacokinetic and pharmacodynamic implications of P-glycoprotein modulation. *Method Mol Biol* 596: 359–384
29. Schinkel AH, Jonker JW (2003) Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: an overview. *Adv Drug Deliv Rev* 55: 3–29
30. Greiner B, Eichelbaum M, Fritz P, et al. (1999) The role of intestinal P-glycoprotein in the interaction of digoxin and rifampin. *J Clin Invest* 104: 147–153

31. Kobayashi M, Saitoh H, Yamaguchi M, et al. (2005) Relationship between loperamide-induced sedative effect and digoxin pharmacokinetics in healthy Japanese subjects. *Pharm Res* 22: 413–418
32. Goda K, Bacso Z, Szabo G (2009) Multidrug resistance through the spectacle of P-glycoprotein. *Curr Cancer Drug Targets* 9: 281–297
33. Luna-Tortos C, Fedrowitz M, Loscher W (2010) Evaluation of transport of common antiepileptic drugs by human multidrug resistance-associated proteins (MRP1, 2 and 5) that are overexpressed in pharmacoresistant epilepsy. *Neuropharmacology* 58(7): 1019–32
34. Liang XJ, Aszalos A (2006) Multidrug transporters as drug targets. *Curr Drug Targets* 7: 911–921
35. Fox E, Bates SE (2007) Tariquidar (XR9576): a P-glycoprotein drug efflux pump inhibitor. *Expert Rev Anticancer Ther* 7: 447–459
36. Kalra BS (2007) Cytochrome P450 enzyme isoforms and their therapeutic implications: an update. *Indian J Med Sci* 61: 102–116
37. McGinnity DF, Riley RJ (2001) Predicting drug pharmacokinetics in humans from in vitro metabolism studies. *Biochem Soc Trans* 29: 135–139
38. Wei P, Zhang J, Egan-Hafley M, et al. (2000) The nuclear receptor CAR mediates specific xenobiotic induction of drug metabolism. *Nature* 407: 920–923
39. Xie W, Barwick JL, Simon CM, et al. (2000) Reciprocal activation of xenobiotic response genes by nuclear receptors SXR/PXR and CAR. *Genes Dev* 14: 3014–3023
40. Tanaka E, Hisawa S (1999) Clinically significant pharmacokinetic drug interactions with psychoactive drugs: antidepressants and antipsychotics and the cytochrome P450 system. *J Clin Pharm Ther* 24: 7–16
41. Flockhart D (2007) Drug Interactions: Cytochrome P450 Drug Interaction Table. Indiana University School of Medicine. <http://medicine.iupui.edu/clinpharm/ddis/table.asp>
42. Brosen K (2007) Sex differences in pharmacology. *Ugeskr Laeger* 169: 2408–2411
43. Snawder JE, Lipscomb JC (2000) Interindividual variance of cytochrome P450 forms in human hepatic microsomes: correlation of individual forms with xenobiotic metabolism and implications in risk assessment. *Regul Toxicol Pharmacol* 32: 200–209
44. Li XQ, Andersson TB, Ahlstrom M, Weidolf L (2004) Comparison of inhibitory effects of the proton pump-inhibiting drugs omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole on human cytochrome P450 activities. *Drug Metab Dispos* 32: 821–827
45. Muck W (2000) Clinical pharmacokinetics of cerivastatin. *Clin Pharmacokinet* 39: 99–116
46. Altmann D, Eggmann U, Ammann P (2008) Drug induced QT prolongation. *Wien Klin Wochenschr* 120: 128–135
47. Zeitlinger M, Muller M (2003) Clinico-pharmacologic explanation models of cerivastatin associated rhabdomyolysis. *Wien Med Wochenschr* 153: 250–254
48. Nivoix Y, Ubeaud-Sequier G, Engel P, et al. (2009) Drug–drug interactions of triazole antifungal agents in multimorbid patients and implications for patient care. *Curr Drug Metab* 10: 395–409
49. Hesse LM, von Moltke LL, Greenblatt DJ (2003) Clinically important drug interactions with zopiclone, zolpidem and zaleplon. *CNS Drugs* 17: 513–532
50. Staatz CE, Tett SE (2004) Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation. *Clin Pharmacokinet* 43: 623–653
51. Kelly P, Kahan BD (2002) Review: metabolism of immunosuppressant drugs. *Curr Drug Metab* 3: 275–287
52. Ruschitzka F, Meier PJ, Turina M, et al. (2000) Acute heart transplant rejection due to Saint John's wort. *Lancet* 355: 548–549
53. Markowitz JS, Donovan JL, DeVane CL, et al. (2003) Effect of St John's wort on drug metabolism by induction of cytochrome P450 3A4 enzyme. *JAMA* 290: 1500–1504
54. Burakgazi E, Harden C, Kelly JJ (2009) Contraception for women with epilepsy. *Rev Neurol Dis* 6: E62–E67

55. Mars H (1974) Levodopa, carbidopa, and pyridoxine in Parkinson disease. *Metabolic interactions. Arch Neurol* 30: 444–447
56. Gallant JE (1998) A review of dual protease inhibitor therapy. *Hopkins HIV Rep* 10: 1, 4–5
57. Bonate PL, Reith K, Weir S (1998) Drug interactions at the renal level. Implications for drug development. *Clin Pharmacokinet* 34: 375–404
58. Frisk AR, Diding N, Wallmark G (1952) Influence of probenecid on serum penicillin concentration after oral administration of penicillin. *Scand J Clin Lab Invest* 4: 83–88
59. Frenia ML, Long KS (1992) Methotrexate and nonsteroidal antiinflammatory drug interactions. *Ann Pharmacother* 26: 234–237
60. Proudfoot AT, Krenzelok EP, Vale JA (2004) Position paper on urine alkalinization. *J Toxicol Clin Toxicol* 42: 1–26
61. Hansten PD, Hayton WL (1980) Effect of antacid and ascorbic acid on serum salicylate concentration. *J Clin Pharmacol* 20: 326–331
62. Zeiler HJ (1985) Influence of pH and human urine on the antibacterial activity of ciprofloxacin, norfloxacin and ofloxacin. *Drugs Exp Clin Res* 11: 335–338
63. Cheer K, Shearman C, Jude EB (2009) Managing complications of the diabetic foot. *BMJ* 339: b4905
64. Tam-McDevitt J (2008) Polypharmacy, aging, and cancer. *Oncology (Williston Park)* 22: 1052–1055, discussion 1055, 1058, 1060
65. Ament PW, Bertolino JG, Liszewski JL (2000) Clinically significant drug interactions. *Am Fam Physician* 61: 1745–1754
66. Hyatt JM, McKinnon PS, Zimmer GS, Schentag JJ (1995) The importance of pharmacokinetic/pharmacodynamic surrogate markers to outcome. Focus on antibacterial agents. *Clin Pharmacokinet* 28: 143–160
67. Toutain PL, del Castillo JR, Bousquet-Melou A (2002) The pharmacokinetic-pharmacodynamic approach to a rational dosage regimen for antibiotics. *Res Vet Sci* 73: 105–114
68. Nicolau DP (2001) Predicting antibacterial response from pharmacodynamic and pharmacokinetic profiles. *Infection* 29(Suppl 2): 11–15
69. Frimodt-Moller N (2002) How predictive is PK/PD for antibacterial agents? *Int J Antimicrob Agents* 19: 333–339
70. Vogelmann B, Gudmundsson S, Leggett J, et al. (1988) Correlation of antimicrobial pharmacokinetic parameters with therapeutic efficacy in an animal model. *J Infect Dis* 158: 831–847
71. Bradley C (2001) Erythromycin as a gastrointestinal prokinetic agent. *Intensive Crit Care Nurs* 17: 117–119
72. Laberge P, Martineau P (1997) Clarithromycin-induced digoxin intoxication. *Ann Pharmacother* 31: 999–1002
73. Morton MR, Cooper JW (1989) Erythromycin-induced digoxin toxicity. *DICP* 23: 668–670
74. Orme ML, Back DJ (1990) Factors affecting the enterohepatic circulation of oral contraceptive steroids. *Am J Obstet Gynecol* 163: 2146–2152
75. Flockhart DA, Desta Z, Mahal SK (2000) Selection of drugs to treat gastro-oesophageal reflux disease: the role of drug interactions. *Clin Pharmacokinet* 39: 295–309
76. Barton TD, Fishman NO, Weiner MG, et al. (2005) High rate of coadministration of di- or tri-valent cation-containing compounds with oral fluoroquinolones: risk factors and potential implications. *Infect Control Hosp Epidemiol* 26: 93–99
77. Neuvonen PJ (1976) Interactions with the absorption of tetracyclines. *Drugs* 11: 45–54
78. Cohen KA, Lautenbach E, Weiner MG, et al. (2008) Coadministration of oral levofloxacin with agents that impair absorption: impact on antibiotic resistance. *Infect Control Hosp Epidemiol* 29: 975–977
79. Van Der Meer JW, Keuning JJ, Scheijgrond HW, et al. (1980) The influence of gastric acidity on the bio-availability of ketoconazole. *J Antimicrob Chemother* 6: 552–554

80. Craig WA, Kunin CM (1976) Significance of serum protein and tissue binding of antimicrobial agents. *Annu Rev Med* 27: 287–300
81. Merrikin DJ, Briant J, Rolinson GN (1983) Effect of protein binding on antibiotic activity in vivo. *J Antimicrob Chemother* 11: 233–238
82. Beer J, Wagner CC, Zeitlinger M (2009) Protein binding of antimicrobials: methods for quantification and for investigation of its impact on bacterial killing. *AAPS J* 11: 1–12
83. Granfors MT, Backman JT, Neuvonen M, Neuvonen PJ (2004) Ciprofloxacin greatly increases concentrations and hypotensive effect of tizanidine by inhibiting its cytochrome P450 1A2-mediated presystemic metabolism. *Clin Pharmacol Ther* 76: 598–606
84. Wen X, Wang JS, Backman JT, et al. (2002) Trimethoprim and sulfamethoxazole are selective inhibitors of CYP2C8 and CYP2C9, respectively. *Drug Metab Dispos* 30: 631–635
85. Weber A, Kaplan M, Chughtai SA, et al. (2001) CYP3A inductive potential of the rifamycins, rifabutin and rifampin, in the rabbit. *Biopharm Drug Dispos* 22: 157–168
86. Glaeser H, Drescher S, Eichelbaum M, Fromm MF (2005) Influence of rifampicin on the expression and function of human intestinal cytochrome P450 enzymes. *Br J Clin Pharmacol* 59: 199–206
87. Niemi M, Backman JT, Neuvonen M, Neuvonen PJ (2003) Effects of gemfibrozil, itraconazole, and their combination on the pharmacokinetics and pharmacodynamics of repaglinide: potentially hazardous interaction between gemfibrozil and repaglinide. *Diabetologia* 46: 347–351
88. Bending MR, Skacel PO (1977) Rifampicin and methadone withdrawal. *Lancet* 1: 1211
89. Kharasch ED, Russell M, Mautz D, et al. (1997) The role of cytochrome P450 3A4 in alfentanil clearance. Implications for interindividual variability in disposition and perioperative drug interactions. *Anesthesiology* 87: 36–50
90. Gascon MP, Dayer P, Waldvogel F (1989) Drug interactions of midazolam. *Schweiz Med Wochenschr* 119: 1834–1836
91. Rieder MJ, Spino M (1988) The theophylline-erythromycin interaction. *J Asthma* 25: 195–204
92. Jokinen MJ, Olkkola KT, Ahonen J, Neuvonen PJ (2003) Effect of ciprofloxacin on the pharmacokinetics of ropivacaine. *Eur J Clin Pharmacol* 58: 653–657
93. Raaska K, Neuvonen PJ (2000) Ciprofloxacin increases serum clozapine and N-desmethylozapine: a study in patients with schizophrenia. *Eur J Clin Pharmacol* 56: 585–589
94. Shakeri-Nejad K, Stahlmann R (2006) Drug interactions during therapy with three major groups of antimicrobial agents. *Expert Opin Pharmacother* 7: 639–651
95. Iannini PB (2002) Cardiotoxicity of macrolides, ketolides and fluoroquinolones that prolong the QTc interval. *Expert Opin Drug Safety* 1: 121–128
96. Ray WA, Murray KT, Meredith S, et al. (2004) Oral erythromycin and the risk of sudden death from cardiac causes. *N Engl J Med* 351: 1089–1096
97. Cox VC, Zed PJ (2004) Once-daily cefazolin and probenecid for skin and soft tissue infections. *Ann Pharmacother* 38: 458–463
98. Pea F, Furlanut M (2001) Pharmacokinetic aspects of treating infections in the intensive care unit: focus on drug interactions. *Clin Pharmacokinet* 40: 833–868

CHAPTER 20

“Non-chemical” drugs: biologicals, protein therapeutics, vaccines and antisense therapeutics

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1 Introduction

It has long been recognized that living organisms have an astonishing ability to develop biochemical survival strategies [1]. One example for such a strategy is the mammalian immune system – an adaptive response to evolutionary challenges by microorganisms. In the past, numerous attempts have been made to exploit these endogenous “biological” survival strategies for medicine. One of the first successful attempts to employ a “biological” in this regard was the introduction of the variola vaccine by Jenner in 1796, at a time when the armamentarium of traditional chemical drugs had been notoriously poor. In the beginning of the 20th century, however, a revolution in chemistry and pharmacology overshadowed the “biological” by a “xenobiotic” concept and led to an explosion of our therapeutic options by providing the more than 10,000 traditional chemicals that we employ in medical practice today. Although our 100 years of experience with traditional chemicals have proven extremely successful, major challenges to our current drug development strategies have arisen by concerns about side effect profiles of many drugs and a perceived reduction in research productivity [1, 2]. Since the dawn of the 21st century we have witnessed the long expected and increasingly successful implementation of biotechnology derived pharmaceuticals (“biologicals”) in the medical practice. The term “biotechnology” was allegedly coined by Karl Ereky, a hungarian engineer in 1919 and related to techniques that had been employed by mankind for thousands of years to produce improved food products e.g. beer by the Sumerians as early as 6000 B.C. In contrast to the more traditional small molecular chemicals, biologicals are derived from living organisms like bacteria, yeast or even larger animals like goat or cow. Biologicals comprise a heterogenous group of pharmaceutical products, notably blood products, recombinant proteins, gene ther-

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apeutic products and cellular products. Due to their specific characteristics, biologicals introduce major challenges to our traditional concepts of drug development and routine practice of therapeutic medicine. Biologicals can be distinguished from traditional chemicals by a number of unique features, e.g. molecule size, low thermostability, species specificity and mode of administration. Therefore, biologicals do not only constitute novel pharmaceutical agents but represent an entirely different class of drugs which, unlike chemicals, do not follow well established paths, both in development and in practical use.

Historically, biologicals have been developed in 3 increasingly bigger waves. Following the identification of restriction enzymes in 197 by Paul Berg, the first recombinant protein, Somatostatin, was brought to the market by the US-company Genentech in 1977, followed by the US-Food and Drug Administration (FDA) approval of recombinant insulin in 1982. This “first” wave of biotechnology products was mostly a substitution strategy for patients lacking endogenous biological counterparts, conceptually similar to the idea of substituting blood components. The “second” wave started with the marketing authorization of the immunosuppressive antibody muromonab-CD3 (OKT3) in 1986, recombinant tissue plasminogen activator (rTPA) in 1987, followed by Interleukin-2 in 1988. These products were not intended to substitute the lack of their endogenous counterparts any more but were aimed at exerting an additional biological effect in a pharmacological sense, mostly in the central blood compartment. The “third” wave started in the mid- 1990s with abciximab, rituximab and infliximab and brought a broader therapeutic base, also targeting tissue pathologies. Today we are in the middle of this 3rd wave which resulted in hundreds of potential therapeutics in development. This development was accelerated by the event of human genome sequencing which – by high-throughput screening for differentially expressed genes – provided a large number of new and potentially clinically important molecular targets, which can be specifically regulated by biologicals, notably monoclonal antibodies.

2 A need for alternatives to traditional chemicals

Despite the obvious and tremendous success of traditional chemical drugs in medicine a number of reports from various stakeholders have recently cast doubt on the efficiency of our current paradigms of practical drug use and drug development ([1–4], Chapter 2). These paradigms have been virtually unchanged for many decades and have led to a long, costly, and seemingly not very efficient path of drug candidates to clinical practice. In the FDA’s view, for instance, “the applied sciences needed for medical product development have not kept pace with the tremendous advances in the basic sciences” [2]. Fuelled by a number of recalled drugs and recent spectacular drug failures, adverse drug reactions have become one of the most important issues in drug development. Adverse drug reactions (ADR) also occur in about 5% of all pharmacologically treated

patients (Chapter 18). Between 2% and 20% of all hospital admissions are caused by ADR, and approximately 10% of all hospitalized patients experience an ADR during their hospital stay [5, 6]. Many problems have been identified as a result of drug action on the HERG K(+) channel and QTc prolongation [7] or drug interactions *via* cytochrome P450. In late clinical development, over 90% of the market withdrawals were caused by drug toxicity with hepatotoxicity and cardiovascular toxicity as the major causes for two out of three market withdrawals [8]. Liver-toxicity problems were recently observed with troglitazone, trovafloxacin, leflunomide, telithromycin, tolcapone or ximelagatran [9].

These issues which are the result of the “pleiotropy” of “dirty” chemicals, as opposed to “clean”, targeted and biological drugs, have resulted in substantial attrition in available drug products. One advantage of biologicals relates to the fact that they do not undergo metabolism in a strict sense and therefore are unlikely to lead to the above described spectrum of drug interactions and adverse drug reactions.

Despite these concerns with traditional chemicals, there is much to win from the traditional small molecular approach. Given the experience in small chemicals development and the fact that only 500 of approximately 10,000 potential molecular targets have yet been exploited [10], there is enormous room for further successes. Driven by product champions like gefitinib and imatinib a targeted small molecule approach based on genomic target identification and high throughput screening has emerged as a novel drug discovery paradigm (Chapters 2 and 15). A targeted approach, however, will be more successful for disease states with “simple” pathophysiologies like chronic myeloid leukemia but will probably not add much to “complex” diseases.

3 Specifics of the development process of biologicals

Although the scientific community has substantial experience with preclinical and clinical development of conventional chemicals it is important to note that those concepts cannot easily be gleaned for the development of biologicals.

In Europe, biologicals have to undergo a centralized approval process at the European Medicines Agency (EMA). Although guidance documents on biological development are evolving [11] there is still considerable uncertainty about different aspects.

Preclinical. Standard preclinical testing, e.g. genotoxicity studies, is not always appropriate for biologics [12]. Inter alia, this relates to the fact that many biologicals exert bell shaped rather than sigmoid dose response relationships. Therefore, the definition of an optimal biological dose “OBD” is more appropriate than definitions of thresholds like “no-observed-adverse-effect-levels” (NOAEL) or “maximum-tolerated-doses” (MTD). Also, toxicological concerns for biologicals are closer related to exaggerated pharmacology than “true” toxicology in a strict sense. A notable example

Table 1 Historical development of “biologicals”. Biologicals have been developed in a number of increasingly productive waves, starting with a 1st waves in the early 1970s

| 1st Wave | 2nd ware | 3rd wave | Present |
|--------------|---------------|--------------------------|----------------------------|
| Insulin | OKT3 | Filgrastim (Neupogen) | Bevacizumab (Avastin) |
| Somatostatin | rTPA | Abciximab (Reopro) | Cetuximab (Erbitlux) |
| | Interleukin 2 | Rituximab (Rituxan) | Omalizumab (Xolair) |
| | Interferon | Infliximab (Remicaide) | Natalizumab (Tysabri) |
| | Erythropoetin | Etanercept (Enbrel) | Adalimumab (Humira) |
| | | Transtuzumab (Herzeptin) | Efalizumab (Raptiva) |
| | | | Alemtuzumab (Campath) |
| | | | Glargine-Insulin (Lantus) |
| | | | Drotrecogin-alpha (Xigris) |
| | | | Anakinra (Kineret) |
| | | | Abatacept (Orencia) |
| | | | Pegaptanib (Macugen) |
| | | | Tositumomab (Bexxar) |

Table 1 adapted from Ref. [1]

was a recent phase I trial in the UK where six volunteers had to be admitted to an intensive care unit after administration of an activating CD28 T-cell super-antibody that was considered “safe” in animals. One of the lessons of this trial is that preclinical toxicity experiments should not only take mere product characteristics into account but also biological effects of the murine equivalent if the compound or its target are not expressed in animals. In the case of the UK trial, the CD28 T-cell surface receptor was shown to share only 68% homology between mouse and man. Thus, lack of severe toxicity in animal models should never be viewed as a guarantee of safety in man [13, 14]. The generation of meaningful preclinical data is therefore crucially dependent on the selection of a relevant biological model and an appropriate species and may not be viewed as a standard battery of tests similar to conventional chemicals. Important selection criteria for a biological model relate to protein homologies in animals, murine counterparts of endogenous molecules and cross-reactivities between species. In contrast to chemicals, primates may be considered as the most appropriate test species for biologicals, rather than rats or dogs.

Clinical. Due to the specific complexities of biological drug development the traditional phase 1–4 concept appears obsolete. In oncology for example the well established concept of dose escalation to the maximum tolerated dose (MTD) is not appropriate for many biologicals [15]. Other approaches like target regulation approaches by *in vivo* imaging or early use of biomarkers to guide dosing and define an optimal biological dose (OBD) might provide a better handle on biological activity. In contrast to chemicals, idiosyncrasies or immunogenicity rather than a sigmoid dose-effect and dose-toxicity

relationship might drive side effects like in the particular case of vaccines [16]. Still, although adverse events might rather be related to idiosyncrasies of the human immune system and are not readily predictable from animal data, a conservative approach to dose escalation is recommended particularly for early development phases as there is a certain probability of a dose-adverse-event relation. For phase I studies, a decision must be made on whether to test the new product in volunteers or patients. In case of targeted biologicals several factors favour selection of patients. Most importantly, the side effect profile in patients who express a target might be different from volunteers without the target. A notable example is the case of Alzheimer vaccines [17] where extracellular beta-amyloid is only expressed in patients and the cases of meningoencephalitis (overall 18/300 (6%)), in an immunization study on patients might have been undetected in volunteers (18, see also case study). Targeted approaches and co-development of biologicals with target biomarkers like her2/neu trastuzumab is likely to become a frequent event.

Side effects. Evolutionary forces have shaped the extremely diverse human cytochrome P450 system as a defense strategy to herbal nutrients and exogenous toxins. Therefore human being are well adapted to protect themselves from side effects of “xenobiotics” like conventional drugs. From a teleological point of view, however, it was never foreseen to administer an “endobiotic”, e.g. an antibody in pharmacological doses to human beings. Therefore, there is much to learn from side effect profiles of biologicals besides their well known immunogenicity. In contrast to chemicals, biologicals display “atypical” side effects. This is illustrated by the wellknown immunosuppressive effects of anti-TNF strategies, but also by more recent examples of progressive multifocal leukoencephalopathy (PMLE) with natalizumab [19] or heart failure with trastuzumab [20, 21]. The term “translational medicine” is thus not a one way “from-bench-to-bedside” ticket for biologicals but also indicates, at times, a need to step back again from the clinic to the bench-side.

In vivo pharmacokinetics and pharmacodynamics. Traditional pharmacokinetics might help to predict human “biological” drug levels in biological fluids from animal data and, thus, serves as an important tool to predict a suitable dose. Biologicals are characterized by specific pharmacokinetic (PK) features [12]. The delivery of biologicals is limited to special routes of administration, mostly the parenteral route and for some cases like insulin also the pulmonary route. This means that often 100% bioavailability is reached, but the volume of distribution might be substantially affected by specific and unspecific binding. Unspecific binding might also have pharmacodynamic (PD) consequences since only the unbound drug fraction confers bio-reactivity. Several biologicals also exhibit non-linear kinetics meaning that the half life of a drug is dose dependent.

This can be explained by specific binding of e.g. an antibody to its target, a process which follows a different rate constant as the elimination process following saturation of the target. Elimination is not driven by hepatic metabolism but mostly by degradation

Table 2 The diverse spectrum of “biologicals”

| Drug class | Example |
|----------------------|--------------------------|
| Blood products | FFP, platelets |
| Recombinant proteins | Insulin |
| Antibodies | Infliximab |
| Soluble receptors | Etanercept |
| Oligonucleotides | Aptameres |
| Vakzines | β -Amyloid-vakzine |
| Gene therapy | PEG-Adenosin Deaminase |
| Stem cells | Embryonic stem cells |

Examples of “biologicals”. Biologicals comprise a heterogenous group of pharmaceutical products, which – in contrast to traditional chemicals – are derived from living organisms like bacteria, yeast or even larger animals like goat or cow (Adapted from Ref. [1])

via enzymes to amino acids, carrier mediated transport mechanisms or endocytosis. For PK–PD correlation studies it is also important to know that biological effects tend to lag behind pharmacokinetic events. This is very unlike the situation with chemicals where usually a close link between PK and PD exists. Unlike chemicals, biologicals are dependent on their conformation i.e. 3D structure for bioactivity. Thus, subtle conformational changes might profoundly affect PD. Therefore generic drugs in a strict sense will never be available for biologics. Interestingly, there is currently an ongoing discussion about “biosimilars”, some of which, e.g. G-CSF or a biosimilar growth hormone, have gained approval [22, 23].

4 Protein therapeutics (PTs)

PTs are the mainstay of biological therapies and can be categorized by function and therapeutic application in group I–IV compounds [24]. Except for group IV, which comprises protein diagnostics, PTs in a strict sense may be classified in 3 groups: Group I represent PTs with enzymatic or regulatory activity (Ia: Replacing a protein that is deficient or abnormal, Ib: Augmenting an existing pathway, Ic: Providing a novel function or activity). Typical class 1a drugs comprise insulin or coagulation factor VIII, i.e. drugs against diseases linked to deficiencies of specific proteins. Group Ib comprises therapies that augment select pathways or immune responses, e.g. colony stimulation factors, interleukins, interferons or growth factor therapies but also rhPTH or Alteplase. Group Ic drugs provide a novel activity like bivalirudin or L-asparaginase.

Group II represent PTs with special targeting activity (IIa: Interfering with a molecule or organism, IIb: Delivering other compounds or proteins). Group IIa therapeutics interfere with molecules or organisms by specific binding and blocking

Table 3 Monoclonal antibodies approved for therapeutic use

| Generic name | Trade name | Antibody format | Antigen | Approved indication | FDA approval | EMA approval |
|--------------|------------------------|------------------------|--------------------------|---|--------------|--------------|
| Muromonab | Orthoclone | Murine, IgG2a | CD3 | Allograft rejection in allogeneic renal transplantation | 86/6/19 | NA |
| Abciximab | ReoPro | Chimeric, IgG1 | CD11b/IIIa r | Maintenance of coronary patency | 94/12/22 | NA |
| Rituximab | Mabthera | Chimeric, IgG1 | CD20 | CD20-positive B-cell non-Hodgkin's lymphoma | 97/11/26 | 98/06/02 |
| Daclizumab | Zenapax | Humanized, IgG1 | CD25 (II2r) | Allograft rejection | 97/12/10 | 99/02/26 |
| Basiliximab | Simulect | Chimeric, IgG1 | CD25 (II2r) | Allograft rejection | 98/05/12 | 98/10/09 |
| Palivizumab | Synagis | Humanized, IgG1 | Protein F | Respiratory syncytial virus (RSV inhibitor) in children | 98/06/19 | 99/08/13 |
| Infliximab | Remicade | Chimeric, IgG1 | TNF α | Crohn's disease and rheumatoid arthritis | 98/08/24 | 99/08/13 |
| Trastuzumab | Herceptin | Humanized, IgG1 | HER2/Neu | Metastatic breast cancer | 98/09/25 | 00/08/28 |
| Etanercept | Enbrel | huFc γ 1/TNFr | TNF α and β | Autoimmune diseases such as ankylosing spondylitis | 98/11/02 | 00/02/03 |
| Gemtuzumab | Mylotarg | Humanized, IgG4 | CD33 | CD33-positive acute myeloid leukemia | 00/05/17 | NA |
| Alemtuzumab | Mabcampath | Humanized, IgG1 | CD52 | B-cell chronic lymphocytic leukemia | 01/05/07 | 01/07/06 |
| Ibritumomab | Zevalin ^{90Y} | Mouse, IgG1 | CD20 | B-cell non-Hodgkin's lymphoma | 02/02/19 | 04/01/16 |
| Adalimumab | Trudexa | Human, IgG1 (PD) | TNF α | Crohn's disease and rheumatoid arthritis | 02/12/31 | 03/09/01 |
| Alefacept | Amevive | huFc γ II/LFA-3 | CD2 | Chronic plaque psoriasis | 03/01/30 | NA |
| Omalizumab | Xolair | Humanized, IgG1 | IgE | Treatment of asthma | 03/06/20 | 05/10/25 |
| Tositumomab | Bexxar ^{131I} | murine, IgG2a | CD20 | CD20-positive B-cell non-Hodgkin's lymphoma | 03/06/27 | NA |
| Efalizumab | Raptiva | Humanized, IgG1 | CD11a | Moderate to severe plaque psoriasis | 03/10/27 | 04/09/20 |
| Cetuximab | Erbix | Chimeric, IgG1 | EGFR | Metastatic colorectal and head and neck carcinoma | 04/02/12 | 04/06/29 |
| Bevacizumab | Avastin | Humanized, IgG1 | VEGF-A | Metastatic colorectal and non-small cell lung carcinoma | 04/02/26 | 05/01/12 |
| Natalizumab | Tysabri | Humanized, IgG4 | Integrin α 4 | Multiple sclerosis | 04/11/23 | 06/06/27 |
| Ranibizumab | Lucentis | Humanized, IgG1 | VEGF-A | Wet-type age-related macular degeneration | 06/06/30 | 07/01/22 |
| Panitumumab | Vectibis | Human, IgG2 | EGFR | Metastatic colorectal carcinoma | 06/09/27 | 07/12/19 |
| Ecilizumab | Sollris | Humanized, IgG2/4 | C5 | Paroxysmal nocturnal haemoglobinuria | 07/03/16 | 07/06/20 |
| Certolizumab | Cimzia | Humanized, IgG1 | TNF α | Crohn's disease | 08/04/18 | NA |

Adapted from: Chames P, Van Regenmortel M, Weiss E, Baty D (2009) Therapeutic antibodies: successes, limitations and hopes for the future. *Br J Pharmacol* 157 (2): 220–233

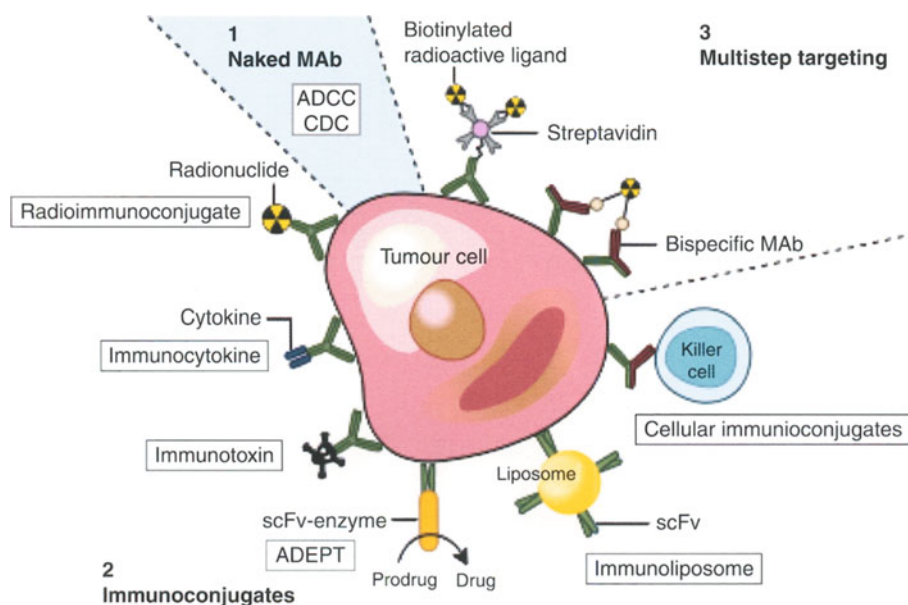


Fig. 1 Potential target-modulating strategies with monoclonal antibodies are presented. These range from immune-dependent target modulation *via* antibody dependent cytotoxicity (ADCC) to targeting of conjugates, e.g. radioisotopes, to target structures. Dependent on sequence variations, monoclonal antibodies may be categorized as chimeric (-ximab), humanized (-zumab), murine (-momab) and human (-umab) antibodies. http://en.wikipedia.org/wiki/File:Monoclonal_antibodies.svg

their function or targeting them for destruction or stimulating a signalling pathway. This group has grown as monoclonal antibody technology has matured and will probably expand further as signalling pathways and aetiologies of disease are more clearly identified. Group IIb therapeutics like the anti-AML drug gemtuzumab-ozogamicin (Mylotarg), which is an anti-CD33 mAb conjugated to the cytotoxin calicheamicin deliver other compounds or proteins to a specific site.

