Markus Müller *Editor*

Clinical Pharmacology: Current Topics and Case Studies

Second Edition

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Part I

Introduction

The Discipline of Clinical Pharmacology

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Markus Müller

In its chapter about "Principles of Clinical Pharmacology," Harrison's textbook *Principles 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 clinical pharmacology was established in several countries as an academic discipline about 40 years ago. Whereas clinical pharmacology was established as a clinical subdiscipline 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 substantial 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 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 USA and the European Association for Clinical Pharmacology and Therapeutics (EACPT) in Europe.

ASCPT stated its vision as follows: "Clinical pharmacology is recognized and serves as the premier discipline at the forefront of the discovery, development,

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regulation, and use (DDRU) of safe and effective medications necessary for the prevention and treatment of illness," whereas the American College of Clinical Pharmacology (ACCP) states: "Promotion of rational use of medications in humans: Innovative research, development and regulation of medications and Education of health care professionals and patients on the optimal utilization of medications." 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 healthcare 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*, *Journal of Clinical Pharmacology*, *British Journal of Clinical Pharmacology*, *European Journal of Clinical Pharmacology*, or the *International Journal of Clinical Pharmacology*.

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 the 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 toward 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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Current Issues in Drug Development

2

Markus Müller

2.1 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 prolongation in the life span of patients infected with HIV by combinating highly active antiretroviral therapies (HAART), (2) the recent breakthroughs in treating hepatitis C by directly acting antiviral agents, (3) the reduction of gastric ulcer due to therapies aiming at the eradication of *Helicobacter pylori* and (4) targeted therapeutic approaches for chronic myeloid leukaemia (CML), kidney cancer or chronic lymphoid leukaemia by means of specific kinase inhibitors. Whereas success rates in (1) and (2) are determined by a combination of powerful drugs which were rapidly developed by industry in response to the challenge posed by the HIV and HCV pandemics, success rates in (3) 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 (4) by intense efforts to address molecular aberrations responsible for unregulated cell growth, e.g. by the fusion protein kinase bcr-abl in CML.

These 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. Given a total number of >20,000 drug products available, 2/3 of these target ten gene families (see Table 2.1) [1]. Interestingly, however, there are

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Table 2.1 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 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
Modified from Ref. [1]
GPCR G-protein-coupled receptor

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].

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 and also success rates for many common cancers are still far from satisfying. Recent years, in particular, have seen major breakthroughs in areas of unmet medical needs, e.g. for the treatment of hepatitis (e.g. sofosbuvir); novel anticoagulation strategies by factor Xa (e.g. rivaroxaban) and II inhibitors (e.g. dabigatran); targeted approaches for rare diseases, e.g. for haemolytics uremic syndrome (e.g. efalizumab); and oncological diseases like kidney and prostate cancer, melanoma and leukemias (e.g. sunitinib, abiraterone, ipilimumab, ibrutinib).

2.2 The Dawn of the Molecular Era, the 'Druggable Genome' and the 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 2.2), this data also led to an important stimulus in drug research. Moore's law for the inflation of computer memory was even surpassed by the increase in sequencing capacities in the last years. Whereas sequencing costs were approximately

Table 2.2 Comparison of the druggable genomes of selected eukaryotes

	Homo sapiens	*Drosophila melanogaster*	*Caenorhabditis elegans*	*Saccharomyces cerevisiae*
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]

1 US$ per base pair in the 1990s, i.e. three billion US$ per genome, this number has come down by a factor of $>10^6$ approaching 1000 US$ per genome in 2015. This dynamic comes hand in hand with the 'big data' revolution and will continue to have profound influences on medicine and drug development by enabling the vision to perceive human beings as 'data sets'.

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].

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 quickly 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 [5]. One notable example is the case of PCSK9, a crucial regulator in LDL metabolism, which was identified in GWAs and has become a prime drug target for atherosclerosis therapies. Overall, genomic medicine has yet failed to provide a 'quick fix' for drug development although an indirect influence of genomics on drug development is clearly visible [6].

Twenty 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'. 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 ('niche busters', 'orphan drugs') and a superior economic growth of companies which have adopted this trend early on (personal communication from IMS health). One indicator for the niche buster concept is the robust growth of oncological pipelines for various subforms of cancer with 397 targets in drug development and an average number of 2–6 drugs per novel target [7].

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. Randomised 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, 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 twentieth century, which has influenced the last century.

2.3 Innovation and Stagnation

The years 1990–2000 have witnessed an increasing focus on codification of every minor technical step – a situation which has produced a 'false sense of control' over drug development [8]. In contrast to this perceived situation of total control, the general view until recently was that drug development faced a crisis in productivity.