Group III PTs are classified in group IIIa compounds, i.e. vaccines protecting against a deleterious foreign agent an autoimmune disease (IIIb) or cancer (IIIc).

5 Vaccines (group III PTs)

Vaccines are undoubtedly one of the most important medical achievements and have led to a substantial increase in life expectancy. Many people alive still remember the threat caused by polio to their generation. Fortunately, diphtheria, tetanus, polio, pertussis, rubella mumps or measles do not present dangers any longer and public interest has shifted to concerns with cancer, heart disease or Alzheimer's. After the first golden era of

vaccines, from the mid-1900s over ~50 years more than 40 vaccines were developed for human use against a variety of viruses, bacteria and toxins including influenza, polio, yellow fever, measles, mumps, rubella, tetanus, pertussis and diphtheria. In 1926 the adjuvant properties of aluminium salts were discovered and the first vaccine adjuvants aluminium salts were approved. Now at the beginning of the 21st century a second golden era of vaccines has been proclaimed [25]. Besides recombinant protein therapeutics, various other forms of vaccines, e.g. DNA vaccines, live and attenuated vaccines, replication defective-, split-, subvirion- or whole virus vaccines are available and alternative modes of administration, e.g. intranasal or transdermal delivery are currently under investigation. Newer production technologies employ cell-based (Vero-, MDCK- cells) rather than a chicken-egg based production [26, 27]. Vaccines and vaccine production facilities in general have come into the public limelight last year when the WHO declared a phase 6 pandemic with influenza A/H1N1/california/2009. A complete list of FDA-approved vaccines may be found at: <http://www.fda.gov/cber/vaccine/licvacc.htm>.

6 Antisense approaches and aptamers

In 2006 the Nobel Price in Physiology or Medicine (<http://nobelprize.org>) was awarded to Andrew Z. Fire and Craig C. Mello for their discovery of RNA interference – gene silencing by double-stranded RNA, a “fundamental mechanism for controlling the flow of genetic information”. In 1998 the two scientists have published their discovery of RNA interference [28], a molecular mechanism, which is “activated when RNA molecules occur as double-stranded pairs in the cell”. “RNA interference occurs in plants, animals and humans. It is of great importance for the regulation of gene expression, participates in defense against viral infections, and keeps jumping genes under control. RNA interference is already being widely used in basic science as a method to study the function of genes and it may lead to novel therapies in the future” (http://nobelprize.org/nobel_prizes/medicine/). RNA interference was first studied in the nematode worm *Caenorhabditis elegans* by injection of ds-RNA which led to specific silencing of the corresponding mRNA and termination of translation and, thus, protein formation. In the following years the components of the RNAi machinery, Dicer and RISC were identified. Today it has become clear that a large part of the non-mRNA-coding and previously-called “junk” DNA might in fact play important roles in gene regulation, above all by transcription of regulatory small RNA (sRNA). Although the endogenous, physiological mechanism of gene silencing by Watson-Crick base pairing of dsRNA to mRNA was new, the strategy of specific silencing and “knock down” has already been introduced earlier to drug development by use of ssDNA antisense oligonucleotides. Two different approaches of “basepairing” therapeutics are currently under investigation in preclinical and clinical trials, i.e. antisense oligonucleotides (ASOs) and RNA interference (RNAi).

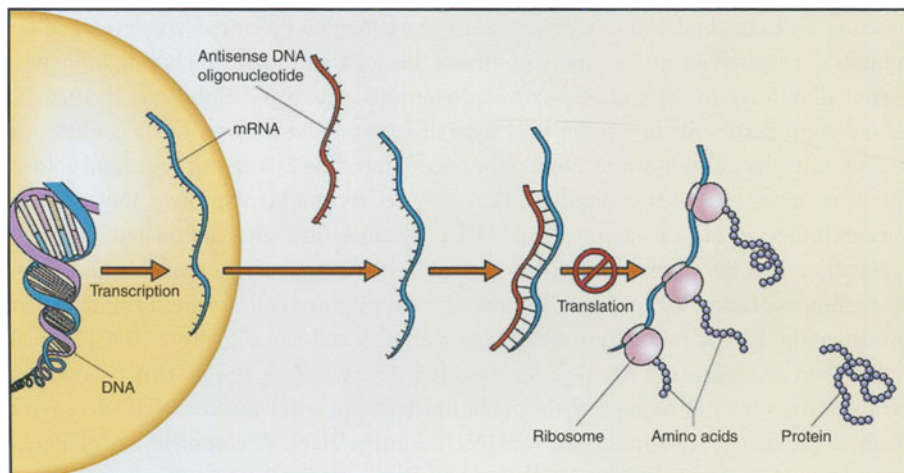


Fig. 2 Schematic showing how antisense DNA strands can interfere with protein translation. <http://en.wikipedia.org/wiki/Antisense> (RNAi Therapeutics: How likely, how soon? Robinson R. Phos Biology Vol. 2, No. 1)

Two other RNA based approaches which may be exploited by drug development are Ribozymes, i.e. autocatalytic RNA which cleaves target sequences in a specific manner and aptamers, RNA molecules which bind to specific targets and act like antibodies. Although several challenges have to be overcome to introduce antisense and RNA based therapeutics into clinical practice in a broad way there are already some products on the market, e.g. fomivirsen (Vitravene) an ASO for the treatment of CMV retinitis and pegaptanib (Macugen) an aptamer against age related macular degeneration (AMD) and several dozen novel ASOs are tested in clinical development [29].

Case Study: Alzheimer vaccine

Alzheimer's disease (AD) was first described in 1901 by the Austrian Neurologist Alois Alzheimer (1864–1915). AD is a progressive form of dementia, generally in patients over the age of 65.

The current working hypothesis on the pathogenesis of AD is that it is caused by a deposition of the fragment "Amyloid- β " ($A\beta$) of the Amyloid Precursor Protein (APP), a cell membrane protein with incompletely understood biological function (Hardy J, *The amyloid hypothesis for Alzheimer's disease: a critical reappraisal*. *J Neurochem* 110: 1129–1134, 2009). $A\beta$ is derived from APP through cleavage by secretases. The gene for APP is localized on chromosome 21 and

patients with trisomy 21 have an increased risk of developing AD. Also, mutations in the Preseniline-gene which codes for γ -Secretase are associated with an increased A β deposition. Accumulation of A β causes toxic damage of neurons and is believed to precede aggregation of the intracellular Tau protein in neurons, which is a further pathognomonic factor of AD.

The current therapy of AD is purely symptomatic and consists of administration of traditional chemicals, i.e. anticholinergic and NMDA-antagonistic drugs which have only modest activity (*Birks J. Cholinesterase inhibitors for Alzheimer's disease. Cochrane Database Syst Rev 25(1): CD005593, 2006*).

Based on the A β hypothesis of AD, A β has been tackled as a therapeutic target by immunotherapy. In animal models, active and passive vaccination was associated with clear effects on the AD disease process and prevented A β disposition. Several mechanisms, e.g. antibody mediated activation of immune and microglial cells, clearance of circulating A β or neutralization of oligomer toxicity are discussed (*Wisniewski and Konietzko. Amyloid- β immunisation for Alzheimer's disease. Lancet 7: 805–811, 2008*).

The success of vaccination in animal studies has led to the start of clinical trials with vaccines that contained preaggregated A β . Trials on an active vaccine – AN1792 – in patients with mild to moderate AD revealed that ~50% of patients developed an anti-A β response. However, a phase II trial had to be stopped early when 6% of vaccinated patients developed symptoms of acute meningoencephalitis. Autopsies of few patients revealed clearance of A β plaques, similar to the response in preclinical studies and, thus, provided support to the overall concept.

Also data from passive immunization studies with the antibody bapineuzumab showed significant clinical benefits on cognitive AD several scales. An important topic in current AD research is the lack of robust biomarkers. Although some markers, most notably ApoE status and CSF concentrations of tau and A β are available there is a lack of markers which correlate with disease severity. Recent interest has focused on PET imaging probes, e.g. 11C-PIB or 18F-FDDNP and other agents which bind to tau or A β .

Unfortunately, recent reports on a follow up of the AN1792 trial did not provide signs of significant clinical benefit. Currently 14 AD vaccine studies are active at <http://clinicaltrials.gov>.

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References

1. Müller M (2006) Biological therapies: concepts and challenges. *Wien Klin Wochenschr* 118(17–18): 508–512
2. FDA (2004) Innovation or stagnation: challenge and opportunity on the critical path to new medical products. www.fda.gov/oc/initiatives/criticalpath/whitepaper.html
3. IBM Business Consulting Services (1998) Pharma 2005 – an industrial revolution in R&D. www-1.ibm.com/services/au/igs/pdf/gw510-9220-pharma-2005-industrial-revolution.pdf
4. Drews J (2000) Drug discovery: a historical perspective. *Science* 287: 1960–1964
5. Lazarou J, Pomeranz BH, Corey PN (1998) Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies. *JAMA* 279: 1200–1205
6. Thurmann PA (2006) Adverse drugs reactions: diagnosis and assessment. *Pathologe* 27: 6–12
7. Shah RR (2005) Drugs, QTc interval prolongation and final ICH E14 guideline: an important milestone with challenges ahead. *Drug Safety* 28: 1009–1028
8. Schuster D, Laggner C, Langer T (2005) Why drugs fail – a study on side effects in new chemical entities. *Curr Pharm Des* 11: 3545–3559
9. Kaplowitz N (2005) Idiosyncratic drug hepatotoxicity. *Nat Rev Drug Discov* 4: 489–499
10. Drews J (1998) Biotechnology's metamorphosis into a drug discovery industry. *Nat Biotechnol* 16(Suppl): 22–25
11. Cavagnaro JA (2002) Preclinical safety evaluation of biotechnology-derived pharmaceuticals. *Nat Rev Drug Discov* 1: 469–475
12. Baumann A (2006) Early development of therapeutic biologics-pharmacokinetics. *Curr Drug Metab* 7: 15–21
13. Goodyear M (2006) Learning from the TGN1412 trial. *BMJ* 332: 677–678
14. Editorial (2006) Urgent changes needed for authorisation of phase I trials. *Lancet* 367: 1214
15. Wachek V (2004) Strategies for designing clinical trials for oligonucleotide therapeutics. *Drug Discov Today* 9: 918–923
16. Saul A (2005) Models of phase 1 vaccine trials: optimization of trial design to minimize risks of multiple serious adverse events. *Vaccine* 23: 3068–3075
17. Gandy S, Heppner FL (2005) Alzheimer's amyloid immunotherapy: quo vadis? *Lancet Neurol* 4: 452–453
18. Gilman S, Koller M, Black RS, Jenkins L, Griffith SG, Fox NC, Eisner L, Kirby L, Rovira MB, Forette F, Orgogozo JM (2005) AN1792(QS-21)-201 Study team. Clinical effects of Abeta immunization (AN1792) in patients with AD in an interrupted trial. *Neurology* 64: 1553–1562
19. Langer-Gould A, Steinman L (2006) What went wrong in the natalizumab trials? *Lancet* 367: 708–710
20. Romond EH, Perez EA, Bryant J, Suman VJ, Geyer CE Jr, Davidson NE, Tan-Chiu E, Martino S, Paik S, Kaufman PA, Swain SM, Pisansky TM, Fehrenbacher L, Kutteh LA, Vogel VG, Visscher DW, Yothers G, Jenkins RB, Brown AM, Dakhil SR, Mamounas EP, Lingle WL, Klein PM, Ingle JN, Wolmark N (2005) Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med* 353: 1673–1684
21. Chien KR (2006) Herceptin and the heart – a molecular modifier of cardiac failure. *N Engl J Med* 354(8): 789–790
22. Anonymous (2006) First biosimilar closer to approval The European Medicines Agency has recommended the approval of Sandoz's version of human growth hormone. *Nat Rev Drug Discov* 5: 178–179
23. Sheridan C (2006) First generic biologics finally approved. *Nat Rev Drug Discov* 5: 445
24. Leader B, Baca QJ, Golan DE (2008) Protein therapeutics: a summary and pharmacological classification. *Nat Rev Drug Discov* 7: 21–39

25. Poland GA (2007) Pharmacology, Vaccinomics, and the second golden age of vaccinology. Clin Pharmacol Ther 82: 623–626
26. Dorner F, Eibl J, Barrett PN (1999) New technologies for vaccines. Wien Klin Wochenschr 111(5): 199–206
27. Dorner F, Barrett PN (1999) Vaccine technology: looking to the future. Ann Med 31(1): 51–60
28. Fire A, Xu SQ, Montgomery MK, Kostas SA, Driver SE, Mello CC (1998) Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature 391: 806–811
29. Sepp-Lorenzino L, Ruddy MK (2008) Challenges and opportunities for local and systemic delivery of siRNA and antisense oligonucleotides. Clin Pharmacol Ther 84: 628–632

CHAPTER 21

Development of Advanced Therapy Medicinal Products – a case for early scientific advice

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Summary

Advanced Therapy Medicinal Products (ATMPs) – gene therapy medicinal products, somatic cell therapy medicinal products, and tissue engineered products – are currently the most innovative drug products and hold promise to offer cure for a variety of diseases for which there are no satisfactory therapies. They have therefore elicited considerable interest and debate. The European Regulation on ATMPs provides a regulatory framework for these innovative medicines, and since 2009 the Committee for Advanced Therapies (CAT) at the European Medicines Agency (EMA) has started its work. The CAT is a multi-disciplinary scientific expert committee, representing all EU member states and EFTA countries, as well as patients' and physicians' associations. This book chapter briefly touches upon some of the difficulties developers of ATMPs may face, and the opportunities to approach the CAT as a regulatory advisor during development.

1 Introduction

Advanced Therapy Medicinal Products (ATMPs) comprise gene therapy medicinal products, somatic cell therapy medicinal products and tissue engineered products

Keywords: Advanced Therapy Medicinal Products (ATMP), Committee for Advanced Therapies (CAT), Cell-Based Medicinal Products (CBMPs), classification and certification procedures, gene therapy medicinal products (GTMP), gene modified organism, risk based approach, scientific advice, stem cells, somatic cell therapy medicinal products

The affiliation of the CAT members and alternates can be found on the EMEA Website (http://www.emea.europa.eu/htms/general/contacts/CAT/CAT_members.html).

(please refer to Table 1 for legal definitions) (Regulation (EC) No 1394/2007 [4]). These highly innovative medicinal products offer treatment opportunities for currently incurable diseases. Thus, ATMPs have elicited considerable interest or even a hype, but they have already generated some worrisome safety concerns as well.

For example, proof of concept for gene therapy of monogenetic diseases has already been observed in humans, which could result in long-term beneficial results [12]. Moreover, cell-based skin substitutes have already been used for several years, and future somatic cell therapy medicinal products and tissue engineered products might also become efficacious therapies. However, despite their promise, ATMPs like conventional drugs can trigger serious adverse events. In some cases these were lethal such as a systemic inflammatory immune reaction or leukaemia due to insertional oncogenesis [2]. Recently, embryonal stem cells caused a tumour after intrathecal injection for spinal cord injury [1]. These examples demonstrate that some cell-based medicinal products also have particular risks that need to be tackled.

With the new European Regulation on ATMPs (Regulation (EC) No 1394/2007), a regulatory framework for these innovative medicines has recently been created. A new Committee has been established at the European Medicines Agency (EMA) in London: the Committee for Advanced Therapies (CAT), which is a multi-disciplinary scientific committee of experts representing all member states of the European Union (EU), Iceland and Norway, as well as representatives from patients' and medical associations. This independent committee started its work in January, 2009. The CAT gathers European experts to review the quality, safety and efficacy of ATMPs according to EMA standards, and to discuss scientific new developments in the field.

The CAT is responsible for the primary evaluation of ATMP Marketing Authorization Applications (MAAs) for the EMA's Committee for Medicinal Products for Human Use (CHMP). The CAT has two new regulatory procedures for companies developing ATMPs: the "classification procedure" and the "certification procedure" (*vide infra*).

Regulatory guidance has already been developed by different EMA/CHMP regulatory groups (e.g. the Biologics Working Party, the Gene Therapy Working Party, or the Cell-based Products Working Party) prior to the CAT, and through the Scientific Advice Working Party. However, the CAT now combines and complements these activities within a single committee to regulate the development of ATMPs in Europe.

As with any other drug, marketing authorization of ATMPs requires, that the product is consistently manufactured to a predefined quality, and that it is safe and efficacious, but the required data can be highly specific [19]. Yet, new strategies for the development and scientific assessment of ATMP's may become necessary. For example, the safety and efficacy of many types of cell-based medicinal products strongly depends on the final performance of the cell preparation administered. Therefore, rigorous control of the manufacturing process and specifications are mandatory, which has limitations inherent to the complex nature of ATMPs.

Likewise, clinical trials may become challenging because clinical efficacy or safety might be apparent only after several years, and necessitates the validation of appropriate surrogate endpoints.

This chapter highlights some major regulatory challenges.

2 Cell-based medicinal products (CBMPs)

Cell-based medicinal products comprise several types of cell therapies, and include somatic cell therapy medicinal products and tissue engineered products, manufactured from viable cells of autologous, allogeneic or xenogeneic origin, which may also be genetically modified. These products are highly heterogeneous due to their origin, starting material, cell population type, differentiation stage and manufacturing process including the degree of *in vitro* manipulation.

Somatic cell therapy medicinal products shall to prevent or treat a disease or to make a diagnosis, by a metabolic, immunological or pharmacological mode of action of the cells (for legal definition see Table 1). Cancer immunotherapy products are one example for such products.

Tissue engineered products are developed for structural repair of tissues, e.g. corneal lesions, liver tissue, cartilage or bone (for legal definition see Table 1). The therapeutic intention is to replace the failing tissue with a functionally equivalent tissue structure. These types of ATMPs are sometimes associated with structural components that promote the formation of a three-dimensional tissue structure. The active substance in these products might be a functionally immature cell preparation (e.g. stem/progenitor cells), or more differentiated cells that form the final tissue (e.g. cartilage).

3 Efficacy and safety challenges

3.1 Patient integration

One of the main challenges of CBMPs is a robust and safe functional and/or structural integration of the product into the patient. The CBMP should yield a stable therapeutic effect, and ideally be able to functionally restore or substitute the affected tissue.

This is not easy to achieve because living cells are fragile and are incredibly complex pharmaceuticals. Their *in vivo* fate and function depends on their micro-environment. However, this is often species- and/or disease-specific, which complicate efficacy and safety studies in animal models and their extrapolation to humans. Notably, cells are reactive to this environment, and are able to change their phenotype. Thus, environmental changes can induce changes in cells. Thus, *in vitro* production will have an impact on the efficacy and/or safety of any CBMP. Prolonged *in vitro* cell culture and

the use of growth factors will alter the cells, which requires adequate subsequent testing of their characteristics. Also, apoptosis will occur in primary cells during long-term *in vitro* culture, which will alter the actual dose and clinical efficacy when implanted into patients. Finding appropriate cell markers is challenging since they are not always specific or directly correlate with cell function. Similarly, robust directed differentiation of stem cells into the desired differentiated cell types is one difficulty in the clinical translation. Additionally, there is a tumourigenic risk of undifferentiated or incompletely differentiated stem cells that needs to be eliminated before clinical use [13].

3.2 Characterization

Poor definition and control of a product during its manufacturing process will decrease safety and efficacy. Thus, appropriate characterization of a product is mandatory.

The required characterization programme, will have to include the functional capability of the cells for the intended clinical use. However, to link specific cell characteristics to the intended function is not an easy task. One of the clinical challenges is how to measure long-term clinical outcome. The differentiation into the desired tissue type, and thus the functional tissue repair, may take several years for some tissues. This requires the conduct of lengthy clinical trials, which may lead to problems including the maintenance of patient follow-up or complications of results due to the underlying natural disease course or other comorbidities [7]. Non-clinical studies in a relevant animal species are required to assess toxicity due to de-differentiation, cell transformation, tumourigenicity, or ectopic engraftment. Also, the animals' immune systems recognize human cells as "foreign" and thus attack them which can lead to artificial immunotoxic effects that may not occur in patients in an autologous setting. Conversely, this immune reaction will rapidly eliminate cells, which could mask potential adverse events that would occur at a later stage in patients.

Nonetheless, several safety aspects of manipulated cells can only be tested in animals, including the biodistribution by invasive techniques or the tumourigenic potential. The use of immuno-deficient animals such as mice with severe combined immunodeficiency may be suitable in some instances. However, due to pronounced interspecies differences between humans and mice the results may need further confirmation in large animals.

Promising for non-clinical testing may be the use of a homologous model, e.g. the use of mouse adult stem cells in mice, resembling the cell-based medicinal product to be used in humans. One can expect that all cellular and molecular interactions are functional due to the homologous setting. As the medicinal product itself is not being tested, this can be used mainly for proof of concept but does not allow the detection of any toxicity arising from potential contaminants in the final product. Sometimes bridging studies to clinical trials may become necessary. In addition, a surgical excision

and *in vitro* culture of cells might lead to contamination with pathogens where simple sterilization is not possible. Hence, new safety methods to improve testing for potential contaminants are needed.

Clinical hurdles are the definition of a target dose as classical dose finding strategies by selecting a dose for confirmatory study from several tested in exploratory studies may sometimes be problematic. Further, in regenerative medicine, suitable comparator treatments or products may not always be available, and a double-blind design can be challenging. Endpoints that were originally validated for other product types may sometimes have to be adapted for a cell-based product [11], e.g. cancer immunotherapies may transiently increase tumour size by T cell influx, edema and swelling which would, represent a “progression” due to an increase in tumour diameter.

Certainly, such challenges are common in the development of ATMPs, and companies are therefore recommended to seek as early as possible scientific advice at the European Medicines Agency. A general guideline on stem cell based products has been developed by the Cell-based Products Working Party (CPWP) [14].

4 Gene therapy medicinal products (GTMP)

Gene therapy medicinal products (GTMP) aim at delivering a gene, and through its expression, a therapeutic effect in patients (for legal definition see Box 1 and Ref. 2). A GTMP typically functions as a sequence of different components, i.e. the vector and the inserted sequence(s), the target cells, and finally the protein encoded by the vector. Each of these factors can induce desired effects as well as adverse effects [20]. This increases the complexity of gene transfer medicinal products.

5 Development challenges and strategies to address them

5.1 Vector manufacture

Currently, viral vectors are most commonly used for gene transfer. However, manufacturing is more difficult with viral than non-viral vectors, which can be assembled synthetically. Only a fraction of viral vector particles are biologically active, and available manufacturing systems often yield a relatively low vector titre which hampers preclinical studies in large animal models or clinical trials. Nevertheless, progress has been made by improving the downstream vector processing, or by alternative production systems that facilitate the large-scale production of vectors [3]. Still, adequate reference standards for testing replication competent vectors have to be found [22], and potency testing regarding transgene expression as well as its bioactivity *in vivo* must be performed.

5.2 Achieving stable gene expression

Treatment of inherited diseases with GTMPs typically requires stable expression of the therapeutic product. However, the duration of gene expression is influenced by various factors including the promoter, cell survival, persistence of the transgene, the immune response against the vector, the patient's cells that were genetically modified and/or the finally expressed protein, which could also elicit an immune response [20, 21].

5.3 Clinical efficacy and safety

Other challenges of GTMPs relate to the clinical efficacy, which depends on the gene transfer efficiency, the ability to target the desired cell type, and the expression levels of the gene [21]. A sufficient quantity of target cells need to be genetically modified, and sufficient gene product needs to be expressed. For example, it is difficult to administer the gene locally in multifocal diseases such as myopathy to distribute its expression in the affected tissue whilst avoiding systemic exposure or inadvertent gene transfer into non-target cells. Tolerability might be hampered by dozens of local injections in each patient. Obviously blinding of such a trial is also difficult if not impossible, and lack of blinding can severely bias clinical results, particularly when soft end points are chosen.

Targeting cancer by gene therapy is particularly challenging since it is virtually impossible to reach each cancer cell in the body. For that reason oncolytic viruses are currently studied [14], and ICH considerations Oncolytic Viruses have recently been released EMEA/CHMP/GTWP/607698/2008.

As far as safety is concerned, insertional mutagenesis, that may lead to insertional oncogenesis, is a concern. The use of strong enhancers/promoters needed to boost the efficacy of a given vector would therefore need to be weighed against the oncogenic risk. To reduce these risks, the vector can be modified to prevent *cis*-activation of genes that flank the integration sites and new assays have been designed to better assess these risks [17]. Alternatively, vectors can be applied that do not integrate, or achieve targeted genomic integration into specific chromosomal loci [16].

Considering these various challenges, the Gene Therapy Working Party (GTWP) has produced various scientific guidelines that address these problems and the CAT will continue to expand this regulatory framework.

6 Combined ATMPs

Combined ATMPs incorporate a medical device and viable cells or tissues. The medical device component must also comply with the requirements of the relevant medical

device directive [5, 6]. This aspect of conformity will usually be assessed by a suitably qualified “Notified Body” for medical devices.

It can be expected that a wide range of combined ATMPs will emerge as science evolves. Existing examples include tissue engineered products incorporated onto an artificial matrix for implantation, or living cells inserted into a special implantation device. The performance of either component may be changed when used in combination. Combined ATMPs pose challenges to find common grounds of scientific principles on which these medicinal products are assessed, whilst meeting both the requirements of the advanced therapy and medical device regulatory frameworks.

7 Involvement of CAT in ATMP development

Well-established regulatory standards covering the quality, safety and efficacy criteria need to be adapted to take into account the specificities of gene- and cell-based medicinal products. The regulators are ready to enter a dialogue with developers and academia to exchange scientific views, whilst at the same time ensuring compliance with the regulatory and legal framework for the authorization of ATMPs.

In Europe, the Committee for Advanced Therapies and the EMEA Secretariat are aware of these challenges and will play an important role in early interactions. Examples are the briefing meetings of individual manufacturers with EMEA’s Innovation Task Force (ITF), CAT’s routine involvement in all scientific advices on ATMPs. The CAT has also published – and will continue to publish papers in scientific journals besides regulatory guidelines.

As an incentive to boost the development of ATMPs, as well as reduced fee applies to all scientific advices on ATMPs [10]. For Small and Medium-sized Enterprises (SMEs), more extensive assistance is offered via EMA’s SME office during the product development but also during the evaluation of the marketing authorization application.

Two new regulatory procedures have been set up specifically for companies developing ATMPs. These are the scientific recommendation from CAT on the regulatory classification as ATMP, and the certification procedure. The purpose of the *classification procedure* is to determine whether a given product meets the scientific criteria which define ATMPs. This shall help to address, as early as possible, questions of borderline with other areas such as cosmetics, medical devices or tissue/cell transplantation [15]. The second new procedure, the *certification procedure* is a scientific evaluation of available quality and non-clinical data. After evaluation by CAT, and certification by EMA, this gives SMEs a possibility to attract financial support for the further development of their product. By scientific input from the CAT, the company will be able to update the quality and non-clinical parts of their dossier. Thus, the certification system gives the SMEs an incentive to develop ATMPs.

Many ATMPs are developed for rare diseases. At the EMA, the Committee for Orphan Medicinal Products (COMP) is responsible for reviewing applications seeking “orphan medicinal product designation” for products for the diagnosis, prevention or treatment of life-threatening or very serious conditions that affect not more than 5 in 10,000 persons in the European Union. Close interactions between CAT and COMP guarantee the exchange of information on orphan ATMPs. Some CAT members were formerly members of the COMP, so there is a clear understanding of the needs of Orphan Drugs in the CAT.

As far as Marketing Authorization Procedures for ATMPs are concerned, the CAT is responsible for the primary evaluation within the framework of the Centralized Marketing Authorization Procedure [10] that is mandatory for ATMPs. In this case the CAT interacts with the Committee for Medicinal Products for Human Use (CHMP), which is EMA’s main scientific Committee for human medicines. A procedure describing the interactions between applicants and CAT, and between CAT and CHMP has been published on the EMEA Website [10].

In conclusion, the Regulation on Advanced therapies provides the Regulatory framework for the approval of ATMPs in the EU. The EMA and CAT are promoting an open dialogue with developers of ATMPs to discuss the scientific challenges. Because of particular difficulties in developing reproducible high quality ATMPs, early scientific advice is recommendable to any company.

Definitions of Advanced Therapy medicinal products in the European Pharmaceutical legislation (see Ref. [1, 2])

Gene therapy medicinal product means a biological medicinal product which has the following characteristics:

- (a) it contains an active substance which contains or consists of a recombinant nucleic acid used in or administered to human beings with a view to regulating, repairing, adding or deleting a genetic sequence;
- (b) its therapeutic, prophylactic or diagnostic effect relates directly to the recombinant nucleic acid sequence it contains, or to the product of genetic expression of this sequence.

Gene therapy medicinal products shall not include vaccines against infectious diseases.

Somatic cell therapy medicinal product means a biological medicinal product which has the following characteristics:

- (a) contains or consists of cells or tissues that have been subject to substantial manipulation so that biological characteristics, physiological functions or structural proper-

ties relevant for the intended clinical use have been altered, or of cells or tissues that are not intended to be used for the same essential function(s) in the recipient and the donor;

- (b) is presented as having properties for, or is used in or administered to human beings with a view to treating, preventing or diagnosing a disease through the pharmacological, immunological or metabolic action of its cells or tissues.

For the purposes of point (a), the manipulations listed in Annex I to Regulation (EC) No 1394/2007 [10], in particular, shall not be considered as substantial manipulations.

Tissue engineered product means a product that:

- contains or consists of engineered cells or tissues, and
- is presented as having properties for, or is used in or administered to human beings with a view to regenerating, repairing or replacing a human tissue.

Cells or tissues shall be considered ‘engineered’ if they fulfill at least one of the following conditions:

- the cells or tissues have been subject to substantial manipulation, so that biological characteristics, physiological functions or structural properties relevant for the intended regeneration, repair or replacement are achieved. The manipulations listed in Annex I of Regulation (EC) No 1394/2007 [10], in particular, shall not be considered as substantial manipulations,
- the cells or tissues are not intended to be used for the same essential function or functions in the recipient as in the donor.

Tasks of the CAT

The main responsibility of the CAT is to prepare a draft opinion on each ATMP application submitted to the European Medicines Agency, before the Committee for Medicinal Products for Human Use (CHMP) adopts a final opinion on the granting, variation, suspension or revocation of a marketing authorization for the medicine concerned. At the request of the Agency’s Executive Director or of the European Commission, an opinion is also drawn up on any scientific matter relating to ATMPs.