An immediate reaction was the steep increase in mergers and acquisitions and an increased activity in noncore 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 [9]. 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 [10]. 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. FDA's 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 and a perceived scientific and regulatory attitude of risk aversion in drug development [11] aimed for radical changes in drug development and approval processes. Besides various 'molecular' and '-omics' approaches, there was an increasing focus on efficacy and toxicology biomarkers and imaging technology to foster drug research. At the level of drug regulation, an increasing awareness emerged that traditional ways of judging drugs and granting market authorization may be outdated and may be one reason for the lack of productivity. Although no leading approach has succeeded so far, the need for an entirely novel conceptual framework is undisputed and also reflected by regulatory documents.

Interestingly, it seems that 'nothing that companies have done has affected their rates of new drug production' [8]. The decline following 1996 was therefore sometimes interpreted as a return to historical normality following an atypical increase in the early 1990s, rather than an absolute decline (Fig. 2.1). What is clear is that from the mid-1990s onwards, development costs have risen steadily, but the number of new chemical entities (NCEs), which have been developed, was in a sharp decline, from a high of 51 in 1996 to a low of 21 in 2005. This trend seems to face a recent reversal [12, 13]. However, when also considering spending per NCE, productivity is still relatively low and seems to follow a trend which – in analogy to 'Moore's law' – has been named 'Eroom's law', i.e. Moore in reverse [14].

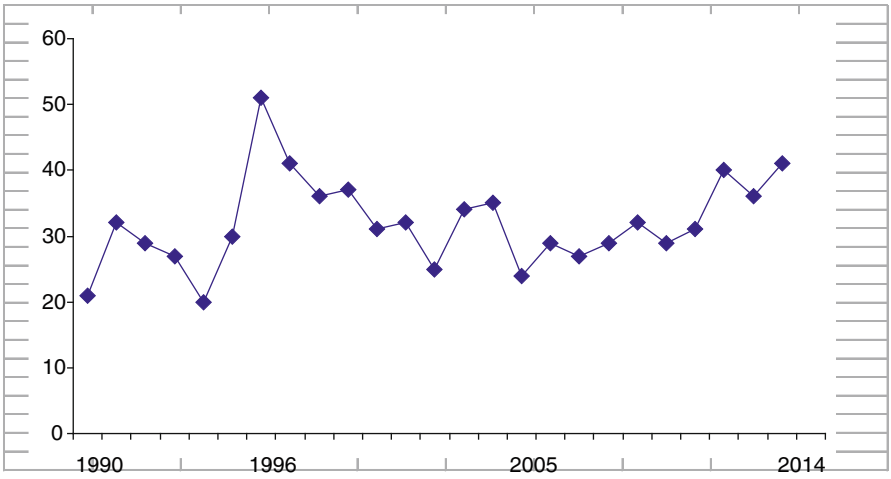

Fig. 2.1 Timeline of approvals of new molecular entities (nMEs) and new biological entities (nBEs) by the US Food and Drug Administration (FDA) between 1990 and 2014. From: http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugInnovation/ucm430302.htm and Ref. [12]

2.4 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, 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, EBM has rightfully raised the bar for successful drug therapy and was wholeheartedly embraced by regulators and reimbursement agencies.

2.5 Issues in Preclinical and Clinical Drug Development

Target 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, automatised assays and large-scale data [15]. 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 [15]. It is likely that future finding strategies will be much more project related, tailor-made and better integrated into the broader drug discovery efforts [15].

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 [16]. Likewise current tools to assess carcinogenicity are under discussion, and there is an agreement that genotoxicity tests in vitro are not very specific and produce a high and unacceptable occurrence of irrelevant positive results [17]. 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. 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 tarnished by preclinical data showing signs of toxicity.

Clinical drug development: The structure of clinical drug development has changed significantly in the last years, both conceptually [18, 19] and structurally [20, 21]. 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 organisations (CROs) and site management organisations (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 [20]. 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. A European Medical Research Council (EMRC) position paper [22] lists the top five recommendations to strengthen independent clinical trials in Europe: (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 authorisation for IDCT and (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'.