Other responsibilities of the CAT include:

- participating in the Agency’s procedures for the certification of quality and non-clinical data for small and medium-sized enterprises developing advanced-therapy medicinal products;

- participating in the Agency's procedures for the provision of scientific recommendations on the classification of advanced-therapy medicinal products in accordance with Article 17 of Regulation (EC) No 1394/2007 [10];
- contributing to the Agency's provision of scientific advice, following relevant procedures established between the CAT and the Scientific Advice Working Party (SAWP);
- involvement in any procedure regarding the provision of advice for undertakings on the conduct of efficacy follow-up, pharmacovigilance and risk-management systems of ATMPs;
- advising, at the request of the CHMP, on any medicinal product which may require, for the evaluation of its quality, safety or efficacy, expertise in ATMPs;
- assisting scientifically in the elaboration of any documents related to the fulfilment of the objectives of Regulation (EC) No 1394/2007 [10];
- providing, at the request of the European Commission, scientific expertise and advice for any Community initiative related to the development of innovative medicines and therapies that requires expertise on ATMPs;

assisting, at the request of the CHMP, in the tasks identified in the work programmes of the CHMP working parties.

EMA information and guidelines

Information on Advanced Therapies from EMEA: http://www.emea.europa.eu/htms/human/advanced_therapies/intro.htm

Information on the Committee for Advanced Therapies (CAT): <http://www.emea.europa.eu/htms/general/contacts/CAT/CAT.html>

Information on Medicines and Emerging Science: <http://www.emea.europa.eu/htms/human/mes/introduction.htm>

EMA Innovation Task Force (ITF): <http://www.emea.europa.eu/htms/human/mes/itf.htm>

EMA SME Office (small and medium-sized enterprises): <http://www.emea.europa.eu/SME/SMEoverview.htm>

EMA Scientific Guidelines for Biologicals, including guidelines specific to ATMPs: <http://www.emea.europa.eu/htms/human/humanguidelines/biologicals.htm>

EMA Multidisciplinary Guidelines, including guidelines specific to ATMPs: <http://www.emea.europa.eu/htms/human/humanguidelines/multidiscipline.htm>

Disclaimer

Although Bernd Jilma has been a member of the Committee of Advanced Therapies (CAT) at the time of writing, the views expressed are personal views and may not reflect the view of the CAT or those of the European Medicines Agency (EMA).

References

1. Amariglio N, Hirshberg A, Scheithauer BW, et al. (2009). Donor-derived brain tumor following neural stem cell transplantation in an ataxia telangiectasia patient. *PLoS Med* 6(2): e1000029
2. Baum C, Kustikova O, Modlich U, et al. (2006) Mutagenesis and oncogenesis by chromosomal insertion of gene transfer vectors. *Hum Gene Ther* 17(3): 253–263
3. Broussau S, Jabbour N, Lachapelle G, et al. (2008) Inducible packaging cells for large-scale production of lentiviral vectors in serum-free suspension culture. *Mol Ther* 16(3): 500–507
4. Commission Directive 2009/120/EC of 14 September 2009 amending Directive 2001/83/EC of the European Parliament and of the Council on the Community code relating to medicinal products for human use as regards advanced therapy medicinal products. *Official Journal of the European Union* L242/3-12 (2009)
5. Council Directive of 20 June 1990 on the approximation of the laws of the Member States relating to active implantable medical devices (90/385/EEC). *Official Journal of the European Union*, L 189, 20.7.1990, p 17 (1990)
6. Council Directive 93/42/EEC of 14 June 1993 concerning medical devices. *Official Journal of the European Union*, L 169, 12.7.1993, p 1 (1993)
7. European Medicines Agency (EMA) (2008) Guideline on safety and efficacy follow-up – risk management of advanced therapy medicinal products EMEA/149995/2008. <http://www.emea.europa.eu/pdfs/human/advancedtherapies/14999508enfin.pdf>
8. European Medicines Agency (EMA): Scientific Guidelines for Human Medicinal Products – Multidisciplinary Guidelines – Gene Therapy. <http://www.emea.europa.eu/htms/human/humanguidelines/multidiscipline.htm>
9. European Medicines Agency (EMA): Committee for Orphan Medicinal Products (COMP): <http://www.emea.europa.eu/htms/general/contacts/COMP/COMP.html>
10. European Medicines Agency (EMA) (2009) Committee for Advanced Therapies (CAT): Procedural Advice on the evaluation of advanced therapy medicinal products in accordance with Article 8 of Regulation (EC) No 1394/2007. EMEA/630043/2008. <http://www.emea.europa.eu/pdfs/human/cat/63004308en.pdf>
11. Hoos A, Parmiani G, Hege K, et al. (2007) A clinical development paradigm for cancer vaccines and related biologics. *J Immunother* 30(1): 1–15
12. Kohn DB, Candotti F (2009) Gene therapy fulfilling its promise. *N Engl J Med* 360(5): 518–521
13. Nishikawa S, Goldstein RA, Nierras CR (2008) The promise of human induced pluripotent stem cells for research and therapy. *Nat Rev Mol Cell Biol* 9(9): 725–729
14. European Medicines Agency (EMA) (2008) Guideline on Human Cell-based Medicinal Products EMEA/CHMP/410869/2006. <http://www.emea.europa.eu/pdfs/human/cpwp/41086906enfin.pdf>
15. European Medicines Agency (EMA) (2007) Committee for Advanced Therapies (CAT): Procedural Advice on the provision of scientific recommendation on classification of advanced

- therapy medicinal products in accordance with Article 17 of Regulation (EC) No 1394/2007. EMEA/99623/2009. <http://www.emea.europa.eu/pdfs/human/cat/9962309en.pdf>
16. High KA (2005) Gene therapy: the moving finger. *Nature* 435(7042): 577–579
 17. Modlich U, Baum C (2009): Preventing and exploiting the oncogenic potential of integrating gene vectors. *J Clin Invest* 119(4): 755–758
 18. Regulation (EC) No 1394/2007 of the European Parliament and of the Council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004. *Official Journal of the European Union* 10.12.2007. L324/121-137 (2007)
 19. Schüssler-Lenz M, Schneider CK (2010) Klinische Prüfung mit Arzneimitteln für Neuartige Therapien [Clinical trials with advanced therapy medicinal products]. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz* 53: 68–74
 20. Somia N, Verma IM (2000) Gene therapy: trials and tribulations. *Nat Rev Genet* 1(2): 91–99
 21. VandenDriessche T, Collen D, Chuah MK (2003) Gene therapy for the hemophilias. *J Thromb Haemost* 1(7): 1550–1558
 22. Wilson CA, Cichutek K (2009) The US and EU regulatory perspectives on the clinical use of hematopoietic stem/progenitor cells genetically modified ex vivo by retroviral vectors. *Method Mol Biol* 506: 477–488

CHAPTER 22

Individualized medicine

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1 The current concept

Historically, the prime goal of drug development has been the development of a blockbuster drug, i.e. a drug generating more than \$1 billion of revenue for its owner (Fig. 1). Classical examples of blockbuster classes of drugs are the statins, non-steroidal anti-inflammatory drugs (NSAIDs) or proton pump inhibitors (PPIs), which can be prescribed to millions of patients for a broad variety of disease states.

The development of blockbusters went hand in hand with the statistical assumptions of randomized controlled trials (RCT; see Chapters 8, 9 and 12) and the concept that large groups of patients could be “lumped” together and compared to test a hypothesis (see Chapter 12). This view has always been challenged by representatives of “alternative” concepts of medicine, who argued that the statistical assumptions of RCTs do not take individual disease and drug response patterns into account. One of Samuel Hahnemanns major homeopathic principles was that illness is specific to the individual. RCTs would therefore be inappropriate tools to test individualized remedies, e.g. homeopathic remedies. It is beyond the scope of this chapter to discuss the weaknesses and downsides of this “alternative” concept *vis-à-vis* RCTs. However, it is interesting to note that a similar reasoning has emerged in the science of medicine, i.e. that greater attention must be paid to individual characteristics of patients.

The current healthcare policy of US President Obama, who introduced as Senator the Genomics and Personalized Medicine Act of 2007 (http://olpa.od.nih.gov/tracking/110/senate_bills/session1/s-976.asp), has a strong focus on individualized medicine and drug safety [1]. Already in September 2008 the Presidential Council of Advisors on Science and Technology (PCAST) presented a report on priorities for personalized medicine (http://www.ostp.gov/galleries/PCAST/pcast_report_v2.pdf) [2]. It stated,

Keywords: Individualized medicine, blockbuster drug, pharmacogenetics, biomarkers, PCAST, effectiveness, benefit–risk profile, maraviroc, TPMT, warfarin, VKOR, abacavir, tamoxifen, clopidogrel, personalized medicine

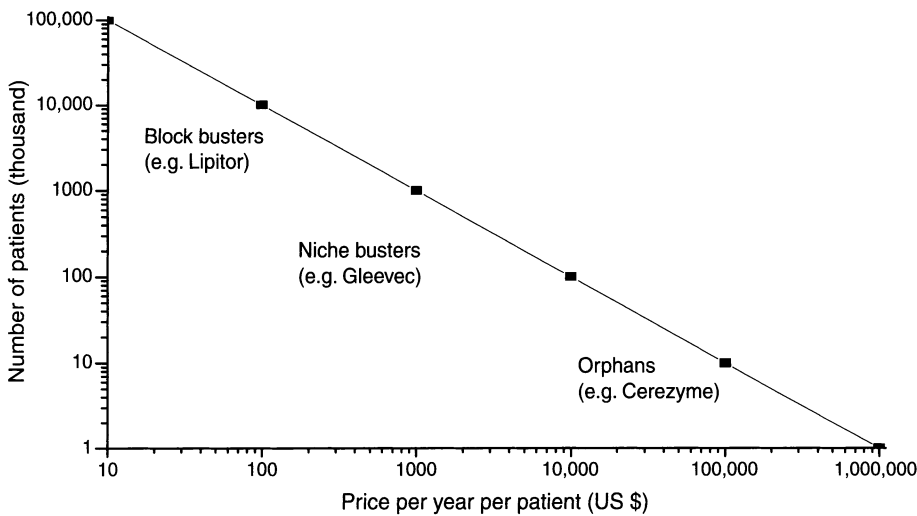


Fig. 1 Different types of high-revenue medicines. Classic blockbusters such as atorvastatin (Lipitor) are prescribed to large patient populations. Stratified medicines that can be demonstrated to be clearly superior to alternatives for a smaller patient population can also achieve high revenues resulting from higher pricing and adoption rates. At one extreme are orphan drugs, which often represent the only therapy for a small population with the disease in question, and which can therefore support even higher pricing if they are highly effective and the condition is sufficiently severe (adapted from Ref. [21])

that ‘*Personalized medicine*’ refers to the tailoring of medical treatment to the individual characteristics of each patient. It does not literally mean the creation of drugs or medical devices that are unique to a patient, but rather the ability to classify individuals into subpopulations that differ in their susceptibility to a particular disease or their response to a specific treatment. Preventive or therapeutic interventions can then be concentrated on those who will benefit, sparing expense and side effects for those who will not”. It was acknowledged that “the current high level of interest in personalized medicine from a policy perspective is attributable not only to the promise of improved patient care and disease prevention, but also to the potential for personalized medicine to positively impact two other important trends – the increasing cost of healthcare and the decreasing rate of new medical product development”. PCAST has, thus, set the agenda of individualized medicine and its goals, i.e. (1) improved patient care and disease prevention, (2) decreasing cost of healthcare and (3) increasing rate of new medical product development.

2 A history of dose individualization

Individualization of drug therapy is by no means an entirely new concept and many aspects of individualization are practiced every day in routine patient care, e.g. drug

dosing dependent on age, sex, body weight or renal function. Also PCAST has acknowledged that “the principle of adjusting treatment to specific patient characteristics has, of course, always been the goal of physicians. However, recent rapid advances in genomics and molecular biology are beginning to reveal a large number of possible new, genome-related, molecular markers for the presence of disease, susceptibility to disease, or differential response to treatment” [2].

Prior to 1900 the development and administration of drugs were mostly based on trial and error; concepts of “dose”, “concentration”, and “therapeutic window” were hardly known [3]. However, the influence of the quantity of a drug on drug response was occasionally reported by observant scientists as early as in the 16th century, e.g. by Theophrastus Bombast von Hohenheim, known as Paracelsus, who stated that “alle ding sind gift und nichts on gift; allein die dosis macht das ein ding kein gift ist” (only the dose makes a remedy poisonous) [4]. Ignorance of the fate of drugs in the human body probably produced a relatively long list of iatrogenic drug victims and it was often not clear whether a patient died due to the disease itself or due to the cure that was administered by the physician [3]. This unfortunate situation led Mark Twain in 1900 to decry “the physician’s grotesque system – the emptying of miscellaneous and harmful drugs into a person’s stomach to remove ailments which in many cases the drugs could not reach at all” [5].

In the subsequent decades physicians struggled with the subject of appropriate drug administration. Dose individualization, i.e. giving the right dose to the right patient, has long been a preoccupation of clinical pharmacologists who justly railed against the common practice of administering a standard dose to all patients. A patient’s biochemical uniqueness generates substantial inter-individual variability and thus profoundly influences the benefit–risk profile in a given individual. Some 20 years ago Sheiner and Tozer first proposed an alternative to the minimalist mentality – the target concentration strategy as a means of optimizing the individual dose [6]. Making better use of clinical PK is based on the hope that signals that would otherwise be lost in statistical noise might be useful to identify covariates that determine drug response.

Although the general usefulness of this approach may be viewed with some skepticism and it is doubtful whether the achievement of this noble goal is feasible, there are several cases in which dose individualization is no longer mere fiction. It was shown, for example, that in children with B-lineage acute lymphoblastic leukemia, the rate of clearance of anti-leukemic agents, which usually differs by a factor of 3–10, is an important predictor of outcome [7]. Outcome could be improved when doses were individualized to prevent low systemic exposure to the drugs in patients with fast drug clearance. Children who received individualized therapy had a significantly better outcome than did those given conventional therapy, and the time dependent systemic exposure to methotrexate was significantly related to the risk of early relapse.

3 Pharmacogenetics and biomarkers

(see also Chapters 15 and 16)

Physicians have long recognized a familial clustering of unusual responses to drugs ([8–12] see also Chapter 15). In 1957 Motulsky published an article on a number of genetic conditions as the cause for a toxic reaction to a drug or an environmental chemical [9]. Vessel and Page showed that the large interindividual variability in PK vanished within sets of monozygotic twins [10–12]. Genetic polymorphisms may occur at different levels in the drug-effect cascade, i.e. at the level of drug targets like receptors, at the level of disease pathways or at the level of drug metabolism. Genetic polymorphisms of metabolic enzymes are important determinants of PK profiles and, thereby, also of toxicity and drug response. The first pharmacogenetic discovery that became a routine aspect of medical practice was the observation that hemolysis was more common among African-American soldiers in the United States Army who were taking the antimalarial primaquine during World War I. Subsequent studies revealed a genetic deficiency of glucose-6-phosphate dehydrogenase as the reason for this serious side effect and it became good medical practice to screen patients for this enzyme defect prior to initiating primaquine therapy [13]. One example for genetic determination of a clinically relevant PK profile is intolerance to 6-mercaptopurine, a standard anti-ALL drug and intolerance was shown among patients with deficiencies in thiopurine-S-methyltransferase (TPMT) enzyme activity [14]. Pharmacogenomics has also provided a number of useful surrogate parameters for disease-drug interactions such as mutations in potassium channel genes and their association with drug induced long-QT syndromes, expression of HER2/neu and benefit of adjuvant chemotherapy in breast cancer or polymorphisms in 5-HT_{2A} receptors, and resistance to antipsychotic drugs [3].

The opportunities created by recent conceptual and methodological advances in molecular biology will have a great impact on drug therapy [15]. Most importantly they may help to select optimal drug candidates for individual patients and avoid unnecessary adverse events by providing a fingerprint of the patient's individual genetic profile. However, the effect of non-genomic variables to overall effects is frequently underestimated.

The current enthusiasm about individualized medicine notwithstanding one has to concede that this concept is also not entirely new. An interesting case was presented recently by Sir Collin Dollery who reported about two conversations [16]: *“The first with a senior, very experienced internist who mused aloud that she thought she had practiced personalized medicine throughout her professional life. She took a careful medical, family, and social history including a detailed account of any current treatment or recent past therapy, alcohol and tobacco intake, and so on. The second conversation was with a younger non-clinical scientist asking why all the discoveries about genetics polymorphisms of the drug-metabolizing enzymes had had so little influence on medicine and why*

CYP2D6 polymorphism were not routinely being tested before prescribing metoprolol, paroxetine, or codeine". According to Dollery both parties have some right on their side, but the reductionist approach has important limitations in very complex realworld situations [16].

4 The unmet need – improving the benefit–risk ration and effectiveness of drugs

Recent reports have shown that current principles of drug therapy might be suboptimal, as adverse drug reactions are a leading cause of death in hospitals ([3], see also Chapter 18). This rather alarming finding was attributed in part to insufficient knowledge of PK principles and individual dosing principles in many clinical specialties. Interestingly, programmes that survey drug therapy according to clearly stated principles were shown to reduce the number of drugs per patient, the number of drugs in hospitals and also the number of ADRs.

Besides the “side effect problem” we also seem to face an “efficacy problem”. The current scientific paradigm of drug development is heavily dependent on statistical assumptions of large RCTs and subsequent EBM or HTA analysis of these results. However, it has to be kept in mind that RCTs represent experimental situations which do not necessarily reflect “real world” conditions. RCTs provide measures of efficacy, i.e. how well an intervention works under the experimental conditions. Effectiveness, i.e. how well an intervention works under “real world” conditions might differ substantially from RCT results. Typically effectiveness is less than efficacy (“efficacy-effectiveness-gap”) because other factors come into play like a mismatch of patient’s characteristics in the RCT *vis-à-vis* the real world condition. The important point is that the tool RCT by itself defines strata of patients dependent on in- and exclusion criteria and therefore stratifies treatment results. An individual patient in a hypertension trial meeting all in- and exclusion criteria of this trial might therefore not be representative of an individual patient with hypertension in an outpatient ward and findings of drug efficacy in this trial might not be representative. To work out, one of the main challenges of EBM is to match real world situations with RCT situations so that the fruits of RCT results can be reaped.

A recent article in *Nature* on GWA of seven common diseases states that “For any given trait there will be few (if any) large effects, a handful of modest effects, and a substantial number of genes generating small or very small increases in disease risk” [17]. The notion that most differences will be small implies a need for large studies. Individualization is a clear turn away from this concept and we may face the inherent tension of a move from an era of large Mega-trials to an era of small Micro-trials, and the concomitant problem that the type 2 error, i.e. the fear of false negative effects, will become more the focus of attention.

5 Improving the benefit/risk profile by biomarker tests

In 2001 Imatinib-mesylate (Gleevec) received regulatory approval for the treatment of patients with chronic myeloic leukemia (CML). *Gleevec* is a well tolerated drug and leads to an overall survival of close to 90% after 5 years. It was developed by screening of chemical libraries for compounds which target specifically the molecule Bcr–Abl, a fusion-protein kinase originating from a translocation between chromosome 9 and 22 giving rise to the “Philadelphia Chromosome”. The development of Gleevec testifies for the power of rational drug development and targeted therapies and provides a good example for an individualized form of medicine, as it is only prescribed for patients positive for the “biomarker” bcr-abl. In recent years similar cases of individualized therapies, based on a combination of a biomarker lab test and a specific drug treatment were developed.

The observation that over-expression of wild-type *VKORC1*, but not *VKORC1* carrying the VKCFD2 mutation, leads to a marked increase in VKOR activity, which is sensitive to warfarin inhibition [18] has led to an increased interest in individualized treatment with warfarin, a notoriously difficult-to-dose drug (<http://www.ama-assn.org/ama1/pub/upload/mm/464/warfarin-brochure.pdf>). It is now recommended to test for polymorphisms of 2 polymorphic enzymes (VKOR and CYP 2C19) before drug administration and it has been shown that the use of a pharmacogenetic algorithm for estimating the appropriate initial dose of warfarin produces recommendations that are significantly closer to the required stable therapeutic dose than those derived from a clinical algorithm or a fixed-dose approach [19].

Also the administration of the monoclonal antibody *trastuzumab* in patients with breast cancer or the small molecule *gefitinib* in patients with lung cancer have increased therapeutic success rates but are dependent on a prior diagnostic test for the presence of the abnormal kinase Her2neu or EGF-receptor mutations, respectively.

In the field of HIV, *maraviroc*, a drug with a novel molecular mechanism at the time of approval, is administered only to patients positive for a test for the chemokine receptor CCR5.

Other examples of potentially useful tests of drug efficacy exist for *tamoxifen* and Cyp 2D6 variants or *clopidogrel* and Cyp 2C19 variants.

Whereas the previous examples mostly pursue the concept of increasing drug efficacy based on the presence or absence or mutation of the target molecule there are also good cases of decreasing drug side effects by a “theragnostic”, i.e. a combined therapeutic and diagnostic approach, e.g. *NAT2* profiling in antituberculous therapy or genotyping for thiopurine-S-methyltransferase (*TPMT*). Extreme intolerance was shown among patients with deficiencies in *TPMT* enzyme activity. Reducing doses of 6-mercaptopurine in *TPMT* heterozygotes and in deficient patients permitted the administration of full protocol doses of other kinds of

chemotherapy while maintaining high thioguanine nucleotide concentrations. Genotyping, or functional enzyme analysis, has now become standard practice in some cancer treatment centres.

In HIV, treatment with abacavir, a highly active NRTI, has become dependent on a prior test for exclusion of HLA-B5701, an allele which carries an 80% risk of allergic reactions in response to *abacavir* (see Case Study Chapter 15).

Other examples of currently available biomarker tests, predictive of response or risk patterns are the *Oncotype DX* test (http://en.wikipedia.org/wiki/Oncotype_DX), a multi-gene expression assay to predict the magnitude of chemotherapy response and likelihood of recurrence of disease in breast cancer or the *AlloMap*-test, a multi-gene expression assay predictive of allograft rejection in heart transplantation (<http://www.xdx.com/allomap>). Despite the recent interest in gene expression signatures, a study which has reanalysed data from the seven largest published studies that have attempted to predict prognosis of cancer patients on the basis of DNA microarray analysis has shown that the list of genes identified as predictors of prognosis was highly unstable and that microarray results should be considered with caution [20]. Another word of caution might be warranted if the Gleevec case is cited as the new paradigm of drug development. Gleevec might be rather the exception than the rule, as it has been shown that for most diseases the patho-physiological pathway is rather complex as in the case of diabetes, coronary heart disease or hypertension and not as straightforward “Mendelian” as in the case of the Philadelphia chromosome. For any given trait there will be few (if any) large effects, a handful of modest effects, and a substantial number of genes generating small or very small increases in disease risk [16].

In conclusion there is ample evidence that current achievements in biomarker research and genomics will have a major effect on clinical practice and development of new drugs, which are better matched to specific patient populations ([21], Fig. 2). To

| <i>Empirical medicine</i> | <i>Stratified medicine</i> | <i>Individualized medicine</i> |
|-------------------------------------|--------------------------------|------------------------------------|
| Vaccines NSAIDs PPIs SSRIs | Imatinib Trastuzumab | Cancer vaccine |

Fig. 2 The patient therapeutic continuum. Individualized medicines, such as cancer vaccines that are based on a particular patient’s tumour, represent one end of a continuum of patient therapy. Empirical medicine is at the other end of this continuum: some agents work for almost all relevant patients, such as non-steroidal anti-inflammatory drugs (NSAIDs), whereas others may only work for a subset of patients but no method is available to identify these patients, such as with antidepressants. In between lies the field of stratified medicine (adapted from Ref. [21])

date, however, translation of genomic science to clinical settings has not kept pace with growing interest in personalized medicine in some fields [22].

Case Study: Warfarin dosing

Oral anticoagulants (OACs) like warfarin or phenprocoumon are the main pillar of treatment for conditions like deep vein thrombosis, pulmonary embolism, atrial flutter or anticoagulation after cardiac valve replacement and, thus, belong to the most widely prescribed drugs. It is estimated that 1% of the population is currently taking OACs.

The mode of action of OACs is the inhibition the polymorphic enzyme Vitamin-K Epoxide Reductase (VKOR) and thereby of clotting factor synthesis in the liver. OACs like Warfarin are metabolized by Cytochrome (CYP) 2C9, a polymorphic enzyme in the liver (Schwarz UI *et al. Genetic Determinants of Response to Warfarin during Initial Anticoagulation. NEJM* 358: 999–1008, 2008). 20%/37% of Caucasians harbour at least one variant copy of CYP 2D9 or VKORC1, which might explain ~15 and ~25%, of variable warfarin dosing, respectively.

After initiation of therapy it typically takes a few days until anticoagulation is complete. Stable anticoagulation in the therapeutic range is critical as underdosing increases the clotting risk whereas overdosing might lead to severe bleeding complications. OACs have a narrow therapeutic range and dosing requirements may vary considerably between patients, e.g. up to tenfold. Therefore, patients need to undergo therapeutic drug monitoring (TDM) by measuring INR levels which should typically range between 2 and 3. However, even under the best conditions only 66% of patients will be in the therapeutic range (*SPORTIF III. Lancet* 362: 1691–1698, 2003).

Unfortunately 21% of patients who receive OAC therapy experience major or minor bleeding (<http://www.ama-assn.org/ama1/pub/upload/mm/464/warfarin-brochure.pdf>) and OACs are among the top drugs leading to hospitalizations due to adverse drug reactions.

Recent studies indicate that appropriate OAC dosing might more readily be achieved after initial measurement of CYP 2C9 and VKORC1 polymorphisms (*The International Warfarin Pharmacogenetics Consortium. Estimation of the Warfarin Dose with Clinical and Pharmacogenetic Data. NEJM* 360: 753764, 2009), as opposed to empiric or age and weight adjusted initial dosing and the greatest benefit may be seen for patients at the extreme ends of the dosage range. Critics of this prospective, individualized approach argue that no RCT has formally tested this hypothesis with

clinical endpoints yet, INR measurements are still the method of choice and many other factors like diet or drug interactions may change INR levels. Moreover it has not been shown whether this approach is cost effective.

In the US the warfarin label has been changed accordingly and recommends genetic testing prior to initiation of warfare therapy and web based dosing estimates are available based on clinical factors and genotypes of *CYP2C9* and *VKORC1* (<http://www.warfarindosing.org/>).

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References

1. Clinton HR, Obama B (2006) Making patient safety the centerpiece of medical liability reform. *NEJM* 354: 2205–2208
2. The Presidential Council of Advisors on Science and Technology (PCAST) (2008) Report on Personalized Medicine. http://www.ostp.gov/galleries/PCAST/pcast_report_v2.pdf
3. Müller M, Müller-Zellenberg U, Hochhaus G, Derendorf H (2001) Current concepts in clinical pharmacokinetics and their implications for clinical medicine. *Wien Klin Wochenschr* 113: 566–572
4. Waite AE (1894) The Hermetic and Alchemical Writings of Aureolus Philippus Theophrastus Bombast of Hohenheim Called Paracelsus the Great, Vol. 2. Elliott & Co., London
5. Ober KP (1997) The Pre-Flexnerian reports: Mark Twain’s criticism of medicine in the United States. *Ann Intern Med* 126: 157–163
6. Sheiner LB, Tozer TN (1978) Clinical pharmacokinetics: the use of plasma concentrations of drugs. In: Melmon KL, Morelli HF (eds.) *Clinical pharmacology: basic principles of therapeutics*. MacMillan, New York, pp. 71–109
7. Evans WE, Relling MV, Rodman JH, Crom WR, Boyett JM, Pui CH (1998) Conventional compared with individualized chemotherapy for childhood acute lymphoblastic leukemia. *N Engl J Med* 338(8): 499–505
8. Müller M (2003) Pharmacogenomics and drug response. *Int J Clin Pharmacol Ther* 41(6): 231–240
9. Motulsky AG (1957) Drug reactions, enzymes and biochemical genetics. *JAMA* 165: 835–836
10. Vesell ES, Page JG (1968) Genetic control of drug levels in man: antipyrine. *Science* 161(836): 72–73
11. Vesell ES, Page JG (1968) Genetic control of drug levels in man: phenylbutazone. *Science* 159(822): 1479–1480

12. Vesell ES (2000) Advances in pharmacogenetics and pharmacogenomics. *J Clin Pharmacol* 40(9): 930–938
13. Carsen PE, Flanagan CL, Iokes CE, Alving AS (1956) Enzymatic deficiency in primaquine-sensitive erythrocytes. *Science* 124: 484–485
14. Relling MV, Hancock ML, Rivera GK, Sandlund JT, Ribeiro RC, Krynetski EY, Pui CH, Evans WE (1999) Mercaptopurine therapy intolerance and heterozygosity at the thiopurine S-methyltransferase gene locus. *J Natl Cancer Inst* 91(23): 2001–2089
15. Mancinelli L, Cronin M, Sadee W (2000) Pharmacogenomics: The Promise of Personalized Medicine. (<http://www.pharmsci.org/ScientificJournals/pharmsci/journal/4.html>) *AAPS Pharmsci* 2(1): article 4
16. Dollery CT (2007) Beyond genomics. *Clin Pharmacol Ther* 82(4): 366–370
17. The Wellcome Trust Case Control Consortium (2007) Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 447: 661–678
18. Rost S, Fregin A, Ivaskevicius V, Conzelmann E, Hörtnagel K, Pelz HJ, Lappegard K, Seifried E, Scharrer I, Tuddenham EG, Müller CR, Strom TM, Oldenburg J (2004) Mutations in VKORC1 cause warfarin resistance and multiple coagulation factor deficiency type 2. *Nature* 427(6974): 537–541
19. International Warfarin Pharmacogenetics Consortium, Klein TE, Altman RB, Eriksson N, Gage BF, Kimmel SE, Lee MT, Limdi NA, Page D, Roden DM, Wagner MJ, Caldwell MD, Johnson JA (2009) Estimation of the warfarin dose with clinical and pharmacogenetic data. *N Engl J Med* 360(8): 753–764. Erratum in: *N Engl J Med* 2009 361(16): 1613 (Dosage error in article text)
20. Michiels S, Koscielny S, Hill C (2005) Prediction of cancer outcome with microarrays: a multiple random validation strategy. *Lancet* 365(9458): 488–492
21. Trusheim MR, Berndt ER, Douglas FL (2007) Stratified medicine: strategic and economic implications of combining drugs and clinical biomarkers. *Nat Rev Drug Discov* 6(4): 287–293
22. Arnett DK, Claas SA, Lynch AI (2009) Has pharmacogenetics brought us closer to ‘personalized medicine’ for initial drug treatment of hypertension? *Curr Opin Cardiol* 24: 333–339

CHAPTER 23

Generics, biosimilars, enantiomers and me-toos

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1 Generics

The term “generic” applies to products containing mostly small molecule chemical active substances, usually produced by chemical synthesis. EU legislation describes a generic product as a product which has the same active substance in the same amount as the originator’s product (the reference product), the same pharmaceutical form, and whose bioequivalence with the reference product has been demonstrated by appropriate bioavailability studies [1]. “Innovative” products in most countries of the world are rewarded and protected from competition in a number of ways, but they are not allowed to keep the market to themselves forever. Generic medicines are basically copies of these innovative medicines which were once new but which have been marketed for several years with proven satisfactory efficacy and safety. The passage of time (10 years in most EU Member States) transforms innovative medicines with new active substances into established medicines, and opens the door to generic competition.

Current EU and US legislation imposes a large regulatory burden on companies developing new molecules – they must provide evidence of satisfactory quality, efficacy and safety, in particular complete details of all toxicology, pharmacology, and clinical trials. In compensation, they are allowed to protect their intellectual property, and to enjoy some measure of regulatory protection from generic competition. Generic medicines are not required to repeat all of these toxicology and clinical studies when the innovator’s product has shown many years of safe and effective use – indeed there is a sense in which it may be regarded as unethical to do so. This is also the situation in the USA and in many other countries worldwide. On the other hand, given this minimal clinical development in the case of a generic, it is the responsibility of the regulatory

* The views expressed in this chapter are the personal views of the authors and may not be used or quoted as being made on behalf of, or reflecting the position of, any national competent authority, the European Medicines Agency (EMA) or one of its committees or working parties.

authorities to ensure that the generic product is equivalent to the originator's product in all important respects, and to assure patients and prescribers that there is no significant difference between the two products in the clinic. In the EU, the term “essentially similar” has been used to describe the relationship between the two, and as expected, the precise meaning of this term has been debated in the courts over many years. In brief, one of the most important ways of providing this assurance is to demonstrate that the generic product is *bioequivalent* to the reference product.

Since generic companies are not required to repeat the large multicentre clinical trials of the innovator, and instead are able to rest mainly on bioequivalence studies, it follows that a generic medicine is usually cheaper than the original on which it is based. We should keep in mind that the population of the EU is bigger than the USA, and within this large market, a very large percentage of all medicines taken by patients are in fact generics. This has important consequences for the healthcare budgets of the Member States.

1.1 Regulatory background

First, before there can be a generic copy of an innovative medicine there has to be an innovative medicine. A description of the detailed particulars of the EU regulatory system for new products is outside the scope of this chapter, but it may be useful to highlight some basic principles.

A medicinal product cannot be marketed in the EU until it receives a Marketing Authorization. Unlike the USA, there are several routes to authorization in the EU – at the national, de-centralized, and centralized (i.e. EU-wide) levels. The centralized procedure was set up in 1995 to deal primarily with new molecules, new technology, biotechnology medicines, new clinical indications, etc., – basically anything containing the word “new”. It is coordinated by the European Medicines Agency (EMA) and involves an assessment by experts in the Member States – the “rapporteurs” – a number of scientific working parties, external experts when necessary, ending in a Scientific Opinion of the main committee of experts – the CHMP. Many new products have been authorized in this way and recent legislation has widened the scope of the Centralized Procedure to include generics, which are coming in increasing numbers. The decision to approve the medicine (or not) is based on the so-called benefit/risk balance for that medicine taking into account all the information presented by the applicant company in their application dossier. After authorization, there is the important business of pharmacovigilance and periodic safety update reports so that the safety profile observed in the rather limited context of the clinical trials can be confirmed in the much wider context of the market.

Generic dossiers are classified as “abridged” and in those cases where some clinical trials are needed they are “hybrid”. In principle the evaluation process for generics seeks to confirm that the benefit/risk balance established for the reference product on the basis

of extensive efficacy and safety studies can also be applied to the generic product on the basis of their abridged/hybrid dossier. The main issues which are considered are:

Quality: The generic applicant must provide full details of manufacturing and control. Generics formulations are not required to be identical to the reference product but they are usually found to be very similar in practice. Obviously the active substance must be the same, but excipients may be different, e.g. different colours, where there is no impact on bioavailability or safety.

Impurities: Since generic manufacturers will normally be using a different source of the active substance, it may be that it is made by a different synthetic process to the one used by the originator and therefore it may contain new impurities not seen before, and for which there is no exposure history in humans. EU legislation requires that all impurities should be “qualified” i.e. shown to be safe, usually with reference to relevant toxicology studies. In practice this is not a problem since today’s synthetic and purification processes can deliver pure substances with individual impurities less than 0.1% in many cases. In the absence of specific structural alerts, e.g. possible genotoxic carcinogen in the worst case, this is below the threshold of toxicological concern for most substances.

Bioequivalence: In the world of generic medicines, the concept of bioequivalence is fundamental. It is a focus of attention for the regulators and will be discussed later in this chapter.

Finally, at the end of the evaluation process, the single most important document which defines the conditions of use of any medicinal product in the EU is the Summary of Product Characteristics – the SPC. This defines the approved clinical indications, population to be treated, dose, precautions, warnings, contra-indications, etc. At first sight, this document may not appear very friendly to clinicians in their everyday practice (they have more useful sources of practical information to guide them in their prescribing decisions). It does, however, summarize the legal justification for the company’s marketing claims, and prescribers need to be aware of it. Having chosen to prescribe a particular medicine, if they depart from the conditions defined in the SPC they may expose themselves to issues of liability. Not surprisingly, the SPC for a generic should be identical to that of the reference product as far as possible, but important differences may exist (see the next section with regard to patented indications).

1.2 Patent protection, data protection and marketing protection for new products

An important consequence of the authorization of new molecules is the concept of “data exclusivity” or “data protection”. Given the high costs of developing new products with new molecules, some form of compensation is considered to be reasonable, in order to protect this investment from generic competition. Apart from obvious measures like

patent protection, new active substances are also protected by EU pharmaceuticals legislation in the form of data and marketing protection. Prior to 2005 this meant that the regulatory authorities could not consider a generic (abridged) application for a period of time 10 years after the authorization of the reference product on which it was based (6 years in some Member States). Since 2005 the legislation has changed slightly to say that authorities can not accept an abridged generic application until after 8 years – the data protection period. After this time, they can evaluate and issue a marketing authorization for a generic, but the company can not market the product until 10 years have elapsed – market protection. All of this is without prejudice to the patent legislation – a generic company would be very unwise to use their marketing authorization and place their product on the market when some form of patent protection was in force.

Concerning patent protection, this can even apply to clinical indications (usage patents) and is applied at a national level. For example, an innovator company may hold a UK patent for the use of their molecule in the treatment of a certain disease, therefore regardless of the favourable benefit/risk balance of any generic product, a generic company would not be able to market their product for that indication in the UK. In practice, the Summary of Product Characteristics, or SPC, for the generic could make no mention of this specific use in the UK. This may expose prescribers to the liability issues referred to in the previous section. Prescribers who use a generic medicine for a specific indication which is “blocked” in the SPC for patent reasons face a slightly different risk compared to their decision to use a medicine based on weak or anecdotal scientific evidence. Nevertheless, as usual they must take responsibility for the use of any product outside the terms of the authorized use.

1.3 Salts, esters

If the innovator has patented certain salts or esters or other derivatives of their molecule, generics may be forced to use a different salt or ester of this active moiety. This is allowed in the legislation, and is an interpretation of the words “same active substance” on the condition that the different salt or ester does not show differences with regard to efficacy and safety compared to the active substance in the reference product, e.g. amlodipine and clopidogrel:

- amlodipine besilate, amlodipine maleate
- clopidogrel hydrogen sulphate, clopidogrel hydrochloride

The common feature in the different forms above is that they are all soluble substances which are probably unlikely to present any bioavailability problems when taken orally, particularly with regard to absorption. However, the generic company will have to provide evidence that the anion/cation/acid/base presents no additional safety problem compared to the reference product. Such solubility differences which may exist

between the generic and the reference substances above are probably not clinically relevant, and this can be shown by means of dissolution results. Comparative dissolution between the generic and reference products performed under standardized conditions can be a useful surrogate bioequivalence marker, a biowaiver. The European Pharmacopoeia has a standard method and applicants are also able to develop their own discriminatory methods. As a very general rule-of-thumb for soluble drugs where there is no intestinal permeability problem, 70% release of drug in 45 min in water is taken to indicate that bioavailability problems will be unlikely. This is too general to indicate bioequivalence, but it is a start. A formal bioequivalence study as described later can provide more convincing evidence of bioequivalence.

1.4 Bioequivalence

Broadly speaking, two medicinal products containing the same active substance are considered bioequivalent if their bioavailabilities after administration in the same molar dose lie within acceptable pre-defined limits. These limits are set to ensure comparable *in vivo* performance, i.e. similarity in terms of safety and efficacy. Oral products delivering systemically-active drugs represent a common context of drug therapy and in this regard bioavailability is linked to the rate and extent of absorption.

In order of increasing confidence, the methods available for investigating bioequivalence are as follows:

- *In vitro* dissolution tests
- Comparative bioavailability (bioequivalence) studies
- Comparative pharmacodynamic studies in humans
- Comparative clinical trials

However, as mentioned above, physicochemical testing may show pharmaceutical equivalence but this does not necessarily mean therapeutic equivalence. For this, some sort of comparative study using human subjects (volunteers or patients) will be required.

Bioequivalence is not normally needed when both the generic and reference product contain a water-soluble drug which is already in solution in the product, but it is particularly relevant for the following types of product where a systemic action is involved:

- Oral immediate release products (e.g. tablets) when one or more of the following criteria apply:
 - indicated for serious conditions requiring assured therapeutic response
 - narrow therapeutic window/safety margin; steep dose-response curve
 - complicated pharmacokinetics

- unfavourable physicochemical properties, e.g., low solubility
- documented evidence for bioavailability problems related to the drug
- where a high ratio of excipients to active ingredients exists
- Non-oral and non-parenteral products, such as transdermal patches, suppositories, etc.
- Modified release products
- Fixed combination products

The most common bioequivalence study design is single-dose, randomized, two-way crossover study (non-replicated) [2]. Two groups of subjects are arranged and randomized to be given either the generic (test) product or the reference product. Plasma levels are measured at intervals. After a suitable washout period the process is repeated with the subjects now receiving the other product. Other designs are indeed possible, e.g. parallel design for drugs with long half-lives or in patients, and steady-state studies for some non-linear drugs. Studies should be carried out in accordance with provisions of EU requirements for Good Clinical Practice, Good Manufacturing Practice, Good Laboratory Practice.

The study protocol must state *a priori* the study objectives and the methods to be used, and the generic formulation to be used must be representative of the product which is intended for the market. Concerning the subjects and other aspects to be defined:

- Subjects
 - number
 - health status
 - age, weight and height
 - ethnicity
 - gender
 - special characteristics e.g. poor metabolisers
 - smoking
 - inclusion/exclusion criteria specified in protocol
- Randomization
- Blinding
- Sampling protocol
- Washout period
- Administration of food and beverages during study

The number of subjects to include is critical, and must be carefully planned to have confidence that the requirements for bioequivalence will be achieved (see later) i.e. the study must be sufficiently powered on the basis of the expected variability in the results. Bioanalytical methods used to measure plasma levels of the drug need to be validated

with regard to specificity, sensitivity, precision, limit of quantitation, etc. and then begins the process of collecting the data. Two sets of data are gathered, one for the generic (test) product and the other for the reference product. According to current EU guidance [3], the most relevant pharmacokinetic parameters should be obtained as follows.

In studies to determine bioequivalence after a single-dose, $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, residual area, C_{\max} and t_{\max} should be determined. In studies with a sampling period of 72 h, and where the concentration at 72 h is quantifiable, $AUC_{(0-\infty)}$ and residual area do not need to be reported; it is sufficient to report AUC truncated at 72 h, $AUC_{(0-72\text{ h})}$. Additional parameters that may be reported include the terminal rate constant, λ_z , and the plasma concentration half-life $t_{1/2}$. In studies to determine bioequivalence for immediate release formulations at steady state (ss), the AUC during a dosage interval (τ) at steady state $AUC_{(0-\tau)}$, $C_{\max,ss}$ and $t_{\max,ss}$ should all be determined.

For immediate-release oral dosage forms like tablets and capsules the two main parameters of interest are C_{\max} and AUC, taken to be indicative of rate and extent of absorption respectively although some authors have suggested C_{\max}/AUC as a better (less variable) estimate of absorption rate [4]. These are compared in terms of the ratio between the mean of these parameters calculated as generic test/reference. In the ideal case where the generic is identical to the reference, this will be 1.00 for both mean C_{\max} and mean AUC. However since biological data are variable in reality, the standard EU acceptance criteria for bioequivalence are set in terms of confidence limits as follows [5]. No particular attention or weighting factor is given to the values of the means themselves; within the confidence interval, all values have equal probability, equal weight, and the confidence interval must be wholly contained within the defined acceptance range.

90% CI around the mean $(C_{\max})_{\text{test}}/(C_{\max})_{\text{ref}}$ should be within 80.00–125.00%

90% CI around the mean $(AUC)_{\text{test}}/(AUC)_{\text{ref}}$ should be within 80.00–125.00%

These standards must be met on log-transformed parameters calculated from the measured data. However, since C_{\max} is inherently more variable than AUC, a wider acceptance range may be justified. Clearly anything that increases variability, for example

- known variability in absorption or clearance,
- too few subjects
- assay imprecision

will widen the 90% confidence intervals measured, and therefore decrease the chances of compliance with bioequivalence requirements. Generic companies have

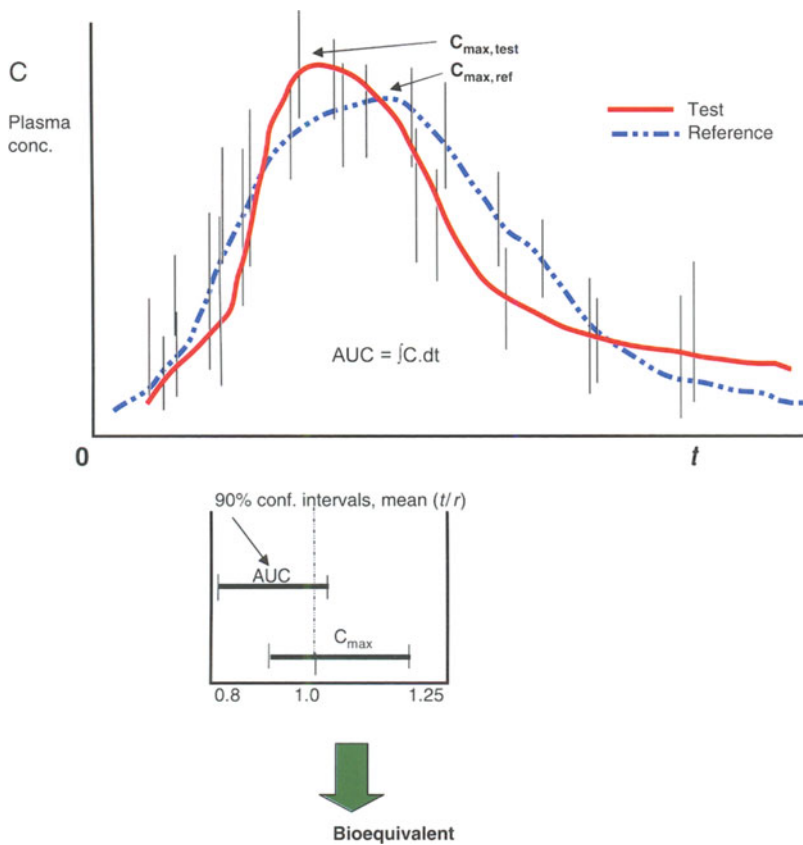


Fig. 1 Oral immediate-release products. Mean drug plasma levels measured in a single-dose crossover study. Generic (test, t) is bioequivalent to the Reference Product (reference, r), even though the mean C_{max} “appears” to be higher and the mean AUC lower. The confidence interval boundaries do not cross the 0.8–1.25 envelope

to decide between too many subjects (increased cost) *versus* too few (increased risk of failure).

1.5 Bioequivalence – some special issues

1.5.1 Narrow therapeutic index drugs (NTIDs)

For drugs with a narrow therapeutic window, e.g. certain antiepileptics, the 80–125% acceptance window may be too generous and allow an unacceptable or even dangerous variability. In these cases the 90% CI and mean AUC is required to be contained in the

range 90.00–111.11%, and this also applies to mean C_{\max} if considered necessary. There is no official list of NTIDs, the judgement is made case by case according to clinical considerations.

1.5.2 Highly variable drug products (HVDP)

Drugs with a known intrasubject variability >30% in a parameter of interest are a special problem in the domain of bioequivalence studies. The number of subjects required is prohibitively high to reach the standard acceptance interval for bioequivalence. Current EU guidance allows for a wider 90% confidence interval to be applied which must be prospectively defined and justified. The acceptance criteria for C_{\max} can be widened to a maximum of 69.84–143.19%. For the acceptance interval to be widened the bioequivalence study must be of a replicate design where it has been demonstrated that the within-subject variability for C_{\max} of the reference compound in the study is >30%. Though many claims are made for drugs as potentially highly variable, surprisingly few products show high variability in tightly-controlled crossover studies.

1.5.3 Chiral drugs, enantiomers

The use of achiral bioanalytical methods is generally acceptable. However, the individual enantiomers are measured when all the following conditions are met:

- the enantiomers exhibit different pharmacokinetics,
- the enantiomers exhibit pronounced difference in pharmacodynamics,
- the exposure (AUC) ratio of enantiomers is modified by a difference in the rate of absorption. The individual enantiomers should also be measured if the above conditions are fulfilled or are unknown. If one enantiomer is pharmacologically active and the other is inactive or has a low contribution to activity, it is sufficient to demonstrate bioequivalence for the active enantiomer.

1.5.4 t_{\max}

It is tempting in some cases to also compare t_{\max} (the time taken to reach C_{\max}), but the statistical techniques for comparison are different, and would probably involve non-parametric methods. The comparison of relative t_{\max} is not common, but may be important in cases where rapid onset of action is important, e.g. in the case of rapid-acting oral hypnotics like temazepam it may be useful for the generic product to show comparability with the reference product in terms of a short t_{\max} .

1.5.5 Drugs which are not orally absorbed

Not all substances given orally are absorbed for systemic use; the case of orlistat may be mentioned as an example. The product Xenical[®] containing orlistat was authorized in the EU on 29 July 1998 with a clinical indication related to the treatment of obesity. The public information available at the EMA website in the form of the summary of the scientific assessment report (EPAR) indicates that orlistat has a predominantly local action in preventing the absorption of fat, and is itself not absorbed to any significant extent. Plasma levels are very low, there may be analytical difficulties concerning sensitivity, limit of quantitation, etc., and therefore a generic company would find it very difficult to perform a standard bioequivalence study as described above. In fact the relevance of such a study as an indicator of comparative efficacy may be questioned, although plasma levels may be important from the point of view of safety.

In this case, the measured end-points of such a bioequivalence study would probably have to be pharmacodynamic in nature, linked to the clinical indication. Since such endpoints are subjectively more variable and lack the analytical precision that exists in measuring plasma levels, it is likely that the number of subjects would have to be increased in order to get sufficient power to establish bioequivalence with acceptable confidence.

1.5.6 Inhaled drugs

In principle, inhaled drugs for systemic absorption and action may be compared by measurement of plasma levels like orally-absorbed drugs. On the other hand, solid particulates like salbutamol present another case. The disposition of inhaled particles is strongly dependent on particle size, the critical size range for delivery to the distal parts of the bronchial tree being ca. 1–5 μm in terms of the mass median aerodynamic diameter (MMAD). Any larger and they impact on the pharynx and are swallowed into the stomach; any smaller and they are lost on exhalation (cigarette smoke). Therefore, tight control of the particle size distribution, is important for generics, and *in vitro* models exist for comparison of the relative disposition of particles in the inhaled cloud of generic and reference products. However, the true indicator of therapeutic equivalence in products used to treat asthma/COPD comes from a human study measuring and comparing a pharmacodynamic endpoint like FEV₁. This becomes mandatory when equivalence is not shown *in vitro* according to the physicochemical tests above, and is not shown convincingly by investigation of pulmonary deposition and systemic safety.

1.5.7 Bioequivalence of intravenous products? complex parenterals

In the case of a soluble systemically-active generic drug given by intravenous injection, there is normally no need for a demonstration of bioequivalence with a reference

product also given in the same way. There are no barriers to absorption of the type that exist with oral products, and the drug may be assumed to be immediately available in both cases. However, a recent revision to the EU bioequivalence guideline foresees the case of “complex-forming” drugs, or formulations which may not be simple solutions and which therefore could also be regarded as complex parenterals, e.g. micelles or liposomes. The issue here is the introduction of an additional phase, e.g. the lipophilic interior of a micelle, which adds another competing equilibrium between the drug and the target and which may influence the kinetics or disposition of the (free) drug.

The field of oncology includes several insoluble drugs which have to be complexed in this way in order to be given intravenously. Docetaxel and paclitaxel are examples. By default, a generic copy of these complex products would have to demonstrate bioequivalence unless they present sound justification for a biowaiver. There is published evidence to suggest that micelles may be short-lived *in vivo*; they are removed by dilution and rapid metabolism of the surfactant, and their ability to significantly affect the *in vivo* kinetics of their cargo drug in reality has been questioned. Therefore, in this regard, it may be possible to develop suitable *in vitro* models to compare the micellar properties of generic and reference products in a way that could be accepted as a “biowaiver”.

By contrast, liposomes tend to be more persistent *in vivo* and their ability to alter the kinetics and disposition of drugs can be clearly seen in plasma level data. *Doxorubicin* is an example where the plasma half-life and distribution of the drug is prolonged in liposomal form as compared to simple solution. Therefore it is unlikely that liposomal forms could be judged to be equivalent on the basis of *in vitro* tests alone. When considering the bioequivalence of liposomal injections, analytical methods are needed which can differentiate between free drug and liposomally-entrapped drug in plasma, at the very least. It is likely that additional studies measuring clinical endpoints would also be necessary unless otherwise justified.

Relevant to both micellar and liposomal injections, there is a mechanism operating in oncology when the drug is intended to treat solid tumours – the EPR effect [6] (Enhanced Permeability and Retention). For example, there are published reports showing prolonged kinetics and an EPR effect for injections of cisplatin solubilized in polymeric micelles. Nanosized structures like micelles and liposomes in the range 10–100 nm are retained by the tight endothelial junctions of normal vasculature but tumour vasculature is abnormal and allows extravasation. Allowing also for reduced lymphatic drainage from malignant tissue, eventually these structures and the associated drug will accumulate in the vicinity of the tumour – this obviously indicates a potential for increased efficacy which may not be reflected in the gross plasma levels of a bioequivalence study. This begs the question – what then is the relevance of a plasma-based bioequivalence study for these complex parenterals? It is possible that *in vitro* models and intracellular

kinetic studies may yield information which could be relevant for the comparison of generic and reference products of this type.

2 Biosimilars

2.1 Complexity

Biological medicines, including large peptides and nucleotides, are complex molecules, not only in terms of primary structure, but also secondary and tertiary. Arising from this complex nature, they are difficult to manufacture and to control, so much so that the manufacturing process itself must be very tightly defined, otherwise small changes in processing conditions may have an impact on the nature of the resulting molecule and alter its pharmacological effect. Historically, many biological medicines have been extracted and purified from a biological source, e.g. Factor VIII from blood, interferon from cell-culture, etc., but in recent years the techniques of recombinant DNA technology have opened the door to the creation of large proteins of medicinal interest (biotech. medicines). Compared to synthetic chemicals, some of these are very large indeed:

| Chemical | MWt (D) | Biological | MWt (D) |
|------------|---------|-----------------------------------|---------|
| Metformin | 166 | Filgrastim, Neupogen [®] | 18,800 |
| Ranitidine | 351 | Etanercept, Enbrel [®] | 75,000 |
| Paclitaxel | 854 | Rituximab, Rituxan [®] | 145,000 |

Biological medicines are generally defined by the following characteristics:

- A biological source, e.g. tissues, blood
- A “non-chemical-synthetic” method of manufacture, e.g. recombinant DNA techniques
- The use of a combination of biological assay and physicochemical methods for full characterization and control

They may include:

- Classical biological products
- Recombinant proteins
- Novel or advanced therapy medicinal products (gene and cell therapies and tissue engineered products)
- Products which cannot be characterized by physico-chemical means

2.2 Biosimilars: General issues

In principle, and following the analogy of generic medicines in the chemical world, when innovative biological medicines have been on the EU market for 10 years, are they also open to competition from generic companies? Can a copy come to the market based on essential similarity and bioequivalence as for generics, without the need for nonclinical and clinical testing? The short answer is no. Such is the complexity of these biological molecules that for many years the possibility of a generic copy was thought to be out of the question. It was believed that it would be impossible for another manufacturer to copy exactly the innovator's manufacture and control of such molecules and end up with a therapeutically equivalent product. However, this view has evolved in recent years, and now current EU legislation [7] allows a competitor company to develop a biological medicine which is claimed to be "similar" to a biological reference product, i.e. a "biosimilar". Biosimilar medicines are not generics and need appropriate preclinical and clinical testing before being delivered to the market and used in patients.

The safety and efficacy profile of a biosimilar is established for each therapeutic indication. Both the European Medicines Agency and the European Generic Medicines Association (EGA) have produced useful Question and Answer documents on their websites [8, 9].

The concepts established for chemical generics, e.g. essential similarity, bioequivalence, do not apply to biosimilars – they are not enough to establish therapeutic equivalence, and extra proof needs to be provided in the form of nonclinical (animal) and clinical studies to establish the "comparability" between the biosimilar and the reference product. The amount of appropriate preclinical and clinical data to provide is decided on a case-by-case basis, depending on the level of complexity of the product, the state of the art of the analytical procedures, the manufacturing processes and clinical and regulatory experience.

In practice, the success of such a development approach will depend on the ability to characterize the product and therefore to demonstrate the similar nature of the concerned products. There is a spectrum of molecular complexity among the various products (recombinant DNA, blood or plasma-derived, immunologicals, gene and cell-therapy, etc.). Moreover, parameters such as the three-dimensional structure, the amount of acido-basic variants or post-translational modifications such as the glycosylation profile can be significantly altered by changes, which may initially be considered to be "minor" in the manufacturing process. Thus, the safety/efficacy profile of these products is highly dependent on the robustness and the monitoring of quality aspects.

As with chemical generics, given the ever-increasing pressure on healthcare budgets across the EU, there have been calls to approve biosimilar medicines, which may be cheaper than the original innovator products and which have an assurance of satisfactory efficacy and safety. The EU has taken the lead in the recognition of these

issues, and has created a regulatory framework for the authorization of biosimilars which are equivalent to an original reference product in all clinically-relevant regards.

2.3 Regulatory experience

The first biosimilar to be authorized in the EU was Omnitrope[®] (Sandoz, somatropin growth hormone), in 2006 which was shown to be similar to the reference product, Genotropin[®] (Pfizer, formerly Pharmacia). Apart from extensive characterization by physico-chemical and biological methods, clinical studies demonstrated similar clinical efficacy for the biosimilar and the reference product. The incidence of anti-somatropin antibodies was initially higher in the biosimilar group. However, these antibodies did not affect efficacy or safety of the biosimilar (application of the principle of benefit/risk balance). Their occurrence was probably linked to the presence of an increased level of host cell proteins. After introduction of additional purification steps, antibody frequency fell to the expected range.

Since 2006, the EMA has released guidelines covering several different types of recombinant proteins, including insulin, granulocyte-colony stimulating factor (G-CSF), somatropin and erythropoietin, as well as low-molecular weight heparins. Up until now, at least thirteen biosimilars have been authorized in the EU, all versions of somatropin, epoetin alfa, epoetin zeta or filgrastim. So for the time being at least, the biosimilar market remains small and focuses mainly on some of the less complex biologicals [10]. Concerning more complex molecules, Christian Schneider, the chairman of the EMA's Biosimilar Medicines Working Party has the following viewpoint:

“... Recent licensing of recombinant somatropins and several erythropoietins as biosimilars has prompted discussions as to whether the same regulatory path could also be applied to more complex biologics, such as monoclonal antibodies (mAbs). Although physicochemical and biological methods for characterization of mAbs are becoming increasingly sophisticated, the ability to compare a biosimilar mAb to a reference mAb on an analytical level remains limited and the design of a clinical development programme for a biosimilar mAb will likely prove a challenge...” [11].

3 Enantiomers

3.1 Sophisticated nonsense?

The stereochemistry of organic molecules has been known and studied since the early 19th century, and terminology has evolved to be quite confusing, with a plurality of systems in use for the differentiation of chiral forms:

| | |
|-------------------------|--|
| <i>d, l</i> or (+), (–) | relating to the effect on polarized light rather than molecular structure, |
| D, L: | (no relation to the above) a convention applied mainly to sugars and amino-acids |
| R, S: | a more recent convention related to 3D structure |

Furthermore, the connection between stereochemistry and biological activity is well-known, with most naturally-occurring molecules, e.g. amino acids, being the *l*-forms. The interactions in the body between a drug and the proteins which elicit a therapeutic response or an adverse effect or the metabolic clearance of the drug require a specific three-dimensional configuration of drug and protein – the “lock and key” hypothesis. Since enantiomers have different three-dimensional configurations, the pharmacodynamics and pharmacokinetics of the two enantiomers which make up a racemic drug may be quite different, especially if the centre of asymmetry of the drug is close to the points of attachment to a receptor.

The connection between stereochemistry and biological activity was dramatically highlighted by the thalidomide tragedy. Tests in mice in the 1960s suggested that only the (S)-enantiomer was teratogenic while the (R)-form possessed the therapeutic activity. Unfortunately, subsequent tests in rabbits showed that both enantiomers had both activities. In view of what is said above, this may seem surprising, but there is evidence that in humans the two enantiomers interconvert *in vivo*. Differences in activity seen *in vitro* may not be seen in small animal models, and the picture in humans may be different again.

In the 1980s, Prof. EJ Ariëns at the University of Nijmegen published an article with the provocative title “Stereochemistry, a basis for sophisticated nonsense in pharmacokinetics and clinical pharmacology” [12]. The main thesis highlighted the neglect of chirality and stereoselectivity in action, and the common practice in the scientific literature of presenting data on mixtures of stereoisomers as if only one compound were involved. This is the nonsense implied in the title.

Since then, there has been more emphasis on the advantages and development of chiral medicines, and the replacement of racemates by chirally-pure forms (chiral switching) which may be expected to have a more uniform action, although many of the claimed benefits have yet to be clearly realised in the clinic.

3.2 Rationale for the development of chirally pure drugs

At first sight the advantages would seem to be as follows:

- an improved safety margin (therapeutic index) through increased receptor selectivity and possibly reduced adverse effects;
- reduced interindividual variability in response linked to polymorphic metabolism;

- a more predictable duration of action due to pharmacokinetic considerations (e.g. half-life) resulting in a more appropriate dosing frequency;
- decreased potential for drug–drug interactions.

But there is usually no point in doing this if there is evidence of rapid interconversion/biotransformation/racemization in plasma.

3.3 Pharmacodynamic and kinetic differences between enantiomers

Since enantiomers have different 3D geometries, the pharmacodynamics and pharmacokinetics of the two enantiomers which make up a racemic drug are not expected to be the same. For example:

- (S)-ibuprofen is over 100-fold more potent an inhibitor of cyclo-oxygenase I (COX-1) than (R)-ibuprofen.
- (R)-methadone has a 20-fold higher affinity for the μ opioid receptor than (S)-methadone.
- (S)-citalopram is over 100-fold more potent an inhibitor of the serotonin reuptake transporter than (R)-citalopram.

The beneficial effects of a drug can therefore reside in one enantiomer (the eutomer), with its paired enantiomer having:

- no activity
- some activity
- antagonist activity against the active enantiomer
- completely separate beneficial or adverse activity from the active enantiomer.

Pharmacokinetic differences may also exist as follows:

- blood levels of (R)-fluoxetine are much lower than (S)-fluoxetine due to a selectively higher rate of metabolic clearance;
- the bioavailability of (R)-verapamil is more than double that of (S)-verapamil due to a selective reduction in hepatic first-pass clearance;
- the volume of distribution of (R)-methadone is double that of (S)-methadone due to a selectively lower binding to plasma proteins, and increased tissue binding;
- the renal clearance of (R)-pindolol is 25% less than (S)-pindolol due to reduced renal tubular secretion.