One key problem of pharmaceutical industry productivity is the increasing cost of conducting large clinical trials. The way clinical trials are conducted nowadays is determined by large trials with clinical end points which are rigid from a design perspective, are 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, Bayesian methodologies, network and pathway analysis, basket trial approaches and individualised treatment protocols to increase flexibility and maximise the use of accumulated knowledge could have an important role in achieving these goals [18, 19, 23, 24]. 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 legislative framework for patient-centred clinical research and (3) declining willingness of the public to pay

Table 2.3 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	Granting of additional (beyond current) extension of exclusivity for predefined high-need, high-risk areas	Achieving consensus independent of commercial and other pressures is key
No encouragement to make a paradigm shift rather than replicative strategies	Use of biologic markers and surrogate markers possible but with limits described above	Use of an independent body such as NAS or IOM[a] to define high-need, high-risk areas Number of designated high-need, high-risk areas restricted to 5–10

Reproduced and adapted from Ref. [21]
[a]NAS denotes the National Academy of Sciences, and IOM the Institute of Medicine [21]

for costly pharmaceuticals in light of available (bio)generics, there has also been a steep increase in regulatory demands on drug development, and there is ongoing discussion on reshaping the drug approval process radically [21], e.g. by granting limited period of exclusivity and an emphasis on post marketing commitments on drug safety and efficacy (Table 2.3).

2.6 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 [25]. 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 [26, 27] and scenarios where academic medicine only flourishes in a private sector as a commercial business activity or succeeds by the public and media certainly not desirable.

2.7 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 [28]. 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* [29] 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 preapproval cost estimate of $ 802 Mio [30].

Conclusion

We currently witness at the end of 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 and new concepts are gradually starting to emerge. International initiatives like IMI, the adoption of novel tools by

regulators, increased cooperation and the fast developing new IT and '-omics' technologies will shape the new landscape of drug development.

Case Study: Pfizer and Gilead

Pfizer (www.pfizer.com) was founded by two German cousins, Charles Pfizer and Charles Erhart in 1849 in Brooklyn, New York, and is currently among the largest biopharmaceutical companies worldwide with revenues of ~ $ 50 billion. During WWII Pfizer became the world's largest producer of penicillin. Important drugs developed by Pfizer are fluconazole, an antifungal; amlodipine for control of hypertension; the antibiotic azithromycin; ziprasidone, a new antipsychotic; voriconazole and anidulafungin, two antifungals; pregabalin, for treatment of neuropathic pain; sunitinib, an oral multikinase inhibitor; and maraviroc, an HIV drug. 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, originally developed by Parke-Davis-Warner-Lambert, became the number 1 branded pharmaceutical worldwide. In September 2008 after a failed $1 billion investment in the trial programme for torcetrapib, an LDL-lowering and HDL-inducing compound, Pfizer, according to a *Wall Street Journal* report, intended to drop efforts to develop medicines for heart disease 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.

Gilead (www.gilead.com) is currently one of the fastest growing companies. It was founded in 1987 by a 29-year-old medical doctor, Michael L. Riordan, and has focused on difficult to treat viral infections like HIV, hepatitis or influenza. Gilead was listed on the NASDQ in 1992. In 1996 cidofovir was launched for CMV infections. Tamiflu, an influenza antiviral, originally discovered by Gilead, was licensed to Roche and achieved approval in 1999. Tenofovir, an HIV drug, was approved in 2001. A major breakthrough for the treatment of hepatitis C was the launch of sofosbuvir (Sovaldi), an extremely costly pharmaceutical with a price ranging between approx. $80,000 and $150,000 for a single course of treatment. The pricing of sofosbuvir has led to worldwide controversy as it may be expected to account, for example, for approx. 20 % of the entire German drug market. In September 2014, Gilead announced that generic versions of sofosbuvir could be sold in several developing countries for a price below $1000.

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Current Issues in Drug Regulation

3

Christa Wirthumer-Hoche and Brigitte Bloechl-Daum

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 authorisation 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 summarises 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.

3.1 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 medicinal product has to pass on

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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]

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).

Drugs are approved by regulatory agencies on the basis of their assessment of whether the available evidence indicates that the benefits of the drug outweigh its risks. Regulatory agencies have been criticised either for being overly tolerant of risks or being excessively risk averse, which reflects the challenge in determining an appropriate balance between benefit and risk with the limited data that is typically available before drug approval. The negative consequences of regulatory tolerance in allowing drugs onto the market that turn out to be unsafe are obvious, but the potential for adverse effects on public health owing to the absence of new drugs because of regulatory risk aversion is less apparent as risk aversion comes with its own risks. A drive towards an excessive focus on avoiding risks and uncertainties will mean that patients pay a price: delay in accessing therapeutics and lost therapeutic options. Good drug regulation is more than just minimising risks; it is about maximising gains in public health [5].

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 3.1). Setting the regulatory evidence requirements very high might not only

Table 3.1 The regulator's dilemma

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

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 authorisation, which determines the time at which patients gain access to new medicinal products. 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 [3, 4]
HTA health technology assessment

stifle innovation but could also delay or inhibit patients' access to effective treatment. Pharmaceutical industry and some patient advocacy groups strongly emphasise the point that these are undesirable consequences, particularly in therapeutic areas characterised 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 authorised 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 authorised in developed countries are debatable. It is difficult to predict how the regulators' dilemma will play itself out in the years ahead.