3.4 Recent regulatory experience of the Chiral Switch: a word of caution

There has been dispute in the regulatory authorities through the 80s and 90s concerning the pressure necessary to obtain only chirally pure drugs and the abandonment of racemates. The argument was that because a chirally pure form of a molecule *can* be developed and delivered to the market it *must*. But this was never taken up as a firm harmonized EU regulatory requirement, although some Member States said it should be “encouraged”. This position rests on the authorities’ main indicator of efficacy and safety – the benefit/risk balance. There are a large number of racemates in clinical use and these have acceptable efficacy and safety – i.e. a positive benefit/risk balance. Nothing more is needed. Of course companies are free to develop pure chiral forms, enantiomers, if they wish to claim certain advantages over the racemate, but nobody is going to make them to do so while the available evidence confirms that the benefit/risk balance of the racemate remains favourable.

An important issue in the racemate to enantiomer trend (“chiral switch”) is linked to patent protection and possible data exclusivity given to the enantiomer product. An enantiomer which has significantly different properties to an established racemate with regard to efficacy and safety can be classified as a New Active Substance in EU legal/regulatory terms, and therefore, apart from patent protection, it will be rewarded with regulatory protection from generic competition usually for 10 years. The authorities will not accept applications for generic copies within 8 years, and between 8 and 10 years generic applications can be accepted and authorized, but not placed on the market. The difficulty is to show a significant difference in efficacy and safety; indeed, what exactly is a “significant difference”? This is the term used in current EU guidance on this subject, and theoretically it allows the enantiomer to be significantly worse. In practice, what is needed is proof of a clinically-relevant advantage in real terms, added-value, otherwise the product will not be protected from generic competition. Even so, the real advantages of some chiral switches are not so clear [13]:

Proton Pump Inhibitors: Omeprazole exist as two inactive enantiomers (prodrugs) that are converted to active moieties which equally inactivate the H^+/K^+ -ATPase pump. Both enantiomers are equipotent, however, their metabolic clearance is quite different, and it has been proposed that (S)-omeprazole would therefore show less interindividual variability, however, clinical data supporting this claim are limited.

SSRIs: It is the (S)-enantiomer that is mainly responsible for the selective serotonin reuptake inhibition of citalopram and its active metabolites. This enantiomer and its metabolites are eliminated slightly faster from the body than the (R)-enantiomer and its metabolites. Since a metabolite of the (R)-isomer has been linked to prolongation of the QT interval and a potential risk of sudden death, it was claimed that development of the (S)-isomer should have a superior benefit/risk balance. Although the clinical advantage

of S-citalopram over racemic citalopram has been questioned in the literature, at the time of writing, escitalopram remains a new active substance protected from generic competition.

Hypnotics: Eszopiclone is the S-enantiomer of racemic zopiclone, submitted to the EMA for evaluation with the proposed clinical indication: *treatment of insomnia, including difficulty falling asleep, nocturnal awakening or early awakening, in adults, usually for short term duration.*

The product received a favourable opinion, and according to the assessment report published on the EMA website:

“... Clinical data presented suggest there is a positive impact in quality of life and day functioning. Overall the efficacy data of the clinical programme support the maintenance of effect and the claimed indication for the treatment of insomnia, including difficulty falling asleep, nocturnal awakening or early awakening, in adults, usually for short term duration...”. Acceptable safety was also shown, so clearly the product has acceptable efficacy and safety *per se*, and could have been authorized.

However, the issue of whether or not S-zopiclone was a new active substance with significant efficacy/safety differences compared to the racemate was a different matter, and received a negative judgement in this regard. It may be that significant and clinically relevant differences do indeed exist, but the company could not provide adequate convincing evidence to demonstrate this, and this is what matters in the regulatory world. Therefore, at the time of writing this chapter, no protection period has been granted under EU pharmaceuticals legislation and the application was withdrawn.

In general, the interface between the pharmaceutical industry and the regulatory authorities can be very heated, especially where disagreements over market protection are concerned, and it is likely that lawyers and clinical pharmacologists on both sides of the regulatory divide will continue to be occupied with such matters for the foreseeable future.

4 Me-toos

4.1 Background

“New control for infections,” – the New York Times headlined its front-page story on 20. December 1936 and marked the beginning of the era of wonder drugs [14]. President Roosevelt’s son had developed severe tonsillitis. As a final measure he was treated with Prontosil and had made a complete recovery. The active ingredient in Prontosil was sulphanilamide, a common industrial chemical that was no longer patented and that no one had ever thought to test against bacteria [15, 16].

Within months, nearly every drug company in the world began synthesizing their own versions of sulphanilamide – the start of the era of “me too”-drugs. These were the first “copycat” drugs which soon led to intense competition among many companies and the price of the new sulfonamides drugs plunged [17]. The pattern was repeated after World War II. The U.S. government licensed penicillin to five firms. Those firms engaged in a fierce competition for sales. Between 1945 and 1950, the price of penicillin plunged from \$3955 to \$282 a pound. The pattern happened yet again with streptomycin.

However, the pharmaceutical industry learned quickly from these experiences and due to alterations in patent law and marketing, drug prices rose dramatically.

Critics of the pharmaceutical industry called this the “era of molecular modification”; once a new effective chemical class was found, most major drug companies tried to come up with their own versions. So in the early 1970s more than 200 sulfonamides, more than 270 antibiotics, 130 antihistamines, and nearly 100 major and minor tranquilizers were on the market. Most of the new drugs “offer the physician and his patient no significant clinical advantages but are different enough to win a patent and then be marketed, usually at the identical price of the parent product or even at a higher price” [18].

The second wave of drug innovation was set off by the biotechnological revolution of the late 1970s and 1980s. As each new class came to market with often similar products from different drug companies, the competition resulted more in a dividing of the market, but there was rarely a competition on price. The raising drug prices of the 1990s increased the pressure on the healthcare system in the western world and the debate about the usefulness of “me-too” drugs got more and more public attention. This ongoing discussion was fuelled by a book from Marcia Angell (former editor of the *New England Journal of Medicine*) called “The Truth about Drug Companies”. She states, that between 1998 and 2002, only 14% out 415 new molecular entities, that were approved were truly innovative, 9% were old drugs that had been improved significantly and 77% were “me-too” drugs [19].

4.2 What are “me-too” drugs?

The term “me-too” drug first came up in the 1960s, following increasing concerns over the above mentioned “molecular modification” of approved drugs that were expressed in US Senate hearings (“Kefauver hearings”) on pricing and monopoly power in the pharmaceutical industry. Historically the term “me-too” has most often referred to a new drug entity with a similar, but not identical chemical structure or the same mechanism of action as that of a drug already on the market. So a “me-too” drug or, more value-neutral, a follow-on drug is a new entrant to a therapeutic class that had already been defined by another drug entity – the “breakthrough drug” that was the first in the class [20].

In the 1970s the median time between Innovator and first “me-too” drug was 10, 2 years, and in the time between 1995 and 1998 it was only 1, 2 years. The median period between first and second “me-too” drug in the 1970s was 4, 2 years and in the 1990 it was 1, 7 years. The interval between second and third me-too drug dropped from 3, 7 years in the 1970s to 0, 9 years in the 1990s [20]. Taking into consideration, that the development of a new drug from bench to bedside is estimated to be between 10 and 15 years [21], it can be safely assumed, that the vast majority of the “me-too” drugs for drug classes that were created recently, were already in the last phases of clinical development at the time of the approval of the class breakthrough drug. Nowadays “the development histories of entrants to new drug classes suggest that development races better characterize new drug development than does a model of *post hoc* imitation” [20]. So the availability of “me-too” drugs does not necessarily mean that imitation has replaced innovation in health care. The product that reaches the market first is the one that won the race, but this does not necessarily reflect who had the idea first or that it is the best drug of the class [22].

4.3 How many “me-too” drugs are enough?

The increasing availability of “me-too” drugs theoretically has provided considerable treatment options for physicians. However in a recent study from Canada, the authors investigated the number of different statins that individual doctors actually used in practice. The mean percentage of prescriptions written for each physician’s preferred statin formulation was 73.7%, the average physician wrote the vast majority of his or her incident prescriptions (94.9%) for only one or two statins. Half of all physicians used, at most, two different statins for all incident prescribing, while 91.3% of physicians used, at most, three different statins for all of their incident prescribing. The authors concluded that physicians issued the majority of their incident statin prescriptions for the same statin formulation. Most physicians required, at most, three different statins for all incident statin prescribing [23].

4.4 Is First-in-class also best-in-class?

Between 1960 and 1998, 72 new drugs were marketed in the USA, which were first in their class. By 2003, 235 follow-on drugs for these therapeutic classes were approved in the USA, resulting in a mean number of 4, 3 drugs per class (range from 2 to 16) [20]. Are these additional drugs all redundant and offer no additional therapeutic benefit? To clarify this issue Dimasi et al. examined the therapeutic ratings that the US FDA has assigned to follow-on drugs. This rating system is a management tool for the FDA to help better allocate resources. The authors found “that approximately one-third of all follow-on drugs have received a priority rating from the US FDA. In

addition, 57% of all classes have at least one follow-on drug that received a priority rating". This could mean that the distinction between first in class and "me-too" drug is not really of clinical relevance.

4.5 Are incentives for drugs that are "first in class" or hurdles for "me-too" drugs called for?

The present drug-approval system gives each component in a class the same period of patent protection. It has been suggested to give later drugs in a class, a shorter period of patent protection unless or until they demonstrate that their drugs had some added therapeutic benefit (such as increased efficacy or improved safety) over other drugs in their class. "This focus on an increased benefit in turn would encourage head-to-head comparisons among drugs – which currently are almost totally lacking" [24]. As often most of the development of a "me-too" drug takes place before the first in class drug is marketed, the request to demonstrate superiority to every other already marketed drug in the class would increase development costs drastically. "What is probably most critical, though, is that, given the way that follow-on drug development often proceeds, this policy will greatly increase uncertainty. A firm can start a development programme in one way, only to find partway through it that it has to change course and do comprehensive head-to-head comparisons with a drug that happened to reach the marketplace before its drug. This can even happen more than once in development. That is, the firm would be required to hit a moving target [20].

Requesting that each "me-too" drug in a class shows superior efficacy over the "first in class" in active controlled-superiority-showing studies could create multiple problems. These studies are often not possible to undertake. In some clinical indications the success rate of standard treatment cannot be surpassed because of the "therapeutic-ceiling effect", which would be the case for example for most antibiotics, or when the "me-too" – drug exerts its pharmacologic effect through the same mode of action as the reference. Even when demonstration of superiority might theoretically be possible, in practice this could raise the evidence requirements to market for new drugs to prohibitive levels [25].

Probably for the above mentioned reasons hardly any of the newly licensed drugs have shown superiority to an active comparator. In a recently published article [25], reviewing the publicly available regulatory assessment reports of all new molecular entities authorized for the first time in the US and EU (for EU: centralized procedure only) during the period from 1 January 2007 to 31 December 2008, the authors state, that only 1 (2.4%) out 42 new drugs in the US and only 10 (21.3%) out of 47 new drugs in the EU showed superiority over active comparator in a head-to-head RCT.

4.6 What is a drug class?

The US Food and Drug Administration (FDA) uses class labelling when “all products within a class are assumed to be closely related in chemical structure, pharmacology, therapeutic activity, and adverse reactions”. The words, “assumed to be closely related”, are not further defined [26].

Criteria for drugs to be grouped together as a class involve some or all of the following:

- Drugs with similar chemical structure.
- Drugs with similar mechanism of action.
- Drugs with similar pharmacological effects [27].

4.7 Is there a “class effect”?

An often discussed question is that of whether a set of drugs forms a class, and whether there is a class effect. “Class effect is usually taken to mean similar therapeutic effects and similar adverse effects, both in nature and extent” [27]. The assumption, that drugs of the same class exhibit similar pharmacological effects and clinical outcomes, can lead to errors of extrapolation with major clinical consequences. The suggestion has been made that . . .” to reduce the risk of faulty extrapolation and to maximize the optimal selection of treatments within a class of drugs, it may be useful to develop and apply a hierarchy of evidence when making decisions about the comparative clinical efficacy and safety of drugs within a class” [28].

As already mentioned the perfect scenario would be that every drug in each class would be evaluated in randomized clinical trials with active comparators from the same class for its effects on clinically relevant outcomes. It is acknowledged that this gold-standard is not always attainable – for example in the case of the statins, such randomized clinical trials would require very large sample sizes and long follow-up to detect significant differences in myocardial infarction or death between two different statins), but to facilitate the discussions about class effects it would be highly useful to cite the levels of evidence and to discuss the strengths and weaknesses inherent of the design of the relevant study [28].

Case Study: Class effect with proton pump inhibitors

For proton pump inhibitors (PPIs) there exists a high level of evidence for a class effect. For example a meta analyses demonstrated that proton pump inhibitors

given at equivalent doses are equally effective for healing esophagitis. No statistically significant difference was detected between the healing rates achieved with standard dose omeprazole compared to the newer PPIs in all grades of esophagitis [29]. In another study the authors found no difference between five PPIs (esomeprazole, lansoprazole, omeprazole, pantoprazole and rabeprazole) – for relief of symptoms and healing of gastro esophageal reflux disease [30].

The British National Institute of Clinical Excellence states that systematic reviews suggest that there is no statistically significant difference between different PPIs at equivalent doses and recommends the use of the cheapest PPI in the approved indication [31].

References

1. The current EU legal definition for generic products is found in Directive 2001/83/EC, Article 10 (2)(b)
2. Chow S-C, Liu J-P (1992) Design and Analysis of Bioavailability and Bioequivalence Studies. Marcel Dekker Inc., New York
3. Guideline on the investigation of bioequivalence, CHMP/EWP/QWP/1401/98 Rev. 1, 29 January 2010
4. Endrenyi L, Yan W (1993) Variation of C_{max} and C_{max}/AUC in investigations of bioequivalence. *Int J Clin Pharmacol Ther Toxicol* 31(4): 184–189
5. The same criteria also apply in the USA and in many other countries worldwide.
6. Duncan R, Sat Y-N (1998) Tumour targeting by enhanced permeability and retention (EPR) effect. *Ann Oncol* 9(Suppl 2): 39
7. Article 10(4) of Directive 2001/83/EC and Section 4, Part II, Annex I to this Directive
8. <http://www.ema.europa.eu/pdfs/human/pcwp/7456206en.pdf>
9. <http://www.egagenerics.com/FAQ-biosimilars.htm#FN>
10. Rachel Chu, Meir Pugatch Biogenerics or biosimilars? Discussing the Present, Considering the Future. The Stockholm Network (2009) http://www.stockholm-network.org/downloads/publications/Biosimilars_FINAL.pdf
11. Schneider CK, Kalinke U (2008) Toward biosimilar monoclonal antibodies. *Nature Biotechnol* 26: 985–990
12. Ariëns EJ (1984) Stereochemistry, a basis for sophisticated nonsense in pharmacokinetics and clinical pharmacology. *Eur J Clin Pharmacol* 26(6): 663–668.
13. Tucker GT (2000) Chiral switches. *Lancet* 355: 1085–1087
14. Waldemar Kaempffert (1936) The week in science: new control for infections. The Chemical Given to F.D. Roosevelt Jr. for Streptococcus Hailed as Important Discovery – New York Times (1857-Current file. New York, N.Y., pp. 1, 24
15. Vikash Kumar (2008) Me-Too Drugs – A Tiny Revolutionize: Latest Reviews, Vol. 6, Issue 3
16. Amyes SGB (2001) Magic Bullets Lost Horizons: The Rise and Fall of Antibiotics. Taylor & Francis Inc., London
17. Goozner M (2004) The \$800 Million Pill: The Truth Behind the Cost of New Drugs. University of California Press, Berkley, California, USA, 297 pp. Chapter 8
18. Silverman MM, Lee PR (1974) Pills, Profits, and Politics, pp. 4–5

19. Angell M (2004) *The Truth about Drug Companies: How They Deceive us and What to Do about It*, Random House
20. Dimasi JA, Paquette C (2004) The economics of follow-on drug research and development: trends in entry rates and the timing of development. *Pharmacoeconomics* 22(s2): 1–14
21. Dimasi JA (2001) New drug development in the United States from 1963 to 1999. *Clin Pharmacol Ther* 69(5): 286–296
22. Lee TH (2004) “Me-too” products – friend or foe? *N Engl J Med* 350(3): 211–212
23. Austin PC, Mamdani MM, Juurlink DN (2006) How many “me-too” drugs are enough? The case of physician preferences for specific statins. *Ann Pharmacother* 40: 1047–1051
24. Alastair JJ, Wood MD (2006) A proposal for radical changes in the drug-approval process. *NEJM* 355(6): 618–623
25. Eichler HG, Bloechl-Daum B, et al. (2010) Relative efficacy of drugs: an emerging issue between regulatory agencies and third-party payers. *Nat Rev Drug Discov* 9: 277–291
26. Furberg C, Herrington D, Psaty B (1999) Are drugs within a class interchangeable. *Lancet* 354(9185): 1202–1204
27. <http://www.medicine.ox.ac.uk/bandolier/booth/glossary/class.html>
28. McAlister FA, Laupacis A, Wells GA, Sackett DL (1999) *Users’ Guides to the Medical Literature: XIX. Applying clinical trial results B. Guidelines for determining whether a drug is exerting (more than) a class effect.* *JAMA* 282(14): 1371–1377
29. Wang WH, et al. (2005) Head-to-head comparison of H₂-receptor antagonists and proton pump inhibitors in the treatment of erosive esophagitis: a meta-analysis. *World J Gastroenterol* 11(26): 4067–4077
30. Standard doses of the oral proton pump inhibitors are clinically equivalent: a comparison Alan B. R. Thomson *Current GERD Reports* 2007, 1: 223–232
31. NICE Guideline Dyspepsia 23. Aug. 2004

CHAPTER 24

Special situations, market fragmentation I: orphan drugs for rare diseases

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1 What are rare diseases?

In the European Union rare diseases have been defined as follows:

“Rare diseases, including those of genetic origin, are life-threatening or chronically debilitating diseases which are of such low prevalence that special combined efforts are needed to address them so as to prevent significant morbidity or perinatal or early mortality or a considerable reduction in an individual’s quality of life or socio-economic potential. As a guide, low prevalence is taken as prevalence of less than 5 per 10,000 persons in the European Union [1]”. While the prevalence number seems relatively small, currently it translates into approximately 250,000 persons in the EU with 27 Member States.

To date, five to eight thousand distinct rare diseases have been described in the medical literature, affecting between 6 and 8% of the population in total, which means that between 27 and 36 million people in the European Union are affected by a rare disease [2].

2 What are orphan drugs?

The lack of drug development for products intended for the prevention, treatment or diagnosis of rare diseases has made necessary the creation of a number of incentives to

* The views expressed in this chapter are the personal views of the authors and may not be used or quoted as being made on behalf of, or reflecting the position of, any national competent authority, the European Medicines Agency (EMA) or one of its committees or working parties.

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stimulate the development of such products. These drugs are known as orphan drugs after fulfilling the criteria for designation and therefore being eligible for incentives to promote development and marketing.

In the EU a medicinal product to treat rare diseases is designated as an orphan medicinal product based on [2]:

- Either a demonstrated insufficient return on investment or the rarity of the condition and
- the absence of satisfactory method of diagnosis, prevention or treatment of the condition concerned is authorized, or, if such method exists, the assumption that the product will be of significant benefit to those affected by the condition.

3 The orphan drug legislation

Under normal market conditions, the pharmaceutical industry has little interest in developing and marketing products intended for only a small number of patients suffering from rare conditions [3]. In the past 25 years, it has been recognized by various authorities that because of the rarity of these diseases, the cost of developing and bringing to the market a medicinal product to diagnose, prevent or treat a rare condition would not be recovered by the expected sales of the medicinal product under normal market conditions [4]. Therefore, specific legislation to stimulate the discovery and development of drugs for rare diseases – the so-called “orphan drugs” – has been introduced: in the USA in 1983 [5], in Japan in 1993 [6], in Australia in 1997 and in the EU in 2000 [7].

4 The Committee on Orphan Medicinal Products (COMP)

In April 2000 a new Committee on Orphan Medicinal Products (COMP) was established at the European Medicines Agency. The COMP is composed of one member nominated by each EU Member State, three patient representatives and three members proposed by the Agency and appointed by the Commission. The Committee meets 11 times per year. The COMP is responsible for reviewing applications from persons or companies seeking “orphan medicinal product designation” for the products they intend to develop for the diagnosis, prevention or treatment of rare diseases [8].

The COMP is also responsible for advising the European Commission on the establishment and development of a policy on orphan medicinal products in the EU, and assists the Commission in drawing up detailed guidelines and liaising internationally on matters relating to orphan medicinal products.

5 Orphan incentives [9]

Sponsors with an orphan designation for a medicinal product may benefit from incentives such as:

- **Market exclusivity:** For 10 years after the granting of a marketing authorization (approval for sale), orphan medicinal products benefit from market exclusivity in all EU member states. During that period, directly competitive similar products cannot normally be placed on the market for the same indication. It is not possible either to extend an existing authorization of a similar product for the orphan indication.
- **Mandatory and direct access to centralized marketing authorization**
- **Protocol assistance:** The Agency can provide scientific advice to optimize development and guidance on preparing a dossier that will meet European regulatory requirements. This helps applicants to maximize the chances of their marketing authorization application being successful. Protocol assistance is considered a priority, as it is a way to both improve and speed up the applications for marketing authorization of orphan medicinal products and therefore to increase the chances for the patients affected by rare diseases to get effective treatments.
Protocol assistance is one of the most utilized incentives for orphan medicines development.
- **Fee reductions:** A special fund from the European Commission, agreed annually by the European Parliament, is used by the Agency to grant fee reductions. Reduction of fees will be considered for all types of centralized activities, including applications for marketing authorization, inspections, variations and protocol assistance. For small and medium sized enterprises (SMEs), additional fee reductions are applicable [10].
- **EU-funded research:** Sponsors developing orphan medicinal products may be eligible for grants from the EU and Member States' programmes and initiatives supporting research and development, including the European Commission framework programme.

6 What are the criteria for orphan designation?

The designation as orphan medicinal product is based either on the prevalence of the condition in the Community or the insufficient return generated by the product to justify the investment. In addition, the application has also to address the seriousness and debilitating nature of the condition and to address the criteria set out in Article 3(1)

(b) of Regulation (EC) No 141/2000 referred to the absence or existence of satisfactory methods [7]. Therefore the criteria for orphan designation are the following:

- Firstly, a criterion is based on the low prevalence (“rarity”) of the condition, i.e. condition affecting not more than 5 in 10,000 persons in the European Union. Alternatively, the sponsor can apply for more frequent conditions if it can be shown that the development would not be covered by sufficient financial return, i.e. if without incentives it is unlikely that the marketing of the medicinal product in the Community would generate sufficient return to justify the investment by the sponsor [3].
- Secondly, it is necessary for designation that the life-threatening or debilitating nature of the condition is justified. The sponsor is invited to provide any scientific and/or medical references that may support the life-threatening or seriously debilitating nature of the condition [3].
- Finally, the sponsors are also required to demonstrate that either there exists no satisfactory method of diagnosis, prevention or treatment of the condition in question or, if such methods exist, that the medicinal product will be of significant benefit to those affected by that condition.

Significant benefit [11] over authorized products is interpreted as “a clinically relevant advantage or a major contribution to patient care” (Article 3.2 of Regulation (EC) No. 847/2000). At the time of designation the significant benefit may have been based for example on an alternative mechanism of action which might result on an improved clinical outcome, more favourable pharmacokinetic properties or potentially better clinical effect. The claims of significant benefit have to be justified and supported by a scientific discussion based on data from the literature, or by any preliminary preclinical and/or clinical results. A justification of significant benefit based on a clinical advantage based on safety may be difficult to accept at the time of designation, since most of the times the product has not been widely used yet and therefore the safety profile remains often largely unknown.

Significant benefit based on major contribution to patient care may be related for example to a new route of administration that is deemed as providing a major contribution to patient care, or a significant decrease in the number of intakes/day with the same clinical outcome, which improves patient care or compliance.

Exceptionally, availability/supply problems have been considered as an opportunity to elaborate on a justification for significant benefit, where there has been a clear long-term shortage in supply of the existing product on the market, which has led to restricted use of the product and consequences for the patients.

So far, more than 60% of positive opinions adopted on orphan designations were based on the assumption of significant benefit [3].

Significant benefit has to be demonstrated at the time of marketing authorization. “If it is established before the market authorization is granted that the criteria laid

down in Article 3 (criteria for designation) are not longer met . . . a designated orphan medicinal product shall be removed from the Community Register of Orphan Medicinal Products” [12].

In most (but not all) situations where products are designated on the basis of significant benefit, the elements, as expected at the time of the designation, will be integrated or will be derived from the data allowing demonstration of quality, efficacy and safety required for the marketing authorization. In some circumstances these elements have to come in addition to these data. Therefore the data to justify significant benefit have to be part of the development plan for an orphan medicinal product and have to be submitted at the time of the application for marketing authorization [11].

7 General requirements for a valid condition for orphan designation [13]

- The characteristics defining a distinct condition should determine a group of patients in whom development of a medicinal product is plausible, based on the pathogenesis of the condition and pharmacodynamic evidence and assumptions.
- Recognized distinct medical entities would generally be considered as valid conditions. Such entities would generally be defined in terms of their specific characteristics, e.g. pathophysiological, histopathological, clinical characteristics.
- Different degrees of severity or stages of a disease would generally not be considered as distinct conditions.

A large proportion of the unsuccessful applications received so far for designation are due to sponsors applying for an artificial sub-set of a condition which on its own has prevalence above the threshold. In addition, different degrees of severity or stages of a condition are not generally considered by the COMP as distinct conditions. As a consequence, the subsets of patients within a condition who have failed first or second-line treatment, or who cannot tolerate standard treatment, are generally not considered as a distinct entity for the purposes of orphan designation [3].

8 What data are necessary at the time of Orphan Drug designation?

A company can apply for orphan designation at any time during the development of the product prior to the application for marketing authorization, but certain minimum of data needs to be presented to justify the designation criteria. A pharmacological concept, not supported by any form of evidence, would generally not be considered

by the COMP as sufficient justification for the designation of the medicinal product in the proposed condition.

- Relevant *in vitro* and *in vivo* data in appropriate preclinical models is usually required for orphan designation.
- If *in vitro* evidence only is provided, then the relevance of these data has to be discussed in the context of the proposed condition.
- When available comparative data or a discussion comparing the results obtained with the product to those obtained with comparators can be submitted, even though this represents a minority of the cases where products have been designated.
- In any case the preclinical data should be discussed even if preliminary results from first administration to humans are available.

8.1 Some examples for a positive opinion for orphan designation

Picoplatin for the treatment of small-cell lung cancer (SCLC) [14]

- SCLC affects less than 75,000 persons in the European Union.
- Picoplatin, a platinum-derivative, has been designed to overcome the onset of resistance to drug treatment and the triggering of important adverse reactions.
- These characteristics of picoplatin may be of potential significant benefit over the existing authorized medicinal products → positive opinion.

Thalidomide for the treatment of multiple myeloma [15].

- Multiple myeloma was considered to affect about 46,000 in the European Union.
- Thalidomide could be of potential significant benefit for the treatment of multiple myeloma. The main reason is that it may offer a new way of killing cancer cells and stopping tumour growth in these patients. → positive opinion

8.2 Negative opinions

“When the outcome for a designation application is negative, the COMP will adopt a negative opinion, unless the sponsor chooses to withdraw the application. The sponsor must inform the Agency in writing of the withdrawal before the COMP adopts an opinion, in other words, before the end of the COMP meeting. When the application is withdrawn, no information on the application is made public. The sponsor can re-apply for orphan designation with additional or complementary data at a later stage. If the sponsor does not withdraw, a negative opinion is adopted by the COMP and is

Table 1 Some examples for negative opinions [16]

| Medicinal product | Condition | Reason for the negative opinion |
|---|---|----------------------------------|
| Capsaicin | Painful HIV-associated Neuropathy | Not considered as a valid subset |
| Histamine dihydrochloride | Malignant melanoma | Prevalence not below 5 in 10,000 |
| Midazolam hydrochloride (for oromucosal use) | seizures which continue for at least 5 min | Prevalence not below 5 in 10,000 |
| Mycobacterial cell wall complex | Superficial bladder cancer | Prevalence not below 5 in 10,000 |
| Sudismase | Active phase of Peyronie's disease | Prevalence not below 5 in 10,000 |
| Tramadol hydrochloride | Painful HIV-associated neuropathy | Not considered as a valid subset |
| Chlorproguanil hydrochloride and dapsone | Acute uncomplicated Plasmodium falciparum malaria | No significant benefit |
| Ibritomomab tiuxetan/ 90Yttrium | B-cell non-Hodgkin's lymphoma | Prevalence not below 5 in 10,000 |
| Chelidonium radix spec. liquid extract – Ukrain | Treatment of pancreatic cancer | No significant benefit |

transformed into a Commission Decision, unless an appeal procedure is triggered. A summary of the negative opinion will be published on the Agency web-site [17]. About one quarter of all applications are withdrawn, before a negative opinion gets adopted and published (see Table 1).

8.3 Reason for negative opinions – subsetting – no significant benefit

8.3.1 Subsetting/valid condition

As said before a valid condition would include a group of patients in whom development of a medicinal product is plausible, based on the pathogenesis of the condition, so distinct medical entities would generally be considered as valid conditions. If an orphan indication refers to a subset of a particular condition, a justification of the medical plausibility for restricting the use of the medicinal product in the sub-set should be submitted, otherwise this would not be sufficient to receive orphan designation, subsetting into different stages and severities of diseases are not allowed.

Example

Infection in cystic fibrosis (CF)

- “Lung infection in CF patients” is clinically and pathophysiologically different than lung infection in non-CF patients.
- Thus, infections in CF can be accepted as a valid subset as CF itself is rare (subset of infection).

Histamine dihydrochloride for treatment of malignant melanoma excluding thin melanomas [18]

- No justification for the exclusion of melanomas of <0.75 mm from the condition, which are a stage of the disease and then has to be included in the definition of the condition
- Without exclusion of thin melanomas the sponsor cannot establish that malignant melanoma affects not more than 5 in 10,000 persons → negative opinion (subsetting)

Tramadol hydrochloride and capsaicin for treatment of painful HIV-associated neuropathy [19, 20]

- No justification for limiting the condition to “painful HIV-associated neuropathy”.
- The Committee considered peripheral neuropathy as the medical condition; painful HIV associated neuropathy would not be a sufficiently justified subset as it is not possible to make a clear distinction based on valid arguments (histology, pathophysiology, etc.) between this and peripheral neuropathy. Thus the proposed condition was not considered as a valid subset of the broader condition “peripheral neuropathy”
- No valid subset of the broader condition “peripheral neuropathy”
- Peripheral neuropathy affects more than 5 in 10,000 persons
→ Negative opinion (subsetting)

Different underlying pathologies sharing a clinical manifestation of the condition

- Treatment of orthostatic hypotension in Pure Autonomic Failure
→ Positive opinion as the condition is rare
- Treatment of orthostatic hypotension in Multiple System Atrophy
→ Positive opinion as the condition is rare
- Treatment of orthostatic hypotension in Parkinson’s disease
→ Negative opinion (withdrawal) as the condition is not rare and orthostatic hypotension is not a condition per se in this case but a manifestation of Parkinson’s disease.

8.3.2 Significant benefit

Significant benefit is defined in Article 3 of Commission Regulation EC 847/2000 as “a clinically relevant advantage or a major contribution to patient care”.

To follow the spirit of the orphan legislation and to have an impact on promotion of drug development applications for orphan designation are accepted at any stage of the development. Therefore the justification for the assumption of “significant benefit” has

to be based on the available evidence at the stage of designation. Many times the early stage of development of a product means that limited data to assess the clinically relevant advantage or major contribution to patient care is available at the time of designation. Thus a critical review comparing authorized treatments and the proposed Orphan Medicinal Product and justifying the assumption of significant benefit should be provided. Orphan status will be reviewed *prior to* the grant of a marketing authorization. At this stage, a higher level of evidence than at the time of designation for the orphan status to be maintained is usually required.

Significant benefit assumptions have to be based on the data contained in the application for marketing authorization and the arguments presented by the sponsor.

More than 70% of the opinions adopted on orphan designation are based on significant benefit. Of them 80% address clinically relevant advantages and approximately 15% are based on justifications on contribution to patient care. The remaining 5% are combinations of the criteria. At the time of marketing authorization more than 65% products required demonstration of significant benefit. Examples of assumptions of significant benefit are:

- Positive outcome on clinically relevant advantage based on a median survival of 24 m vs. 15 m in the (active) control group. Absolute difference in (overall) survival is 9 m ($p = 0.0001$)."
 - Positive outcome on major contribution to patient care for oral vs. iv application: "the burden of iv infusion and the difficulties in venous access" (contribution to patient care ~ "disutility").
- Negative opinion for a product that offers once daily oral vs. twice daily oral.

9 Confirmation of orphan status at the time of marketing authorization

Designated orphan medicinal products have mandatory access to the centralized procedure marketing authorization. The assessment of the benefit/risk balance of the applications for marketing authorization is done by the Committee of Human Medicinal Products (CHMP) and is based on the same standards applied to products intended for non-rare disease. The quality, safety and efficacy of the medicinal products are evaluated by the experts from the Member States contributing to CHMP and coordinated by the Agency. The COMP reviews the orphan designation criteria at the time of marketing authorization application and checks the following:

- The proposed therapeutic indication falls within the scope of the designated orphan indication for the medicinal product.
- The condition is still being judged life-threatening or chronically debilitating.

- The prevalence of the condition is no more than 5 in 10,000 at the time of the review of the designation criteria.
- When the designation is based on significant benefit, the assumption that the product might be of benefit to those affected by the orphan condition is established.

If the orphan criteria are still fulfilled, the COMP will issue a (positive) opinion recommending “not to remove” the product from the Community Register of Orphan Medicinal Products. Upon the grant of the marketing authorization by the European Commission, orphan medicinal products will benefit from 10 years of market exclusivity for the authorized indication.

If the criteria are no more fulfilled, the COMP may issue an opinion recommending removing the medicinal product from the Register, so the product is marketed without right to access the incentives offered by the Regulation.

Additionally, as laid down in Regulation (EC) No. 1901/2006 on medicinal products for paediatric use [21], orphan medicinal products may be granted a two-year extension of the market exclusivity if they have agreed and complied with a Paediatric Investigation Plan (PIP) and the information arising from the PIP is incorporated into the summary of product characteristics.

10 Challenging marketing exclusivity for orphan medicinal products

A potential similarity between two medicinal products and the possible implication in the product development should be taken into account. Once a first orphan medicinal product is currently under market exclusivity, no further MA or extension of an existing MA can be granted for a similar medicinal product in the same therapeutic indication.

Similarity is defined in the Commission regulation 847/2000:

- “Similar medicinal product” means a medicinal product containing a similar active substance of substances as contained in a currently authorized orphan medicinal product, and which is intended for the same therapeutic indication;
- “Similar active substance” means an identical active substance, or an active substance with the same principal molecular structural features (but not necessarily all of the same molecular structural features) and which acts *via* the same mechanism.

Furthermore in the Guideline on aspects of the application of Article 8(1) and (3) of Regulation (EC) No 141/2000 the principles for similarity assessment are explained. According to this guideline the assessment of similarity between two medicinal

products takes into consideration principal molecular structural features, mechanism of action and therapeutic indication. If significant differences exist within one or more of these criteria, then the two products will be considered as not similar.

In regulation 141/2000 three derogations to break market exclusivity for similar products are paid down. These are

- If the holder of the marketing authorization for the original orphan medicinal product has given his consent to the second applicant.
- If the holder of the marketing authorization for the original orphan medicinal product is unable to supply sufficient quantities of the medicinal product.
- If the second applicant can establish in the application that the second medicinal product, although similar to the orphan medicinal product already authorized, is safer, more effective or otherwise clinically superior.

One of the public examples of non similarity for a designated orphan medicinal product is presented below:

Temsirolimus (Torisel) [22]

- Sorafenib (Nexavar) is a receptor tyrosine kinase inhibitor and targets the RAS/RAF/MEK/ERK pathway as well as the c-KIT, FLT-3, PDGFR and VEGFR signalling pathways.
 - Sunitinib (Sutent) is a tyrosine kinase inhibitor which targets VEGFR, PDGFR, c-KIT and FLT-3 signalling pathways.
 - Temsirolimus (Torisel) is a selective inhibitor of mTOR (mammalian target of rapamycin), . . . The anti-tumour effect of temsirolimus may also in part stem from its ability to depress levels of HIF and VEGFR, thereby impairing vessel development.
- TORISEL has a different molecular structure and a different mechanism of action
- TORISEL is not similar to Sutent or Nexavar.

11 Success of the orphan programme

11.1 Designated orphan medicines

In the first 10 years since the EU orphan legislation more than 690 medicinal products were officially designated as orphan by the European Commission.

Table 2 provides an overview of the status of orphan designation applications since 2000, the first year of implementation of the Orphan legislation. This table shows that the number of applications is increasing over time, and has reached its highest value so far with 164 applications received in 2009.

Table 2 Overview of the status of orphan designation applications since 2000 [3]

| | 2000 | 2001 | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | Total |
|-------------------------------|------|------|------|------|------|------|------|------|------|------|-------|
| No. of applications submitted | 72 | 83 | 80 | 87 | 108 | 118 | 104 | 126 | 119 | 164 | 1061 |
| Positive COMP opinions | 26 | 64 | 43 | 54 | 75 | 88 | 81 | 97 | 86 | 113 | 727 |
| Final negative COMP opinions | 0 | 1 | 3 | 1 | 4 | 0 | 2 | 1 | 1 | 2 | 15 |
| Withdrawals | 6 | 27 | 30 | 41 | 22 | 30 | 20 | 19 | 31 | 23 | 249 |

Furthermore, 74 designated products have been removed from the Register on request of sponsors, for administrative reasons, or when development was discontinued.

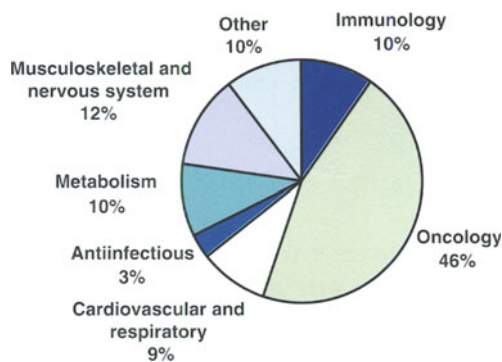


Fig. 1 Distribution of positive COMP opinions on designation by therapeutic area [3] (Reprinted from Drug News & Perspectives by kind permission of Thomson Reuters)

The distribution of positive COMP Opinions by therapeutic area is provided above in Fig. 1.

11.2 Marketing authorizations for orphan medicines

To date, 58 designated orphan medicinal products have received marketing approval in the EU so far. More than two-thirds (38%) of the authorized orphan medicinal products were antineoplastic and immuno-modulating agents (Fig. 2).

Of the 58 orphan medicinal products authorized, 40% of the marketing authorizations were granted “under exceptional circumstances” and 5% as “conditional approval” (Table 3). “Exceptional circumstances” means that at the time of the evaluation, it was deemed unreasonable to expect the applicant to provide comprehensive evidence on the safety and efficacy of the medicinal product. In case of “conditional approval”,

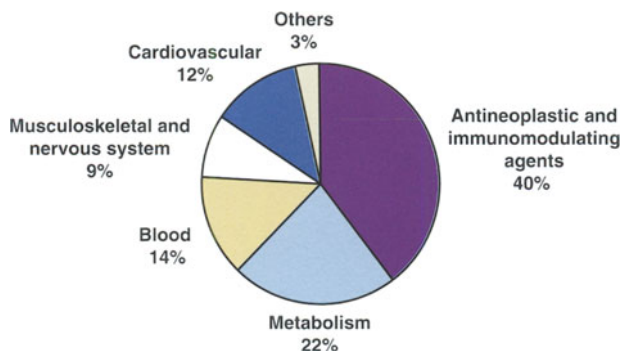


Fig. 2 Distribution of orphan marketing authorizations per therapeutic area [3] (Reprinted from Drug News & Perspectives by kind permission of Thomson Reuters)

further studies will be needed to maintain the marketing authorization; this will be reviewed annually by the Agency.

48 of the above orphan medicinal products that have received marketing authorization received protocol assistance or scientific advice prior to the submission of the marketing authorization application.

12 Discussion

Despite the obvious benefits of the orphan legislation, some criticism has been raised. The most common reproach of the orphan-product legislation has been the very high cost of treatment with some of the drugs. Drugs such as imiglucerase, an enzyme replacement therapy developed by Genzyme to treat Gaucher's disease, and other orphan blockbuster drugs have led to calls for modification of the legislation. The suggestion has been raised to place a cap on revenues from orphan drugs, to shorten exclusivity provisions, or to review of exclusivity provisions, when a drug becomes profitable [23].

However, the development of any new medication is a long, risky, and costly undertaking, and drug companies have to recover their investment once the drug is marketed. There are many examples of orphan drugs that provide valuable treatment, but which have little prospect of commercial return (e.g. zinc acetate for Wilson's disease). Although every effort should be made to prevent any unfair advantage from orphan-product legislation, changes that might stifle essential enthusiasm for development of rare-disease products should be avoided. Without the well considered incentives of the Orphan Drug Act, development of drugs for many rare diseases might well not have taken place [6].

So despite all misgivings, ten years after the inception of the orphan regulation in Europe, there is a clear evidence for success, with 58 new medicinal products having

Table 3 List of the 58 orphan medicinal products approved through the centralized procedure since 2001 [3] (Reprinted from Drug News & Perspectives by kind permission of Thomson Reuters)

| Year | Orphan MA | Significant benefit | Type of MA | Prevalence ≤1/10,000 | Received PA/SA |
|------|---|---------------------|---------------------------|-------------------------|-------------------|
| 2001 | <i>Fabrazyme</i> for Fabry disease | No | Exceptional circumstances | Yes | Yes |
| | <i>Replagal</i> for Fabry disease | No | Exceptional circumstances | Yes | Yes |
| 2002 | <i>Glivec</i> for chronic myeloid leukaemia | Yes | Exceptional circumstances | No | Yes |
| | <i>Tracleer</i> for pulmonary arterial hypertension | Yes | Exceptional circumstances | No | Yes |
| | <i>Trisenox</i> for acute promyelocytic leukaemia | Yes | Exceptional circumstances | No | No |
| | <i>Somavert</i> for acromegaly | Yes | Normal | No | Yes |
| | <i>Zavesca</i> for Gaucher disease | Yes | Exceptional circumstances | Yes | Yes |
| 2003 | <i>Carbaglu</i> for hyperammonaemia | No | Exceptional circumstances | Yes | No |
| | <i>Aldurazyme</i> for Mucopolysaccharidosis | No | Exceptional circumstances | Yes | Yes |
| | <i>Busilvex</i> for haematopoietic progenitor cell transplantation | Yes | Normal | Yes | Yes |
| | <i>Ventavis</i> for pulmonary arterial hypertension | Yes | Exceptional circumstances | No | Yes |
| 2004 | <i>Onsenal</i> for Familial Adenomatous Polyposis | No | Exceptional circumstances | No | No |
| | <i>Litak</i> for Hairy cell leukaemia | Yes | Normal | No | No |
| | <i>Lysodren</i> for adrenal cortical carcinoma | Yes | Normal | No | No |
| | <i>Pedea</i> for Patent Ductus Arteriosus | Yes | Normal | No | No |
| | <i>Photobarr</i> for Barrett's oesophagus | No | Normal | No | Yes |
| | <i>Wilzin</i> for Wilson's disease | Yes | Normal | Yes | No |
| | <i>Xagrid</i> for Thrombocythaemia | Yes | Exceptional circumstances | No | Yes |
| | <i>Orfadin</i> for Hereditary tyrosinemia type 1 | No | Exceptional circumstances | Yes | No |
| | <i>Prialt</i> for chronic pain requiring intrathecal (IT) analgesia | Yes | Exceptional circumstances | No | No |
| 2005 | <i>Xyrem</i> for cataplexy in patients with narcolepsy | Yes | Normal | No | Yes |
| | <i>Revatio</i> for pulmonary arterial hypertension | Yes | Exceptional circumstances | No | No |

| | | | | | |
|------|---|-----|---------------------------|-----|-----|
| 2006 | <i>Naglazyme</i> for replacement therapy in patients with mucopolysaccharidosis VI | No | Exceptional circumstances | Yes | No |
| | <i>Myozyme</i> for glycogen storage disease type II (Pompe's disease) | No | Normal | Yes | No |
| | <i>Evaltra</i> for acute lymphoblastic leukaemia | Yes | Exceptional circumstances | Yes | No |
| | <i>Nexavar</i> for advanced renal cell carcinoma | Yes | Normal | No | Yes |
| | <i>Sutent</i> for gastrointestinal stromal tumour and metastatic renal cell carcinoma | Yes | Conditional Approval | No | Yes |
| | <i>Savene</i> for anthracycline extravasation | No | Normal | Yes | No |
| | <i>Thelin</i> for idiopathic pulmonary arterial hypertension or pulmonary arterial hypertension | Yes | Normal | No | No |
| | <i>Exjade</i> for chronic iron overload due to blood transfusions | Yes | Normal | No | No |
| | <i>Sprycel</i> for acute lymphoblastic leukaemia and chronic myeloid leukaemia | Yes | Normal | No | Yes |
| | <i>Diacomit</i> for severe myoclonic epilepsy in infancy | Yes | Conditional Approval | Yes | No |
| 2007 | <i>Elaprase</i> for mucopolysaccharidosis type II (Hunter syndrome) | No | Exceptional circumstances | Yes | Yes |
| | <i>Inovelon</i> for Lennox-Gastaut syndrome | Yes | Normal | No | No |
| | <i>Cystadane</i> for homocystinuria | Yes | Normal | Yes | No |
| | <i>Revlimid</i> for multiple myeloma | Yes | Normal | No | Yes |
| | <i>Soliris</i> for paroxysmal nocturnal haemoglobinuria | No | Normal | Yes | Yes |
| | <i>Siklos</i> for sickle cell syndrome | No | Normal | Yes | Yes |
| | <i>Atrinelex</i> for acute lymphoblastic leukaemia | Yes | Exceptional circumstances | No | No |
| | <i>Increlex</i> for primary insulin-like growth factor-1 deficiency due to molecular or genetic defects | No | Exceptional circumstances | No | No |
| | <i>Glialan</i> for intra-operative photodynamic diagnosis of residual glioma | Yes | Normal | Yes | Yes |
| | <i>Yondelis</i> for soft tissue sarcoma | Yes | Exceptional circumstances | Yes | No |
| | <i>Tasigna</i> for chronic myeloid leukaemia | Yes | Normal | No | Yes |
| | <i>Torisel</i> for renal cell carcinoma | Yes | Normal | No | Yes |
| | | | | | |
| | | | | | |
| | | | | | |

(continued)

Table 2 (Continued)

| Year | Orphan MA | Significant benefit | Type of MA | Prevalence ≤1/10,000 | Received PA/SA |
|-------|--|---------------------|--|------------------------------|------------------------|
| 2008 | <i>Thalidomide Celgene</i> for multiple myeloma | Yes | Normal | No | Yes |
| | <i>Volibris</i> for pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension | Yes | Normal | No | Yes |
| | <i>Firazyr</i> for angioedema | Yes | Normal | No | Yes |
| | <i>Ceplene</i> for acute myeloid leukaemia | Yes | Exceptional circumstances | No | No |
| | <i>Kuvan</i> for hyperphenylalaninaemia | Yes | Normal | No | No |
| | <i>Mepact</i> for osteosarcoma | Yes | Normal | Yes | Yes |
| | <i>Vidaza</i> for acute myeloid leukaemia and myelodysplastic syndromes | Yes | Normal | No | No |
| 2009 | <i>Nymusa</i> for primary apnoea in premature newborns | Yes | Normal | No | No |
| | <i>Afinitor</i> for renal cell carcinoma | Yes | Normal | No | No |
| | <i>Mozobil</i> for mobilize progenitor cells prior to stem cell transplantation | Yes | Normal | Yes | No |
| | <i>Cayston</i> for gram negative bacterial lung infection in cystic fibrosis | Yes | Conditional Approval | No | Yes |
| | <i>Arcalyst</i> for Cryopyrin-Associated Periodic Syndromes (CAPS), including Familial Cold Autoinflammatory Syndrome (FCAS) and Muckle-Wells Syndrome (MWS) | No | Exceptional circumstances | Yes | No |
| | <i>Ilaris</i> for Cryopyrin-Associated Periodic Syndromes (CAPS), including Familial Cold Autoinflammatory Syndrome (FCAS) and Muckle-Wells Syndrome (MWS) | No | Exceptional circumstances | Yes | No |
| | <i>Nplate</i> for idiopathic thrombocytopenic purpura (ITP) | Yes | Normal | No | Yes |
| Total | 58 | SB 73% | Normal: 55% Exceptional circumstances: 40% Cond. App: 5% | Prevalence ≤1/10,000: 40% | Received PA/SA: 48% |

MA Marketing authorization; PA/SA protocol assistance/scientific advice

reached the market and being available for patients with rare diseases. These medicines may be used for the treatment of up to 2.6 million patients in Europe. Furthermore, with 690 products for orphan conditions designated in Europe, and several ongoing MA applications, more orphan medicinal products are expected to be authorized in the following years. More than one third of the designated products are intended for patients affected by very rare diseases, and until 2000 the pharmaceutical industry was unlikely to develop medicines for these conditions [3]. However, as there is an estimate of five to eight thousand rare diseases, many patients are still untreated and continuous joint efforts from researchers, industry and regulators are needed to improve the treatment options for these patients.

13 Useful links

- European Medicines Agency: website: www.ema.europa.eu
- The Community Register of medicinal products: http://ec.europa.eu/enterprise/sectors/pharmaceuticals/documents/sol/community-register/html/index_en.htm
- European Commission Directorate-General for Health and Consumer Protection: http://ec.europa.eu/health/ph_threats/non_com/rare_diseases_en.htm
- European Organization for Rare Diseases (Eurordis): <http://www.eurordis.org>
- Orphanet: <http://www.orpha.net/>
- Community Register of orphan medicinal products for human use: <http://ec.europa.eu/enterprise/pharmaceuticals/register/index.htm>
- Common EMEA/FDA application form for Orphan Medicinal Product Designation (Published November 2007): http://www.emea.europa.eu/pdfs/human/comp/EMEAFDA_Application_Form_for_Orphan_Medicinal_Product_Designation.doc

References

1. http://ec.europa.eu/health/ph_threats/non_com/rare_diseases_en.htm
2. <http://www.ema.europa.eu/htms/human/orphans/intro.htm>
3. Butlen-Ducuing F, Riviere F, Aarum S, Llinares-Garcia J (2010) European medicines agency support mechanisms fostering orphan drug development. *Drug News & Perspect* 23(1): 71–81
4. Heemstra HE, et al. (2009) Translation of rare disease research into orphan drug development: disease matters. *Drug Discovery Today* 14: 23–24, 1166–1173
5. The Orphan Drug Act (1983) <http://www.fda.gov/orphan/oda.htm>
6. Haffner M, Torrent-Farnell J, Maher P (2008) Does orphan drug legislation really answer the needs of patients? *Lancet* 371(9629): 2041–2044
7. Regulation (EC) No. 141/2000 of the European Parliament and of the Council of 16 December 1999 on Orphan Medicinal Products. *Official Journal of The European Communities*
8. <http://www.ema.europa.eu/htms/general/contacts/COMP/COMP.html>

9. http://www.ema.europa.eu/pdfs/human/comp/leaflet/orphan_designation_leaflet.pdf
10. <http://www.ema.europa.eu/pdfs/human/comp/6320009en.pdf>
11. <http://www.ema.europa.eu/pdfs/human/sciadvise/426001en.pdf>
12. Article 5.12 of Regulation EC No. 141/2000
13. Guideline on the format and content of applications for designation as orphan medicinal products and on the transfer of designations from one sponsor to another (ENTR/6283/00 Rev 3, July 2007)
<http://www.ema.europa.eu/pdfs/human/comp/628300en.pdf>
14. Doc.Ref.: EMEA/COMP/473073/2007
15. Doc.Ref.: EMEA/COMP/11249/2003 Rev.2
16. <http://www.ema.europa.eu/htms/human/orphans/opinions.htm>
17. <http://www.ema.europa.eu/pdfs/human/comp/71091709en.pdf>
18. Doc.Ref.: EMEA/COMP/17/04
19. Doc.Ref.: EMEA/COMP/223605/2007
20. Doc.Ref.: EMEA/COMP/223599/2007
21. Regulation (EC) No. 1901/2006 of the European Parliament and of the Council of 20 December 2006 on medicinal products for paediatric use
22. <http://www.emea.europa.eu/humandocs/PDFs/EPAR/torisel/H-799-en6.pdf>
23. Cheung RY, Cohen JC, Illingworth P (2004) Orphan drug policies: implications for the United States, Canada and developing countries. *Health Law J* 12: 183–200

CHAPTER 25

Special situations, market fragmentation II: sex differences

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1 Expanding scope of gender and sex differences

Delivering the right drug at the right dose to the right patient is one of the basic tenets of clinical pharmacology and personalized medicine. Recognition of the disparities in men's and women's health, including shortcomings in traditional medical practice and unmet scientific needs, has provided the necessary springboard for substantial therapeutic advances and will continue to pave the way for further advances in the field. Detecting sex differences in drug trials is a step toward an era of personalized medicine.

Traditionally, women's health focussed on reproductive health issues, such as contraception, pregnancy, menopause and breast cancer and was relegated mainly to obstetricians and gynaecologists. Medical Research has historically assumed that any sex differences in medicine outside of reproductive could be explained by body weight and/or percent fat. Medical research to investigate sex- and gender based differences has facilitated a better understanding of the influence of sex and gender on health. Female gender has been shown to be a risk factor for the development of adverse drug reactions. Although the underlying reasons have to be elucidated, hormonal and immunological factors, in addition to differences in pharmacokinetics and pharmacodynamics, have been discussed.

Women in Austria live 5.6 years longer than men reflecting data of life expectancy in Europe and northern America, comprising more than 50% of the population [1]. By 2025, the number of postmenopausal women worldwide is expected to rise to 1.1 billion [2]. The aging population is predominantly women, and one could argue that geriatric medicine is essentially women's medicine. Female consumers are an increasing force likely to have substantial influence in the prescription marketplace.

2 Sex versus gender

The substantial understanding of the two terminologies is important to be used appropriately in the understanding of scientifically relevant terms. Biologically, every cell has a sex, and differences are not necessarily a result of the variations in the hormonal regimen, but can be a direct result of genetic differences between the two sexes. Sex affects health since males and females have different patterns of diseases and conditions that affect the approaches to diagnosis, prevention and/or treatment, including pharmacologic agents [3, 4]. In the medical research the two definitions of “sex” and “gender” are given as follow:

- **Sex:** The classification of living things, generally as male or female according to their reproductive organs and functions assigned by chromosomal complement.
- **Gender:** A person’s self-representation as male or female, or how that person is responded to by social institutions based on the individual’s gender presentation. Gender is rooted in biology and shaped by environment and experience.

3 Sex matters

3.1 Physiologic variability

Gender-specific physiological differences include lower body mass index, and smaller organ size in women compared with men, resulting in larger distribution volumes in men. Beside a lower gastric acid secretion in women compared with men, importantly women have a higher proportion of body fat which may increase the distribution volume for lipophilic drugs [5]. In women, the percentage of tissue-water fluctuates throughout the menstrual cycle, as high estradiol concentrations are associated with sodium and water retention. Women have a lower glomerular filtration rate and lower creatinine clearance. In men, testosterone-induced increase in muscle metabolism is associated with augmented creatinine clearance [6].

Physiologic variability among research subjects increases with inclusion of both males and females and may necessitate larger sample sizes [7]. The desire for homogeneity extends to animal studies, encompassing not only species and breed but also sex [8]. The lack of data from animal assays compounds the problem of exclusion of women from phase 1 and 2 drug studies. Sex-specific variations in dose-response and adverse reactions to drugs are discovered late in drug development or not at all. The absence of women in early studies of drugs fosters dismissal of women’s symptoms and side effects of medications, ranging from headaches to hot flushes. A better understanding of unexplained differences in pharmacodynamic endpoints could result from the application of innovative clinical pharmacology methodologies and tools to better understand

these differences and optimize therapeutics. The developing technologies of pharmacogenomics, proteomics, metabolomics will allow identification of sex-specific gene expression or other patterns that can be used further to identify populations at risk for adverse events, delayed complications, lack of efficacy, and sex-specific complications [9, 10]. The participation of women in clinical research varies with the types of research studies included, the years of research publications included, and the outcomes used for comparisons by sex [11–14].

3.2 Pharmacokinetics – sex differences

From pharmacokinetic studies there is evidence of gender-specific differences for a number of drugs. Drug absorption, either orally or transdermally, does not differ significantly between women and men. The same applies for plasmaprotein binding of drugs. Relevant differences between women and men in the unbound fraction of highly plasmaprotein-bound drugs have not been shown [15]. The differences in the activity of drug-metabolizing enzymes are possibly of clinical relevance. Women often exhibit a higher hepatic clearance for CYP2D6 and CYP3A4 (an enzyme involved in the metabolism of >50% of all medications) substrates than men [16]. Many cardiovascular drugs are metabolized by enzymes of the cytochrome P450 system. Endogenous hormones, including estrogens and progestins are also metabolized via these enzymes. Drug concentrations are dependent on the volume of distribution and clearance. Both parameters are dependent on body weight for most drugs independent of sex differences. Renal clearance of unchanged drug is decreased in females due to a lower glomerular filtration. Sex differences in activity of the cytochrome P450 (CYP) and uridine diphosphate glucuronosyltransferase (UGT) enzymes and renal excretion will result in differences in clearance. There is evidence for females having lower activity of CYP1A2, CYP2E1 and UGT; higher activity of CYP3A4, CYP2A6 and CYP2B6; and no differences in CYP2C9 and CYP2D6 activity [15–18].

3.3 Pharmacodynamic – sex differences

Pharmacodynamic is the response of the body to a given dose of a drug over time. Analysis for pharmacodynamic differences though is rare. Pharmacodynamic changes can affect both the desired therapeutic effect of a drug as well as its adverse effect profile. Regarding the cardiovascular system, resting heart rate in women is three to five beats higher than in men. Length of the cardiac cycle in men is longer. In women, length of the cardiac cycle varies throughout the menstrual cycle and is prolonged during menstruation. These cyclic fluctuations no longer appear following complete autonomous blockade. Women have a longer corrected QT interval and a shorter sinus node recovery time.

The most widely reported sex difference is the higher risk in females for drug-induced long QT syndrome, with two-thirds of all cases of drug-induced torsades occurring in females [19–21]. Furthermore, sex-related differences in the density of ion channels may partially explain this phenomenon. Females also have a higher incidence of drug-induced liver toxicity, gastrointestinal adverse events due to NSAIDs, and allergic skin rashes. There are still large gaps in our knowledge of sex differences in clinical pharmacology and significantly more research is needed.

3.4 Female specific aspects

Further female-specific aspects must be considered in the administration of drugs. Menstrual cycle, pregnancy and menopause can be associated with changes in the pharmacokinetics of drugs, mostly as a result of changes in sex steroid concentrations and alterations in total body water (e.g. expansion of total body water, increase of renal plasma flow, and glomerular filtration during pregnancy). It has been reported that menstruation, pregnancy and ovariectomy can modulate CYP2D6 activity [22–24]. The clinical relevance of these changes is not clear. In addition, interactions with exogenous hormone therapy such as oral contraception and hormone replacement therapy must be taken into account. Estrogens and progestins interact with a number of cardiovascular drugs, possibly by inhibiting CYP enzymes or increasing drug glucuronidation. *In vivo* data have shown that oral contraceptives can increase or decrease drug concentrations of co-administered medications [15].

3.5 Adverse drug reactions

Up to 5% of all hospital admissions are the result of adverse drug reactions (ADRs). Identifying those factors which may predispose to ADRs is essential for risk management. Amongst the known risk factors for adverse reactions are increasing age, polypharmacy, liver and renal disease as well as being female. Female patients have a 1.5- to 1.7-fold greater risk of developing an ADR, compared with male patients. The reasons for this increased risk are not entirely clear but include gender-related differences in pharmacokinetic, immunological and hormonal factors as well as differences in the use of medications by women compared with men. Women generally have a lower lean body mass, a reduced hepatic clearance, have differences in activity of cytochrome P450 (CYP) enzymes (40% increase in CYP3A4, varied decrease in CYP2D6, CYP2C19 and CYP1A2), and metabolize drugs at different rates compared with men. Other important factors include conjugation, absorption, protein binding and renal elimination, which may all have some gender-based differences. However, how these differences result in an increased risk of ADRs is not clear. There are pharmacodynamic differences between men and women, seen particularly with cardiac and psychotropic medications. There is no doubt that chlorpromazine, fluspirilene and

various anti-psychotics appear more effective in women than men for the same dosage and plasma concentration. Similarly, women are at increased risk of QT prolongation with certain anti-arrhythmic drugs compared with men even at equivalent serum concentrations.

Increasingly the evidence is that idiosyncratic drug reactions, particularly cutaneous reactions, appear to have an immunological etiology. It is possible that gender difference in T-cell activation and proliferation account for this as well as the increased prevalence of skin diseases such as systemic lupus erythematosus and photosensitivity. Whatever the mechanism(s), it is important to be aware that gender is a significant factor in ADRs [25].

An internal FDA project that examined 300 new drug applications between 1995 and 2000 determined that 72 drugs out of the 300 examined were metabolized *via* the cytochrome P450 3A4 pathway and exhibited a sex difference in pharmacokinetics. One hundred and sixty-three of those studies included sex analysis. Eleven drugs showed greater than 40% difference in pharmacokinetics between men and women as listed in the product label. Despite these differences, no dosing recommendations were made. Ten medications withdrawn from the market between 1997 and 2000 had a greater adverse profile in women, it was shown that 4 of the drugs were associated with the primary health risk of torsades de pointes [26].

3.6 Clinical trials – women's representation

3.6.1 History

Women had limited opportunities to participate in medical research as a result of 2 medical disasters. In the 1950s and early 1960s, thalidomide use by pregnant women resulted in children with birth defects worldwide. Even though it had not been approved for use in the United States, thalidomide focussed public and political attention on the approval of new drugs. In the early 1970s, research revealed that the daughters of women who took diethylstilbestrol during pregnancy had an increased risk of vaginal cancer. Together, these medical disasters led the FDA, industry, researchers, and the public at large to conclude that women who could become pregnant were not appropriate subjects in clinical drug trials. In 1977, the FDA issued guidelines that required women of childbearing potential to be excluded from drug trials (except for drugs used in the treatment of life-threatening or serious diseases) until teratogenicity data from animal studies of the drug were available [27]. Since most of these teratogenicity studies were not completed until after phase 2 and 3 trials were under way, the guideline effectively barred women from most early-phase clinical trials.

The European medicines agency (EMA) stated in 2006 that females and males are expected to be represented in cardiovascular clinical trial in a proportion that mimics the prevalence of the disease. According to investigations preformed by regulatory bodies, women are, in general, adequately represented in clinical trials reflecting gender

prevalence of the disease studied (Review by EMEA of pivotal trials for products filed 2000–2003).

3.6.2 Barriers of participation in clinical trials

Recruitment and retention of women in clinical trials are considered more complex and more expensive than recruitment and retention of men. Some obstacles to participation, such as the need for child or other dependent care, lack of transportation, lack of health insurance or inadequate health insurance, and lack of time for healthcare visits because of joint demands of work and family, can be problems for both women and men but disproportionately affect women [28–31]. The successful recruitment and retention to large studies, such as the Women’s Health Initiative and the Breast Cancer Prevention Trial [32, 33] and others, have resulted in many reports on methods for recruitment of women.

4 Regulatory changes to include women in clinical trials

The FDA issued a Gender Guideline that ended restrictions on premenopausal women’s participation in early-phase drug trials, encouraging investigators, and potential research participants to evaluate risks and benefits of inclusion of women of child-bearing potential on a study-by-study basis [34]. Progress in the inclusion of women in clinical trials has followed the changes in policies and regulations over the past 15 years. Sex-specific analyses are required to ensure that differences in response rates, adverse events, or drug interactions are recognized. Sex-specific analyses are equally important so that drugs that are effective in only one sex are not rejected because of pooling of data.

5 Pregnancy

Taking into account the pharmacokinetic differences in sex, it is not surprising that differences also arise in pregnancy. A wide array of physiological and hormonal changes occur during pregnancy; most begin in the first trimester and increase linearly until parturition [35, 36]. Prescription and over-the-counter drug use in pregnancy is necessary for many women today. For some women, this is because they become pregnant with pre-existent conditions that require ongoing or intermittent pharmacotherapy. For others, this is because pregnancy itself can give rise to new medical conditions such as gestational diabetes and preeclampsia. The principal concern of prescribing physicians is whether or not agents will harm the fetus (i.e. have teratogenic effects). This concern rose to prominence primarily as a result of the thalidomide disaster. Marketed for use in morning sickness, thalidomide was found to be a potent

teratogen capable of producing a variety of birth defects relating to development. Regulations such as those established by the World Medical Association's Declaration of Helsinki Principles in 1964 include the guidelines for vulnerable populations such as children, mentally disabled persons, prisoners, and pregnant women. Consequently, determining the teratogenicity of new drugs currently dominates the objectives of pregnancy-relevant experiments conducted throughout drug development. Clinical trials of drug therapies for AIDS and HIV infections precipitated new guidelines for clinical trials of pregnant women [37]. The revised regulations of the FDA intended to "enhance the opportunity for participation of pregnant women in research" issued in 2001. However, the overriding challenge is obtaining adequate information on drug safety during pregnancy as quickly as possible after a new drug is marketed.

6 From sex differences to individual differences: where the science is taking us

Is stratifying studies solely on phenotypes (such as male/female) a matter of the past? And is the increase in the use of genetic subtypes in diagnosis and therapeutic efficacy the substance for the future trials? Currently, single nucleotide polymorphism (SNP) technology is leading the movement toward individualized therapy. The human genome is made of 3 billion base pairs, and for every thousand base pairs there is a variable base pair that gives rise to an SNP, resulting in 3 million SNPs in the human genome. SNPs serve as markers for mapping the genome. Pharmacogenomics is the use of genetic information to predict the safety, toxicity, and/or efficacy of drugs in individual patients or groups of patients. Pharmacogenetics analysis can be used to develop a medicine response profile for individual patients. As the field of pharmacogenomics advances, clinical trial design and statistical analysis will become even more important as we move into an era of personalized medicine.

Case Study: Cardiovascular disease and women

Disparities in presentation and outcomes: Coronary heart disease (CHD) tends to appear later in women than it does in men (10 years later for total CHD and 20 years later for its most serious manifestations such as myocardial infarction [MI] and sudden cardiac death) but becomes the leading killer of US and European men by age 45 and of women by age 65 [38]. About 55% of all females' deaths are caused by CVD, especially coronary heart disease and stroke (Fig. 1). Cardiovascular death rates tend to slightly decline in men within the past two decades but are constantly

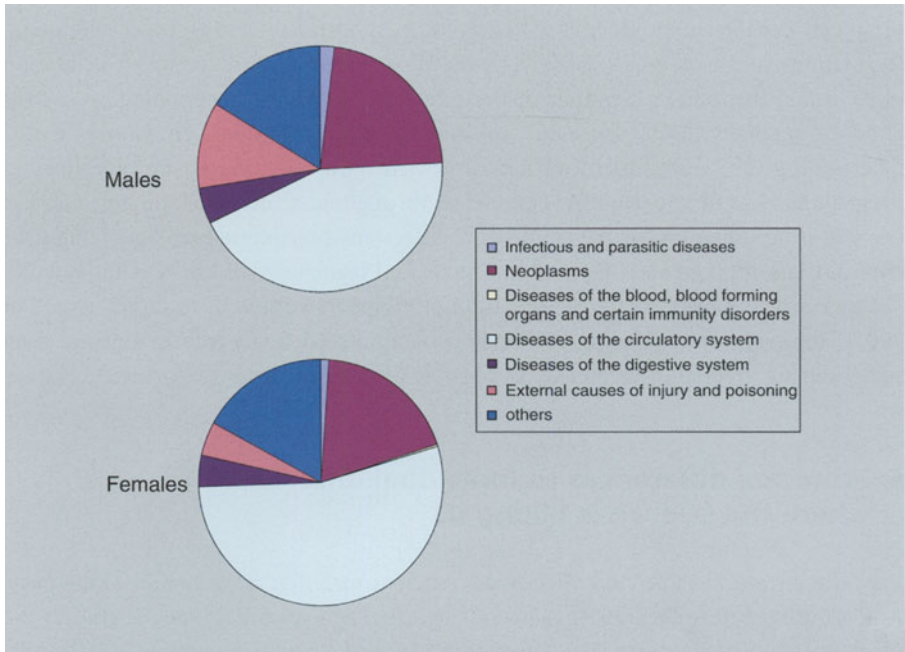


Fig. 1 Causes of death in Europe in the year 2006 (<http://data.euro.who.int/hfamdb>)

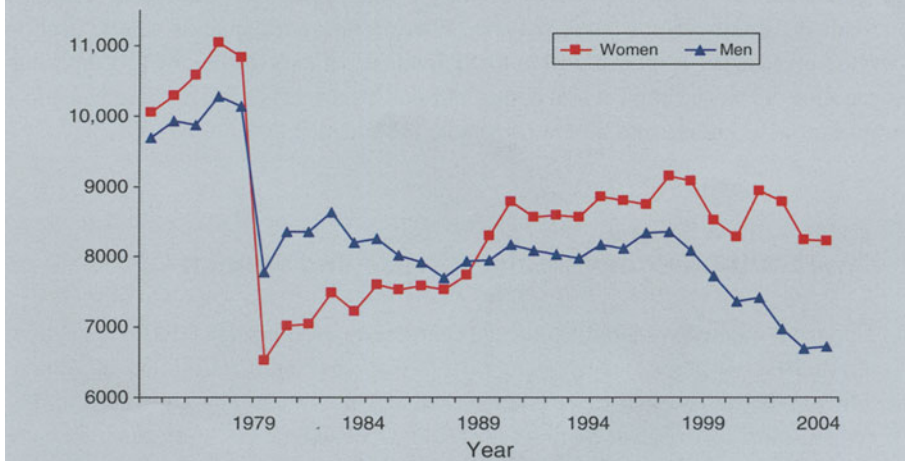


Fig. 2 Mortality from CHD in Austria 1975–2004 (Statistik Austria)

high in women (Fig. 2) [39]. Some of this discordance may be due under use of aggressive evidence based therapy. What is not fully understood is that women during the fertile age have a lower risk of cardiac events, but this protection fades after menopause thus leaving women with untreated risk factors vulnerable to develop myocardial infarction, heart failure, and sudden cardiac death. Furthermore, clinical manifestations of ischaemic heart disease in women may be different from those commonly observed in males and this factor may account for under-recognition of the disease. The outcomes after treatment for coronary artery disease, particularly acute myocardial infarction, are different for women compared with men. Women have a well-documented higher mortality after acute myocardial infarction [40–42]. Much of this disparity has been attributed to differences in age and comorbidities. Additionally, women appear to be at higher risk than men when diabetes, hypertriglyceridemia, and metabolic syndrome are present. The under use of revascularization procedures in women has been suggested as an explanation, but it has not been uniformly demonstrated to explain increases in mortality [43].

Sex specific differences in vascular physiology and pathology

Underlying hormonal changes:

- Incidence of CHD is increased in patients with early menopause, gestational diabetes, peripartum vascular dissection, preeclampsia and eclampsia, polycystic ovarian syndrome, low-birth-weight children and hypothalamic hypoenestrogenemia [44].
- Higher prevalence of vascular abnormalities such as Raynaud's phenomenon, migraines, vasospastic disorders, and other vasculitides in women.
- The aging process heralds a reduction in estrogen to about 1/10th premenopausal levels. The predominant source of estrogen before menopause is estradiol. After menopause, a lower level of estrogen is produced primarily from the conversion of androgens to estrone in adipose tissue. This might explain the rise in risk for CHD for women that occur after menopause. It is supported by the fact that younger women with endogenous estrogen deficiency have a >7-fold increase in coronary artery risk [44].

Underlying differences in vascular structure

- Women have smaller and less compliant conduit arteries than men.
- During pregnancy changes in arterial size occur (not elucidated if physiological or pathological remodelling).

- Little evidence of changes in the caliber of the coronary arteries over time after female-to-female heart transplantation.
- After a female heart is transplanted to a man, there is progressive enlargement of the coronaries after accounting for body habitus and left ventricular hypertrophy [45].
- Women on androgens have been found to have larger arteries than control subjects.
- Reduction in size of brachial arteries when genetic men have been taking estrogen [46]. Enlargement with androgens, consistent with positive remodelling.
- Women who present with acute coronary syndromes have a higher incidence of non-obstructive coronary artery disease in the Women's Ischemia Syndrome Evaluation (WISE) study [47].
- Estrogen exerts anti-apoptotic effects, thereby increasing circulating endothelial progenitor cells [48]: it can be hypothesized if aging, leaves women vulnerable to a decreased ability to sustain adequate vascular repair.
- Increased frequency of coronary artery spasm.

Pharmacotherapy and CHD

In the following section we will highlight the existing pharmacokinetic differences in evidence based drug therapy recommended for first line therapy in CHD with respect to pharmacodynamic differences. Administration of fixed doses, not adapted to body weight, frequently result in higher plasma concentrations in women, owing to their lower distribution volume compared with men [49]. Further influencing factors in women are hormonal changes and different activities of a number of drug metabolizing enzymes.

1. *Betablockers*: Myocardial beta 1-receptors are up-regulated in case of estrogen deficiency, without effects on binding affinity [49]. Hormone supplementation with estrogens and progestins can prevent such up-regulation. Reduced cardiac sympathetic response to catecholamines results, on the whole, under endogenous estrogens. Women have greater drug exposure than men, with higher maximum concentration and larger area under the plasma concentration-time curve for metoprolol. Women also have a greater reduction in exercise heart rate and systolic blood pressure, as described for the beta 1-cardioselective blocker metoprolol which is primarily metabolized via CYP2D6. With respect to mortality reduction after

myocardial infarction or heart failure, beta-blocker therapy exhibits similar benefits for women and men.

2. *ACE-Inhibitors*: Premenopausal women demonstrate lower ACE activity than post menopausal women: a difference abolished by hormone replacement therapy. Relevant gender specific pharmacokinetic differences have not been described for the ACE-inhibitors. Meta-analyses of ACE-inhibitor therapy in heart failure revealed a reduction of mortality rate in men by 37%, but only 22% in women [50]. A further meta-analysis investigating the effects of ACE-inhibitor therapy early after myocardial infarction complicated by left-ventricular dysfunction, found comparable favourable effects for both genders with respect to prognosis and hospitalization rate [51]. Women with asymptomatic left-ventricular dysfunction appear not to profit from ACE-inhibitor therapy with regard to morbidity and mortality [52]. ADRs in the form of ACE-inhibitor cough occur twice as frequently in women. On the basis of the small proportion of women included in ACE-inhibitor studies, data from women are less advantageous than for men. Regarding the lack of data for prospective analysis of gender differences the question of whether women basically profit less from ACE inhibitor therapy has not been definitely elucidated.
3. *Calcium channel blockers*: Despite appreciable gender-specific pharmacokinetic differences under calcium channel blockers, the impact on pharmacodynamics is slight. These substances are subject to considerable first pass metabolism in the liver, and are substrates of CYP3A4, for which higher activities have been described in women than in men, accordingly women show faster clearance and lower levels of calcium channel blockers, than do men [53]. Reduction in blood pressure is more pronounced in women than in men. Clinical endpoint studies have revealed no relevant differences between women and men with regard to mortality and morbidity for cardiovascular diseases.
4. *Digitalis*: For digitalis, there is evidence of higher mortality in female patients with chronic heart failure. The cause is assumed to be excessive dosage for women, despite lower administered digoxin doses, women demonstrated higher serum levels than did men in the DIG trial. Other aspect of these evident differences are suspected gender specific differences in cellular sodium and calcium handling [54]. In a subgroup analysis of HERS (Heart and Estrogen-Progestin Replacement Study), which investigated the effect of post menopausal hormone replacement therapy (HRT) in secondary prevention of cardiovascular disease, evidenced that women under HRT, who additionally received digitalis, experienced elevated incidence of coronary events in the first year of the study [55]. This prognostically unfavourable

effect of hormone replacement therapy did not occur in women who took no digitalis. As digitalis therapy in this study had not been randomized, it remains to be elucidated whether women taking digitalis had been sicker and whether this explains the higher incidence of cardiac events.

5. *Antiarrhythmics*: Gender-specific differences in myocardial repolarization have been long known. The fact that QT time in childhood is of equal length in both sexes, and that it shortens after puberty in young men with elevated androgen levels, speaks for effects of sex steroids. Also cyclic fluctuations of the female QT time have been reported, with maximum prolongation during ovulation and menstruation [56]. Pro-arrhythmic effects in the form of torsades de pointes tachycardia, as the expression of an acquired long-QT syndrome, occur in women under antiarrhythmic therapy significantly more frequently than in men. This syndrome can also be induced by a great number of other drugs especially psychotropic drugs and antibiotics [21, 57]. The significance of these more frequent pro-arrhythmias on prognosis of women has not been fully elucidated.
6. *Aspirin*: The bioavailability of acetylsalicylic acid is greater in women than in men, owing to slower clearance and, in turn, significant prolongation of half life [58]. This gender-specific difference is assumably the result of greater activity of the degradation pathway via conjugation with glycine and glucuronic acid in men. As oral contraceptives can stimulate these degradation pathways, the difference in bioavailability of acetylsalicylic acid disappears in women under hormonal contraception. In secondary prevention of cardiovascular diseases, therapy with acetylsalicylic acid is equally well documented for women and men. The benefit of aspirin in primary prevention of myocardial infarction is less clear for women [59].
7. *Statins*: Pharmacokinetic gender-specific differences with respect to statins are slight. With the exception of pravastatin, rosuvastatin (both without significant CYP metabolism), and fluvastatin (predominantly CYP2C9 metabolism), all statins are primarily subject to hepatic metabolism via CYP3A4 and cerivastatin additionally to metabolism via CYP2C8. Consequently, drug interactions with substances also metabolized via CYP3A4 have to be considered. Despite higher plasma concentrations in women for a number of statins, there have been no recommendations for dose adjustment in women. Nevertheless, the risk of ADRs appears greater in women. Administration of cerivastatin (since taken off the market) was associated with unacceptable frequencies of myopathy and rhabdomyolysis, especially in older, thin women [60]. Primary and secondary prevention studies have revealed beneficial effects that are comparable for women and men.

Summary

The percentage of women participating in studies on CHD has risen since the mid-1980s, with the result that the percentage of women covered by such investigations now coincides with the actual prevalence of CHD in women. Gender-specific differences have not been investigated for many cardiovascular drugs. If such gender-specific analyses have been performed, pharmacokinetic differences for women and men became apparent. The higher plasma concentrations in women may be one explanation why female sex is associated with a greater risk of ADRs. Despite these often relevant pharmacokinetic differences between female and male patients, the impact on pharmacodynamics is generally moderate. There are only slight differences concerning the prognostic significance of primary and secondary preventive cardiovascular therapeutic strategies for women and men. It must be emphasized, however, that women have been often under-represented in endpoint studies of coronary heart disease. Statements for women are mostly reached via subgroup, *post hoc*, or meta-analyses. No statistically significant gender differences in terms of efficacy and safety of most of the drugs are found, however, most of the trials are not prospectively designed to detect gender differences. Further discussions may be needed to determine if and for what products and/or product indications gender analyses should be performed and during what stage in clinical development this information should be collected.

References

1. Statistik Austria (www.statistik.at)
2. Uhl K (2008) Advancing Women's health in the 21st century: applying the tools of clinical pharmacology. *Clin Pharmacol Ther* 83: 3–7
3. Anonymous (2001) Exploring the biological contributions to human health: does sex matter? *J Womens Health Gend Based Med* 10: 433–439
4. Sapienza C. Institute of Medicine Report – “Exploring the biological contributions to human health: Does sex matter?” Gender analysis of medications: challenges to the sciences and profession of pharmacy. Presented at the 62nd FIP Congress, September 1–5, 2002; Nice, France. Abstract #AS-S-002.
5. Meibohm B, Beierle I, Derendorf H (2002) How important are gender differences in pharmacokinetics. *Clin Pharmacokinet* 41: 329–342
6. Gross JL, Friedman R, Azevedo MJ, Silveiro SP, Pecis M (1992) Effect of age and sex on glomerular filtration rate measured by 51Cr-EDTA. *Braz J Med Biol Res* 25: 129–134
7. Bennett JC and the Board on Health Sciences Policy of the Institute of Medicine (1993) Inclusion of women in clinical trials – policies for population subgroups. *N Engl J Med* 329: 288–292
8. Institute of Medicine, Committee on the Ethical and Legal Issues Relating to the Inclusion of Women in Clinical Studies (1994) *Women and Health Research: ethical and Legal Issues of Including Women in Clinical Studies*, Vol. 1. National Academy Press, Washington, DC

9. Haack S, Seeringer A, Thürmann PA, Becker T, Kirchheiner J (2009) Sex-specific differences in side effects of psychotropic drugs: genes or gender? *Pharmacogenomics* 10(9): 1511–1526
10. Aerssens J, Paulussen AD (2005) *Pharmacogenomics and acquired long QT syndrome. Pharmacogenomics* 6(3): 259–270
11. Meinert CL, Gilpin AK, Unalp A, Dawson C (2000) Gender representation in trials. *Control Clin Trials* 21: 462–475
12. Vidaver RM, Lafleu B, Tong C, Bradshaw R, Marts SA (2000) Women subjects in NIH-funded clinical research literature: lack of progress in both representation and analysis by sex. *J Womens Health Gend Based Med* 9: 495–504
13. Harris DJ, Douglas PS (2000) Enrollment of women in cardiovascular clinical trials funded by the National Heart, Lung, and Blood Institute. *N Engl J Med* 343: 475–480
14. Müllner M, Vamvakas S, Rietschel M, van Zwieten-Boot BJ (2007) Are women appropriately represented and assessed in clinical trials submitted for marketing authorization? A review of the database of the European Medicines Agency. *Int J Clin Pharmacol Ther* 45(9): 477–484
15. Schwartz JB (2003) The influence of sex on pharmacokinetics. *Clin Pharmacokinet* 42: 107–121
16. Anderson GD (2008) Gender differences in pharmacological response. *Int Rev Neurobiol* 83: 1–10
17. Cotreau MM, von Moltke LL, Greenblatt DJ (2005) The influence of age and sex on the clearance of cytochrome P450 3A substrates. *Clin Pharmacokinet* 44: 33–60
18. Wolbold R, Klein K, Burk O, et al. (2003) Sex is a major determinant of CYP3A4 expression in human liver. *Hepatology* 38: 978–988
19. Hreiche R, Morissette P, Turgeon J (2008) Drug-induced long QT syndrome in women: review of current evidence and remaining gaps. *Gend Med* 5(2): 124–135
20. Ebert SN, Liu XK, Woosley RL (1998) Female gender as a risk factor for drug-induced cardiac arrhythmias: evaluation of clinical and experimental evidence. *J Womens Health* 7(5): 547–557
21. Makkar RR, Fromm BS, Steinman RT, Meissner MD, Lehmann MH (1993) Female gender as a risk factor for torsades de pointes associated with cardiovascular drugs. *JAMA* 1; 270(21): 2590–2597
22. Hogstedt S, Lindberg B, Rane A (1983) Increased oral clearance of metoprolol in pregnancy. *Eur J Clin Pharmacol* 24: 217–220
23. Llerena A, Cobaleda J, Martinez C, Benitz J (1996) Interethnic differences in drug metabolism: influence of genetic and environmental factors on desrisoquine hydroxylation phenotype. *Eur J Drug Metabol Pharmacokinet* 21: 129–138
24. Wadelius M, Darj E, Frenne G, Rane A (1997) Induction of CYP2D6 in pregnancy. *Clin Pharmacol Ther* 62: 400–407
25. Rademaker M (2001) Do women have more adverse drug reactions? *Am J Clin Dermatol* 2(6): 349–351
26. Keitt SK, Wagner CR, Tong C, Marts SA (2003) Understanding the biology of sex and gender differences: using subgroup analysis and statistical design to detect sex differences in clinical trials. *MedGenMed* 5(2): 39
27. Pregnancy discrimination act of 1978, 92 Stat. 2076; 42 USC 2000e(k). 39
28. Recruitment and Retention of Women in Clinical Studies (1995) Office of Research on Women's Health. Bethesda, MD, NIH Pub No. 95-3756
29. Killien M, Bigby JA, Champion V, et al. (2000) Involving minority and underrepresented women in clinical trials: The National Centers of Excellence in Women's Health. *J Womens Health Gend Based Med* 9: 1061–1070
30. Underwood SM (2000) Minorities, women and clinical cancer research: the charge, promise, and challenge. *Ann Epidemiol* 10: S3–S12
31. Brown DR, Fouad MN, Basen-Engquist KB, Tortolero-Luna G (2000) Recruitment and retention of minority women in cancer screening, prevention, and treatment trials. *Ann Epidemiol* 10: S13–S21

32. Wilcox S, Shumaker SA, Bowen DJ, et al. (2001) Promoting adherence and retention to clinical trials in special populations: a women's health initiative workshop. *Control Clin Trials* 22: 279–289
33. Fisher B, Costantino JP, Wickerham DL, et al. (1998) Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. *J Natl Cancer Inst* 90: 1371–1388
34. Food and Drug Administration (1993) Guideline for the study and evaluation of gender differences in the clinical evaluation of drugs, notice. *Fed Reg* 58: 39405–39416.
35. Anger GJ, Piquette-Miller M (2008) Pharmacokinetic studies in pregnant women. *Clin Pharmacol Ther* 83(1): 184–187
36. Dawes M, Chowienzyk PJ (2001) Drugs in pregnancy. *Pharmacokinetics in pregnancy. Best Pract Res Clin Obstet Gynaecol* 15(6): 819–826
37. Connor sperling gelber (1994) Reduction of maternal-infant transmission of HIV with zidovudine treatment. *N Engl J Med* 331: 1173–1180
38. World Health Organization Statistical Information System 2004. www.who.int/whosis/
39. Shafazand M, Schaufelberger M, Lappas G, Swedberg K, Rosengren A (2009) Survival trends in men and women with heart failure of ischaemic and non-ischaemic origin: data for the period 1987–2003 from the Swedish Hospital Discharge Registry. *Eur Heart J* 30(6): 671–678
40. Vaccarino V, Krumholz HM, Berkman LF, Horwitz RI (1995) Sex differences in mortality after myocardial infarction: is there evidence for an increased risk for women? *Circulation* 91: 1861–1871
41. Milcent C, Dormont B, Durand-Zaleski I, Steg PG (2007) Gender differences in hospital mortality and use of percutaneous coronary intervention in acute myocardial infarction: microsimulation analysis of the 1999 Nationwide French Hospitals Database. *Circulation* 115: 833–839
42. Anderson RD, Pepine CJ (2007) Gender differences in the treatment for acute myocardial infarction: bias or biology? *Circulation* 20; 115(7): 823–826
43. Chandra NC, Ziegelstein RC, Rogers WJ, et al. (1998) Observations of the treatment of women in the United States with myocardial infarction: a report from the National Registry of Myocardial Infarction-I. *Arch intern Med* 158: 981–988
44. Bairey Merz CN, Johnson BD, et al. (2003) Hypoestrogenemia of hypothalamic origin and coronary artery disease in premenopausal women: a report from the NHLBI-sponsored WISE study. *J Am Coll Cardiol* 41: 413–419
45. Herity NA, Lo S, Lee DP, et al. (2003) Effect of a change in gender on coronary arterial size: a longitudinal intravascular ultrasound study in transplanted hearts. *J Am Coll Cardiol* 41: 1539–1546.
46. Holubkov R, Karas RH, Pepine CJ, et al. (2002) Large brachial artery diameter is associated with angiographic coronary artery disease in women. *Am Heart J* 143: 802–807
47. Sharaf BL, Shaw L, Johnson BD, et al. (2004) Any measurable coronary artery disease identified in women presenting with ischemic chest pain is associated with an adverse outcome: findings from the NIH-NHLBI-sponsored Women's Ischemia Syndrome Evaluation (WISE) study angiographic core laboratory. *J Am Coll Cardiol* 43: 292A
48. Rauscher FM, Goldschmidt-Clermont PJ, et al. (2003) Aging, progenitor cell exhaustion, and atherosclerosis. *Circulation* 108: 457–463
49. Jochmann N, Stangl K, Garbe E, Baumann G, Stangl V (2005) Female-specific aspects in the pharmacotherapy of chronic cardiovascular diseases. *Eur Heart J* 26: 1585–1595
50. Garg R, Yusuf S (1995) Overview of randomized trials of angiotensin-converting enzyme inhibitors on mortality and morbidity in patients with heart failure. Collaborative Group on ACE Inhibitor Trials. *JAMA* 273: 1450–1456
51. Shekelle PG, Rich MW, Morton SC, et al. (2003) Efficacy of angiotensin-converting enzyme inhibitors and beta-blockers in the management of left ventricular systolic dysfunction according to race, gender, and diabetic status. *J Am Coll Cardiol* 41: 1529–1538

52. Flather MD, Yusuf S, Kober L, et al. (2000) The ACE inhibitor myocardial infarction collaborative group. Long-term ACE inhibitor therapy in patients with heart failure or left-ventricular dysfunction: a systematic overview of data from individual patients. *Lancet* 355: 1575–1581
53. Krecic-Shepard ME, Park K, Barnas C, Slimko J, Kerwin DR, Schwartz JB (2000) Race and sex influence clearance of nifedipine: results of a population study. *Clin Pharmacol Ther* 68: 130–142
54. Blaustein MP, Robinson SW, Gottlieb SS, Balke CW, Hamlyn JM (2003) Sex, digitalis, and the sodium pump. *Mol Interv* 3: 68–72
55. Furberg CD, Vittinghoff E, Davidson M, et al. (2002) Subgroup interactions in the Heart and Estrogen/Progestin Replacement Study: lessons learned. *Circulation* 105: 917–922
56. Rodriguez I, Kilborn MJ, Liu XK, Pezzullo JC, Woosley RL (2001) Drug-induced QT prolongation in women during the menstrual cycle. *JAMA* 285: 1322–1326
57. Bednar MM, Harrigan EP, Ruskin JN (2002) Torsades de pointes associated with nonantiarrhythmic drugs and observations on gender and QTc. *Am J Cardiol* 89: 1316–1319
58. Ho PC, Triggs EJ, Bourne DW, Heazlewood VJ (1985) The effect of age and sex on the disposition of acetylsalicylic acid and its metabolites. *Br J Clin Pharmacol* 19: 675–684
59. De Berardis G, Sacco M, Pellegrini F, Graziano G, Tognoni G, Nicolucci A (2009) Aspirin for primary prevention of cardiovascular events in people with diabetes: meta-analysis of randomised controlled trials. *BMJ* 339: b4531.
60. FDA. CDER. Report no.: <http://www.fda.gov/cder/foi/label2001/207S6lbl.pdf>.

CHAPTER 26

Special situations III: Medicines for Children

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Summary

The majority of drugs have never been evaluated for use in children. Developmental differences between adults and children of different ages affect pharmacokinetics and pharmacodynamics, and the safety profile of drugs. Use of drugs without paediatric information carries risks such as inappropriate dosing, lack of efficacy, and unexpected adverse events. Paediatric drug studies have been hampered by ethical and legal restrictions, methodological challenges and economical restraints. Recently, regulatory initiatives to stimulate paediatric drug development have been implemented in the US and EU. The *EU Paediatric Regulation* “Better Medicines for Children” requires paediatric development according to a *Paediatric Investigation Plan (PIP)* for all new drugs and on-patent drugs when applying for an authorization extension. Paediatric development is rewarded with a 6-months patent extension. PIPs are reviewed and amended by a *Paediatric Committee* at the European Medicines Agency. Certain collateral measures are included that are intended to improve information, transparency, and to stimulate research into paediatric medicines. Key points to consider for a PIP such as the definition of relevant paediatric indications(s), development of age-appropriate formulation(s), juvenile animal studies, paediatric PK and PD studies, and clinical efficacy and safety studies are discussed. A case study on a PIP is provided.

1 Children as therapeutic orphans

For decades, medicines have not been evaluated for use in children [1]. Thus, the majority of medicines currently used to treat sick children are used off-label or off-

Keywords: Developmental pharmacological differences, EU Paediatric Regulation, Paediatric Investigation Plan, inventory of paediatric therapeutic needs, age-appropriate formulation, juvenile animal studies, physiologically-based PK model, population PK, extrapolation

licence. About 40% of drugs prescribed to children in the outpatient setting, 70% at paediatric intensive care units, 80% in haemato-oncology, and 90% at neonatal intensive care units are off-label/off-licence [2–6]. The frequency of off-label/off-licence use increases with the complexity of disease, the number of drugs prescribed, and with decreasing age.

To treat children with drugs licensed only for adults implies that data on quality, efficacy and safety are extrapolated from adults to children. Such extrapolation is usually not appropriate because of physiologic, pathophysiologic and pharmacologic differences between adults and children of various ages. In the developing child rapid changes occur in the activity of body functions affecting the pharmacokinetics of drugs, such as gastrointestinal uptake, disposition in various body compartments, metabolism and excretion [7]. Moreover, pharmacodynamic effects are also age-dependent for some drugs although the mechanisms involved are less understood [8]. Drug effects and adverse effects may affect body growth and development, e.g. corticosteroids, effects not occurring in adults. Finally, many diseases or disease manifestations are specific to children, e.g. neonatology, heart failure, leukaemia.

Use of unlicensed drugs in children carries risks such as ineffective dosing, overdosing, lack of efficacy, and unknown safety profiles in children. A classical example was the ‘grey baby syndrome’, resulting from chloramphenicol given to neonates in doses downscaled from adults. Because of immature metabolism, these doses lead to accumulation and life-threatening toxic effects [9]. Child-appropriate formulations are usually lacking and extemporaneous formulations are used instead, e.g. crushed tablets, pharmacy-prepared liquid formulations, which carry risks of contamination and unprecise dosing. Finally, marketing authorization holders cannot be held liable for problems occurring during unlicensed drug use. There is empirical evidence that adverse drug reactions in children are more frequent with drugs used off-label/unlicensed than with licensed drugs, 6% vs. 3.9% in the out-patient setting [10], and 3.4% vs. 1.4% in the in-patient setting [11].

2 Hurdles to drug development for children

2.1 Ethical and legal aspects

Clinical research in children comprises a fine balance between the need for special protection of children and the imperative to generate valid data for treatment of children. Special protection of children is required because of their inability to decide themselves about study participation, because of their increased vulnerability against adverse effects and their consequences, and because of an increased burden to children (distress and pain) through study procedures. This is counterbalanced by the current deficit in paediatric drug data and the resulting risks.

For decades, the legislation had focused on protection of children, thus clinical research in children was severely restricted. There has been a paradigm shift over the last decade with the recognition that properly conducted drug studies in children are preferable as they carry less risk and yield more information than uncontrolled off-label drug use in children. As a result, the recent *EU Clinical Trials Directive* (2001/20/EC) makes provisions for studies in children [12]: they are permitted if there is benefit for the group of children, not necessarily for the individual, which now allows for controlled and placebo studies in children. Moreover, the directive ensures special protection of the child, e.g. by permitting only research questions that cannot be addressed by studying adults, that are related to the child's disease; requiring documented informed consent by proxies, and minimization of risks and burden to the child.

2.2 Lack of public acceptance

In public perception and the attitude of parents concerned, the necessity to perform controlled clinical studies to evaluate drugs has not yet become widely accepted. There is still a major gap of knowledge and consequent reluctance to subject children to experimentation like "guinea pigs". This attitude is a major hurdle for recruitment of children into clinical studies.

2.3 Methodological challenges of clinical studies in children

Recruiting children in sufficient numbers into studies is challenging because many paediatric diseases are rare, and children are heterogenous with respect to age, development, and comorbidity. In addition, children and parents are reluctant to participate in studies, and consent rates are notoriously low. During study conduct, non-compliance with study measures or complete drop-out is common. Thus, paediatric protocols need to be pragmatic to adapt to the needs of children at different stages of development and with severe underlying disease. Moreover, for many therapeutic areas, age-appropriate study endpoints have yet to be defined and validated [13].

Paediatric drug development requires development and testing of age-appropriate drug formulations that allow accurate dosing and reliable administration in all age groups. Pharmacokinetic studies are challenging because of difficulties to obtain blood in children and the limited sample volumes that can safely be obtained, particularly from small children.

2.4 Lack of research infrastructures and paediatric research networks

The special methodological challenges of clinical studies in children require special expertise, infrastructures, and organizational structures. However, such structures are

currently far less developed than study centres for adults. Paediatric clinical study centres must provide good paediatric care and, in addition, have dedicated study personnel with specific know-how. There is a need for paediatric research networks to facilitate multicentre studies. Finally, there is a need for research grants dedicated to paediatric studies, and improved cooperation between paediatric academic researchers and the pharmaceutical industry.

2.5 Low economic potential of paediatric indications for pharmaceutical industry

Until recently, the biggest hurdle to paediatric drug development has been the lack of commitment by the pharmaceutical industry. Paediatric studies require higher resources but frequently paediatric indications have lower economic potential than adult indications. Thus, paediatric development was considered unprofitable by companies.

3 Regulatory initiatives to improve paediatric drug development

Regulatory initiatives focus on improving paediatric drug development by the pharmaceutical industry. They apply the principles of legal requirements for paediatric studies and financial rewards or incentives (“stick and carrot principle”).

3.1 Paediatric initiatives in the USA

In 1997, the *Food & Drug Agency Modernization Act (FDAMA, Paediatric Exclusivity Rule)* introduced a 6 months prolongation of market exclusivity for authorized drugs when paediatric studies were performed (incentive). This was followed in 1998 by the *Final Paediatric Rule* which authorized FDA to request paediatric studies and paediatric product information for new drugs with expected benefit for children (requirement). In 2002, paediatric exclusivity was extended in the *Best Pharmaceuticals for Children Act (BPCA)*, and in 2003 FDA’s authority to request paediatric studies in the *Paediatric Research Equity Act (PREA)*. In 2007, BPCA and PREA were both renewed and improved [14].

The US paediatric legislation was found to be very successful: From 1998 to 2004, 253 paediatric drug studies were performed by the pharmaceutical industry as a result of the Paediatric Exclusivity Rule. More than 200 Studied lead to additional paediatric information in the product information of the respective drug [15].

3.2 EU Paediatric Regulation

The *EU Regulation on Medicinal Products for Paediatric Use* (EC No 1901 & 1902/2006) came into force in January 2007 [16]. It build on the experience of the US legislation and, thereby, has become an even more powerful legislative tool to enforce paediatric drug development. The objectives of the Paediatric Regulation (“*Better Medicines for Children*”) are to (i) ensure high quality, ethical research into medicines for children, (ii) increase availability of authorized medicines for children, (iii) improve information available on medicines for children. These objectives should be achieved, (iv) without unnecessary trials in children and (v) without delaying authorization of medicinal products for other age populations.

The main pillars of the Paediatric Regulation are

1. Requirement for a Paediatric Investigation Plan (PIP);
2. Reward for compliance with the PIP in the form of a patent extension;
3. Paediatric Committee (PDCO) at the European Medicines Agency (EMA);
4. Some collateral measures.

3.2.1 Requirements and rewards

The requirements are similar for all new medicinal products (article 7) or for authorized on-patent medicinal products when applying for a new indications, new pharmaceutical forms and new routes of administration (article 8): applicants must submit, at the time of marketing authorization application, the results of all studies performed and details of all information collected in compliance with a previously agreed PIP, or a decision granting a waiver or a deferral. The reward for articles 7 and 8 applications is a 6 months patent extension, on the conditions of compliance with PIP, inclusion of the results of the PIP in the product information, and approval of the medicinal product in all Member States.

For orphan drugs, there is also the requirement to submit a PIP. However, orphan drugs can get two additional years of exclusivity for compliance with the PIP.

For medicinal products already off-patent, the Paediatric Regulation established a new type of marketing authorization, the *Paediatric Use Marketing Authorization* (PUMA) which applies for medicinal products developed exclusively for use in the paediatric population (article 30). PUMAs are optional but there is the requirement to cover a paediatric indication and formulation, to comply with an agreed PIP, and to include paediatric information into the product information. The incentive for a PUMA is 10 years data protection and that the existing brand name may be retained.

Certain drugs are exempted from the requirement for a PIP, such as generics, hybrids, biosimilars, drugs with “well-established use”, homeopathic drugs, and traditional herbal medicines.

3.2.2 Paediatric Investigation Plan

A *Paediatric Investigation Plan* is basis for the development and authorization of a medicinal product for the intended paediatric population subsets. It includes details of the timing and the measures to demonstrate quality, safety, and efficacy for use of the drug in the paediatric population. It must include development of (an) age-appropriate formulation(s). A PIP needs to be proposed by the applicant by end of phase 1 of development for a new product. It is scientifically reviewed, amended, and agreed or refused by the PDCO. An agreed PIP is binding for the applicant. However, if new information evolves during development of the drug which impacts on the PIP, a modification of the PIP may be submitted to the PDCO.

A *waiver* may be granted by the PDCO for a class of drugs, or an indication, or for a specific product, based on (i) expected lack of efficacy or safety concern, (ii) if the condition only occurs in adults, (iii) lack of significant therapeutic benefit over existing therapies for paediatric patients.

A *deferral* may be granted by the PDCO for initiation and/or completion of studies of the PIP when it appears appropriate to conduct studies in adults first, e.g. for safety reasons, or when studies in the paediatric population will take longer to conduct than studies in adults. Paediatric development is most often a combination of a PIP with deferrals and waivers for population subsets.

3.2.3 Paediatric Committee

The PDCO is a multidisciplinary scientific committee consisting of academics and agencies’ employees. It is composed of five members of the Committee for Medicinal Products for Human Use (CHMP), one member per EU member state (if not represented through a CHMP member), three representatives of health professionals and three representatives of patient organizations; and an alternative per each representative. The PDCO’s tasks are to assess, amend, and formulate an opinion on PIPs, deferrals, and waivers based on scientific grounds. It also performs compliance checks of PIPs. Further tasks are to assist in scientific advice, write guidelines for paediatric aspects of drug development, establish and revise an inventory of paediatric needs, a priority list of off-patent paediatric drugs, and support the Agency on establishing a European Network of paediatric research networks. After 2 years and 9 months of existence, the PDCO has received 701 PIP/waiver applications, covering a total of 1057 indications. It has adopted 229 positive opinions on PIPs, 20 negative opinions on PIPs, and 135 opinions on full waivers. The three most frequent

therapeutic areas covered by PIPs were pulmonology-allergology (30%), oncology (12%), and cardiology (8%) [17].

3.2.4 Collateral measures

The Paediatric Regulation includes a number of *collateral measures* intended to improve information, transparency on paediatric studies and to stimulate research into paediatric medicines. The measures include (i) free scientific advice for paediatric studies at the EMA, (ii) a survey on drug use in children performed in all EU states (article 42), (iii) an inventory of paediatric therapeutic needs, (iv) public assess to paediatric studies in the EudraCT clinical trials database, (v) grants for paediatric studies on off-patent drugs (as special call in the EU 7th framework programme) and (vi) a European paediatric research network co-ordinated by EMA.

4 Points to consider for a Paediatric Investigation Plan

4.1 Paediatric indications

Defining the relevant paediatric indications for a drug is fundamental for each PIP and decisions on any deferrals or waivers. This must take into account the expected therapeutic benefit of the drug for children, the timing of paediatric development, and how much extrapolation is possible from adults to subsets of the paediatric population.

Whether a significant therapeutic benefit may be expected for the drug depends on the frequency and seriousness of the condition to be treated, the expected effect and safety issues of the drug, and the availability of alternative treatments for children. The need can be judged by referring to the *Inventory of Paediatric Therapeutic Needs* for several therapeutic areas which was compiled by Paediatric Working Party at the EMA [18]. Based on the current survey of actual drug use in children in all EU member states (mandated by the Paediatric Regulation, article 42), the inventory will be updated.

The timing of paediatric studies in relation to adult development depends on the urgency of paediatric need and safety issues regarding the drug. Paediatric development should start early if the drug targets a primary paediatric indication or a life-threatening disease, or if a significant therapeutic benefit is expected with no alternatives currently available. Paediatric development should start later if therapeutic alternatives already exist or if safety concerns for the drug mandate that more adult data should be available before children are exposed [19].

Extrapolation of data from adults to children or between paediatric age groups may allow avoiding certain studies in children. Possibilities for extrapolation should always be explored as performing unnecessary studies in children is ethically not acceptable. Whether extrapolation is possible depends on the similarity of indications between adults and children, similarity of disease (aetiology, manifestation, progression), response to treatment, and clinical outcome measures. For an example of extrapolation, see the PIP case study on clopidogrel.

The International Conference of Harmonization has defined the following *paediatric age groups* in relation to developmental stages (ICH E11): prematures (<37th wks of gestation), term newborns (age 0–27 days), infants and toddlers (age 2–23 months), children (age 2–11 years), adolescents (age 12–17 years). However, the choice of age groups for a specific PIP depends on the pharmacological properties of the drug and the target disease(s).

4.2 Child-appropriate formulations

There is a need for age-appropriate formulations that assure accurate dosing and reliable administration across all targeted paediatric age-groups [20]. Several formulations are usually needed for children of different ages. Oral administration is used most commonly and multiple oral dosage forms are available (solutions, syrups, suspensions, powders, granules, effervescent tablets, orodispersible tablets, chewable tablets, conventional immediate release and modified release tablets and capsules) that are suitable for different ages. A range of strengths is usually required to allow exact dosing. Color and taste must also be considered to optimize adherence in children. The toxicity of some excipients varies across paediatric age groups and between paediatric and adult populations, e.g. benzyl alcohol is toxic in the preterm newborn. The magnitude of doses used in neonates may be 100-fold lower than in adults. Therefore, injectable formulations should have appropriate drug concentrations to minimize the risk of medication errors.

4.3 Juvenile animal studies

The aim of non-clinical studies to support the development of drugs for children is to obtain information on potentially different safety profiles from those seen in adults [21, 22]. Standard non-clinical studies using adult animals, or safety information from adult humans, cannot always adequately predict these differences for all paediatric age groups. Juvenile animal studies should be considered when human safety data and previous animal studies are insufficient for a safety evaluation in the intended paediatric age group, for example if non-clinical studies indicate target organ or systemic toxicity relevant for developing systems, possible effects on growth and/or development in the target age group or if a pharmacological effect of

the drug will affect developing organ(s). Or if substantial differences between the adult and young populations with respect to pharmacokinetic characteristics of the active substance are indicated. In addition, potential differences between the mature and immature systems for the potential target organs must be taken into account, including whether the end-points investigated are similar and/or relevant for the intended paediatric population. Furthermore, effects related to delayed or altered development must be considered which may be evident even after treatment termination. Finally, novel aspects of the intended paediatric formulation may require additional safety data to support the specific formulation.

4.4 Clinical Pharmacology studies in children

Pharmacokinetic studies in children are performed to support formulation development and to determine pharmacokinetic parameters in different age groups to support dosing recommendations [23]. Children cannot be subjected to dose ranging studies as those used in adults, therefore some initial estimation of dose in paediatrics should be obtained via extrapolation approaches [8, 24]. *Physiologically-based PK models (PBPK)* use PK data from adults, existing PK data from children, and physiological information (organ size, compartments, enzyme activities, hepatic and renal function, etc.) for different ages [25]. PBPK models allow to map the complex mechanistic drug movements in the body to a physiologically realistic structure. Some drug specific input parameters can be obtained from in vitro studies. PBPK are used to estimate PK parameters and predict appropriate doses for different paediatric age groups. However, depending on the data input into the model, prediction will have variable degrees of uncertainty. In any case, PBPK predictions must be confirmed by real PK or PK–PD studies in all targeted paediatric age groups. However, by incorporating existing data, the model allows to significantly reduce the sample size of paediatric studies, and to estimate sampling schemes in advance. Paediatric PK and PD studies commonly use a stepwise approach, usually starting with adolescents and proceeding to younger age groups. Data generated in older age groups is incorporated into the PBPK model in staggered fashion, thereby gradually improving the prediction for younger age groups.

A useful approach to paediatric PK/PD studies is *Population PK modelling* [26]. In contrast to standard PK studies where large numbers of samples are taken at fixed time points, population PK approaches obtain few (sparse) samples at random time points from larger, more heterogenous populations. Population PK is usually applied in children during treatment with the drug, and may use incidental samples, i.e. obtained as part of routine blood sampling. The population PK approach requires larger patient groups to be studied but allows to analyse the influence of co-variates, e.g. age, on PK parameters, and thereby, to built prediction models, e.g. for younger age groups. Population PK models can be used in combination with PBPK models.

For a rational approach to clinical pharmacology studies in children, the Centre for Drug Evaluation and Research (CDER) at the US Food and Drug Administration has designed the *Paediatric drug development decision tree for types of PK–PD studies required in children* [27]. The decision tree recommends different types of paediatric studies based on existing knowledge of the disease and PK/PD of the drug in adults. The prerequisite to use adult data is similarity of disease and response to treatment between adults and children. If the PK–PD relationship of a drug is similar between adults and children or the PK–PD relationship can be determined, then only PK studies and safety studies are recommended for the bridging and dose determination. This approach is designed to minimize the number of children subjected to studies.

Practical aspects of paediatric PK/PD studies are barriers to blood collection in children and limited volumes and numbers of samples that can safely be obtained, particularly from small children. Sampling should be performed by experienced staff in child-friendly environment. Research blood samples should be taken during routine sampling where possible. To minimize pain, local anaesthetic cream and oral saccharose (in infants), and for repeated samples, indwelling lines should be used. Blood samples volumes can be minimized by use of microassays, or alternative samples (urine, exhaled air and saliva), biomarkers (stable isotopes) or biosensors (microdialysis). Sample numbers can be reduced by population PK approaches.

4.5 Clinical efficacy and safety studies

Clinical trial protocols as used in adults cannot simply be imposed on paediatric trials but protocols must be appropriately designed for children of different age groups [13, 19]. Key challenges of clinical studies in children are recruitment, including the consent process, child-appropriate study designs, validated age-appropriate endpoints, and assessment of long-term safety.

Recruitment of sufficient numbers of children into clinical trials is difficult because many paediatric diseases are rare and children are heterogenous with respect to age, development, and comorbidity. Therefore, inclusion and exclusion criteria must not be too restrictive. A major challenge is obtaining consent for study participation. Careful attention must be given to both the parents and the child in the consent/assent process to provide information in age-appropriate fashion and address any concerns. Compliance of children during study conduct can be improved by pragmatic, child-appropriate protocols that accommodate the needs of the child and its family. Study staff should be experienced in the care of children and study contacts should be in a child-friendly environment. Assessment schedules should allow flexibility and should be minimally invasive and burdensome.

Study endpoints appropriate for each age-group need to be developed and validated which may require separate studies. This is particularly challenging for patient-reported outcome measures which need to be adapted to the child's mental capacity. Subjective

endpoints, e.g. pain, cannot be assessed directly in small children but behavioural scales or proxy assessments are used instead.

Drugs may affect physical and cognitive growth and development, and the adverse event profile may differ in paediatric patients. The dynamic processes of growth and development may not manifest an adverse event acutely, but at a later stage of growth and maturation. Therefore, long-term safety studies or surveillance data, either while patients are on chronic therapy or during the post-therapy period, may be needed to determine possible effects on skeletal, behavioral, cognitive, sexual and immune maturation and development [19].

Case Study: Paediatric Investigation Plan for Clopidogrel

A Paediatric Investigation Plan for Clopidogrel hydrogen sulphate was submitted to the PDCO by the marketing authorization holder in 2007. Clopidogrel is a thienopyridine which selectively inhibits the binding of adenosine diphosphate (ADP) to its P2Y₁₂ platelet receptor and the subsequent ADP-mediated activation of the glycoprotein GPIIb/IIIa complex, thereby inhibiting platelet aggregation [28].

Clopidogrel is authorized in adults for the indications: *Prevention of atherothrombotic events in (i) patients suffering from myocardial infarction, ischaemic stroke or established peripheral arterial disease and (ii) patients suffering from acute coronary syndrome*. Since atherothrombosis is extremely rare in children, the applicants requested a waiver for the adult indication. Instead they proposed the following paediatric indication: *Prevention of shunt thrombosis in infants (0–12 mo) with cyanotic heart disease palliated with a systemic-to-pulmonary artery shunt*.

Systemic-to-pulmonary shunts are used in children with cyanotic congenital heart disease such as right heart obstruction (e.g. pulmonary atresia), or single ventricle anatomy (hypoplastic right or left heart syndrome) as a means to provide blood flow to the lung for oxygenation. These shunts (modified Blalock-Taussig shunt, Sano shunt, central shunt or ductal stents) are connections between the systemic circulation and the pulmonary vasculature of limited caliber which have a significant risk of narrowing and shunt thrombosis. Adequate shunt flow is vital for these children. Therefore, antiplatelet therapy, usually acetylsalicylic acid (ASA), is used as thromboprophylaxis by most centres [29].

The applicants chose to focus paediatric development of clopidogrel on children with systemic-to-pulmonary shunts as a paediatric model of thrombosis where the need for antiplatelet therapy is supported by existing data and reflected in guidelines [30]. Moreover, this is a relatively homogenous paediatric population with a frequency of outcome events sufficiently high to make a clinical trial

feasible. A waiver was requested for the proposed indication for children from 1 to 18 years, as the indication prevention of shunt thrombosis was considered specific to neonates and infants. This paediatric development was already ongoing in the context of an FDA paediatric written request [31].

The PIP proposal included the following measures [32]:

1. Development of a paediatric liquid oral solution of clopidogrel.
2. Relative bioequivalence study of the authorized 75 mg tablet vs. 75 mg oral solution in healthy adults.
3. Dose-finding and pharmacodynamic study assessing platelet aggregation inhibition with clopidogrel in children 0–24 months of age at risk for thrombosis.
4. Clinical efficacy and safety of clopidogrel in children 0–12 months of age with systemic-to-pulmonary shunts.

The dose-finding and PD study (#3) had been completed at the time of PIP submission and its results were published in 2008 [33]. The PICOLO trial was a prospective, multicenter, randomized, placebo-controlled trial evaluating the pharmacodynamics of clopidogrel in children (0–24 months) with a condition at risk for arterial thrombosis, e.g. systemic-to-pulmonary shunts, Kawasaki syndrome, intravascular stent. The objectives of the study were to (i) determine the dose of clopidogrel needed in infants and young children to achieve a mean 30% to 50% inhibition of platelet aggregation (i.e. similar to that observed with 75 mg in adults) and (ii) assess the safety and tolerability of clopidogrel in infants and young children. Patients were randomized to clopidogrel versus placebo in sequential dose groups. Platelet aggregation was assessed at baseline and steady state. Ninety-two children were randomized, and 73 completed the study. A total of 79% of patients were taking aspirin. Compared with placebo, clopidogrel at the highest dose resulted in platelet inhibition as targeted. No serious bleeding events occurred. The study conclusions were that clopidogrel 0.2 mg/kg/d in children 0–24 months of age achieves a platelet inhibition level similar to that in adults taking 75 mg/d. Clopidogrel is well tolerated in infants and young children at this dose.

The clinical efficacy and safety study of clopidogrel in children with systemic-to-pulmonary shunts is still ongoing as of today and no details can be provided.

The PDCO viewed the PIP indication, prevention of systemic-to-pulmonary shunt thrombosis, as a good paediatric model indication for antiplatelet therapy and addressing a significant paediatric need. The PDCO also agreed with the waiver for the adult indication (atherothrombosis) because of its rarity in childhood. However, the PDCO did not agree with a waiver for development of clopidogrel for children

from 1 to 18 years. First, the PICOLO study had actually included children up to the age of 2 years and generated dosing information for this age range. Second, systemic-to-pulmonary shunts may remain in place beyond 12 months of age in some patients, and the PDCO requested that clopidogrel treatment should be evaluated in these patients. Third, there is a variety of other paediatric conditions where antiplatelet therapy is currently used but systematic evidence on efficacy and safety is lacking. These conditions include Fontan shunts (total cavo-pulmonary anastomosis), intravascular stents, Kawasaki syndrome with coronary aneurysms, and paediatric stroke, which affect all paediatric age ranges. The PDCO considered that clopidogrel could be of significant benefit in these conditions. However, each of these conditions is so rare that clinical efficacy studies would be highly challenging or unfeasible. Therefore, an additional PD study was requested in children aged 2 to <18 years requiring antiplatelet therapy for any reason, e.g. shunts, stents, Kawasaki syndrome, stroke. This was understood as bridging study between adults and children up to 2 years. Given the efficacy of clopidogrel in adults and on the condition that efficacy could be shown in children with systemic-to-pulmonary shunt, clinical efficacy in children 2 to <18 years would be extrapolated based on demonstrating similar PD effects.

The final agreed PIP contained the following studies, in addition to the proposed measures listed above [32]:

5. Clinical safety extension phase of study #4 for children aged 1 year and older.
6. Pharmacodynamic, safety, and descriptive efficacy study of clopidogrel in children aged 2 to less than 18 years at risk of thrombosis.

In summary, this PIP is an interesting example for the following reasons: The adult indications for clopidogrel (atherothrombosis) are practically non-existent in children and, accordingly, a waiver was granted. At first sight, there would appear to be no therapeutic benefit for children. The applicant proactively proposed to develop clopidogrel for a completely different paediatric indication (prevention of thrombosis in infants with systemic-to-pulmonary shunts) as model for antiplatelet therapy in children and addressing a significant paediatric need. The PIP included development of a paediatric liquid formulation for use down to neonatal age, a dose-finding and PD study (in children up to 2 years), and a pivotal efficacy and safety study in children with systemic-to-pulmonary shunts up to 1.5 years of age. This development left a gap regarding children 2 to <18 years of age in whom antiplatelet therapy is used in several other conditions too rare to allow separate paediatric studies. Therefore, a PD and safety study in children 2 to <18 years at risk of thrombosis was added, bridging between adults and children up to 2 years.

No formal efficacy study was requested in this setting for feasibility reasons. Thus, the PIP is also an interesting example for the concept of extrapolation of clinical efficacy in certain paediatric age groups.

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References

1. Wilson JT (1999) An update on the therapeutic orphan. *Pediatrics* 104: 585–590
2. Turner S, Gill A, Nunn T, Hewitt B, Choonara I (1996) Use of ‘off-label’ and unlicensed drugs in paediatric intensive care unit. *Lancet* 347: 549–550
3. Conroy S, McIntyre J, Choonara I (1999) Unlicensed and off label drug use in the neonate. *Arch Dis Child Fetal Neonatal* Ed 80: F142–F145
4. Conroy S, Choonara I, Impicciatore P, Mohn A, Arnell H, Rane A, Knoepfel C, Seyberth H, Pandolfini C, Raffaelli MP, Rocchi F, Bonati M, Jong G, de Hoog M, van den Anker J (2000) on behalf of the European Network for Drug Investigation in Children. Survey of unlicensed and off label drug use in paediatric wards in European countries. *BMJ* 320: 79–82
5. ‘T Jong GW, Eland IA, Sturkenboom MC, van den Anker JN, Stricker BH (2002) Unlicensed and off-label prescription of drugs to children: a population based cohort study. *BMJ* 324: 1313–1314
6. Lindell-Osuagwu L, Korhonen MJ, Saano S, Helin-Tanninen M, Naaranlahti T, Kokki H (2009) Off-label and unlicensed drug prescribing in three paediatric wards in Finland and review of the international literature. *J Clin Pharm Ther* 34: 277–287
7. Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE (2003) Developmental pharmacology: drug disposition, action, and therapy in infants and children. *N Engl J Med* 349: 1157–1167
8. Johnson TN (2005) Modelling approaches to dose estimation in children. *Br J Clin Pharmacol* 59(6): 663–669
9. Sutherland JM (1959) Fatal cardiovascular collapse of infants receiving large amounts of chloramphenicol. *AMA J Dis Child* 97: 761–767
10. Horen B, Montastruc J, Lapeyre-Mestre M (2002) Adversedrug reactions and off-label drug use in paediatric outpatients. *Br J Clin Pharmacol* 54: 665–670
11. Turner S, Nunn AJ, Fielding K, Choonara I (1999) Adverse drug reactions to unlicensed and off-label drugs on paediatric wards: a prospective study. *Acta Paediatr* 88: 965–968
12. European Parliament (2001) Directive 2001/20/EC of the European Parliament and Council of 4 April 2001. *Official Journal of the European Communities* L121: 34–44

13. Kauffman RE (2000) Clinical trials in children: problems and pitfalls. *Paediatr Drugs* 2: 411–418
14. US Food and Drug Administration (2007) Pediatric Research Equity Act (PREA) and Best Pharmaceuticals for Children Act (BPCA). <http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/DevelopmentResources/UCM049870.pdf> (accessed 2010-04-24)
15. Benjamin DK Jr, Smith PB, Murphy MD, et al. (2006) Peer-reviewed publication of clinical trials completed for pediatric exclusivity. *JAMA* 296: 1266–1273
16. Regulation (EC) No1901/2006 and 1902/2006 of the European Parliament and of the Council of 12 December 2006 on medicinal products for paediatric use (2006). Paediatric regulation. Official J Eur Union 18: L378/1
17. European Medicines Agency, PDCO press release March 2010, <http://www.ema.europa.eu/pdfs/human/pdco/15139110en.pdf> (accessed 2010-04-24)
18. European Medicines Agency (2007) Inventory of Paediatric Needs. <http://www.emea.europa.eu/htmls/human/paediatrics/inventory.htm> (accessed 2010-04-24)
19. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (2000) Clinical Investigation of Medicinal Products in the Pediatric Population, E11
20. European Medicines Agency, Committee for Medicinal Products for Human Use (2006) Reflection paper: formulations of choice for the Paediatric population, EMEA/CHMP/PEG/194810/2005
21. European Medicines Agency, Committee for Human Medicinal Products (2008) Guideline on the Need for Non-clinical Testing in Juvenile Animals on Human Pharmaceuticals for Paediatric Indications, EMEA/CHMP/SWP/169215/2005
22. De Schaepdrivjer LM, Bailey GP (2009) Preclinical juvenile toxicity assessments and study designs. In: Mulberg AE, Silber SA, van den Anker JN (eds.) *Paediatric Drug Development, Concepts and Applications*. Wiley-Blackwell, Hoboken, New Jersey
23. European Medicines Agency, Committee for Medicinal Products for Human Use (2006) Guideline on the role of pharmacokinetics in the development of medicinal products in the paediatric population. EMEA/CHMP/EWP/147013/2004
24. Manolis E, Pons G (2009) Proposals for model based paediatric medicinal development within the current EU regulatory framework. *Br J Clin Pharmacol* 68: 493–501
25. Björkman S (2004) Prediction of drug disposition in infants and children by means of physiologically based pharmacokinetic (PBPK) modelling: theophylline and midazolam as model drugs. *Br J Clin Pharmacol* 59: 691–704
26. Anderson BJ, Allegaert K, Holford NH (2006) Population clinical pharmacology of children: general principles. *Eur J Pediatr* 165: 741–746
27. US Food and Drug Administration, CDER. Pediatric Decision Tree. <http://www.fda.gov/cder/mapp/4000.4.pdf> (27 April 2004)
28. Savi P, Nurden P, Nurden AT, Levy-Toledano S, Herbert JM (1998) Clopidogrel: a review of its mechanism of action. *Platelets* 9: 251–255
29. Li JS, Yow E, Berezny KY, Rhodes JF, Bokesch PM, Charpie JR, Forbus GA, Mahony L, Boshkov L, Lambert V, Bonnet D, Michel-Behnke I, Graham TP, Takahashi M, Jaggars J, Califf RM, Rakhit A, Fontecave S, Sanders SP (2007) Clinical outcomes of palliative surgery including a systemic to–pulmonary artery shunt in infants with cyanotic congenital heart disease: does aspirin make a difference? *Circulation* 116: 293–297
30. Monagle P, Chalmers E, Chan A, deVeber G, Kirkham F, Massicotte P, Michelson AD (2008) Antithrombotic therapy in neonates and children: American college of chest guidelines (8th edition) physicians evidence-based clinical practice. *Chest* 133: 887–968
31. US Food and Drug Administration (2010) Written requests issued. <http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/ucm050002.htm> (accessed 2010-04-24)

32. European Medicines Agency (2008) Clopidogrel (Plavix) PDCO opinion, EMEA-000049-PIP01-07-M01 P/122/2008. <http://www.ema.europa.eu/htms/human/paediatrics/decisions.htm> (accessed 2010-04-24)
33. Li J, Yow E, Berezny KY, Bokesch PM, Takahashi M, Graham TP, Sanders SP, Sidi D, Bonnet D, Ewert P, Jennings LK, Michelson AD, for the PICOLO Investigators (2008) Dosing of clopidogrel for platelet inhibition in infants and young children: primary results of the Platelet Inhibition in Children On cLOpidogrel (PICOLO) Trial. *Circulation* 117: 553–559

Further reading

Rose K, van den Anker JN (eds.) (2007) *Guide to Paediatric Clinical Research*. Karger, Basel

Mulberg AE, Silber SA, van den Anker JN (eds.) (2009) *Paediatric Drug Development, Concepts and Applications*. Wiley-Blackwell, Hoboken, New Jersey

Yaffe SJ, Aranda JV (eds.) (2005) *Neonatal and Pediatric Pharmacology, Therapeutic Principles in Practice*. Lippincott Williams & Wilkins, Philadelphia

Jacqz-Agrain E, Choonara I (eds.) (2006) *Paediatric Clinical Pharmacology*. Fontis Media, Lausanne, and Taylor & Francis, New York

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