The Innovative Medicines Initiative (IMI) [6] is a public–private partnership established by the European Union and the European Federation of Pharmaceutical Industries and Associations (EFPIA). The core mission of IMI is to address many diverse issues in order to foster pharmaceutical innovation for the benefit of citizens, as well as enhance the competitiveness of the healthcare sector in Europe. Since 2008 IMI has catalysed the formation of many consortia to address challenges in drug development and regulation. With the recent launch of its second phase, which will run until 2024, the IMI will have committed more than €5 billion to create multi-stakeholder, cross-disciplinary consortia.

The adaptive pathway approach (formerly known as 'adaptive licensing') is part of the European Medicines Agency's (EMA) efforts to improve timely access for patients to new medicines.

The concept of adaptive pathways foresees either an initial approval in a well-defined patient subgroup with a high medical need and subsequent widening of the indication to a larger patient population, or an early regulatory approval (e.g. conditional approval) which is prospectively planned, and where uncertainty is reduced through the collection of post-approval data on the medicine's use in patients.

This approach is particularly relevant for medicines with the potential to treat serious conditions with an unmet medical need and may reduce the time to a medicine's approval or to its reimbursement for targeted patient groups. It involves balancing the importance of timely patient access with the need for adequate, evolving information on a medicine's benefits and risks [7, 8].

3.2 Authorising 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 weight. Currently, only around 30 medicinal products containing a new active substance (NAS; the definition includes new chemical and new biological entities) are authorised 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. 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.

Negative clinical outcome results seem to contribute most significantly to current non-approval rates. Relevant learning-phase studies are valuable in reducing the number of failed dossiers and speeding up pharmaceutical innovation [9]. Drug developers are encouraged to increase investments in such studies before moving to large and more costly phase III trials.

For medicinal products containing a NAS, evaluation of safety and efficacy is paramount. However, when assessing generics, the main issues are quality and bioequivalence [10]. This topic is addressed in more detail in Chap. 23. Another rather hard to delineate area refers to so-called biosimilars. Biosimilars are replicas of authorised biologicals. As biologicals are large complex molecules, the bioequivalence approach as mentioned above is not sufficient. Biosimilars never will be identical with the originator, and like small molecules, they are comparable to the originator; therefore, comparability studies have to be performed. Apart from quality data, the applicant also needs to submit clinical data on efficacy and to a certain extent also on safety and immunogenicity.

There are currently four regulatory pathways how a medicinal product can obtain a market authorisation in the EU. On one end of the regulatory spectrum is the centralised procedure where a single submission of a marketing authorisation application is followed by a single assessment procedure and – if favourable – results in a single marketing authorisation valid in all EU member states. On the other end is a purely national process. In between are the mutual recognition procedure and the decentralised procedure.

3.2.1 The Centralised Authorisation

The legal time frame for an authorisation procedure in the EU typically takes 210 days, excluding a clock-stop period where the marketing authorisation 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 authorisation procedure (Table 3.2) [11]. The advantage of this procedure is that the best available expertise in Europe can be acquired and that an approach fully harmonised across all member states can be established. The advantage for pharmaceutical companies is a single point of entry and that finally authorisation is issued by the European Commission which is binding for all member states.

The central authorisation procedure is coordinated by the European Medicines Agency (EMA). The working body, which assesses the marketing authorisation dossier, elaborates the opinion and recommends 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

Table 3.2 Medicinal products requiring a central authorisation 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
Autoimmune diseases and other immune dysfunctions
Viral diseases
(c) Medicinal products that are designated as orphan medicinal products [Regulation (EC) No 141/2000]

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 and pharmacovigilance in the Pharmacovigilance Risk Assessment Committee (PRAC) and also in the Committee for Advanced Therapies (CAT) [12].

At predefined time points, the applicant receives a list of questions, which need to be addressed 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 authorisation. Therefore, procedures for provision of scientific advice by regulatory agencies to sponsors of development programmes for medicinal products have been established both in the EU and USA. The EU scientific advice procedure is carried out by the Scientific Advice Working Party (SAWP) 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 [13]. Sponsors of a medicinal product development programme can discuss the suitability of their planned development including details of non-clinical and clinical study designs, as well as pharmaceutical quality-related questions. The majority of requests for scientific advice refer to phase 3 clinical trials.

3.2.2 The Mutual Recognition Procedure (MRP) and the Decentralised Procedure (DCP) [14]

If a new medicinal product is not legally required to go through the central authorisation procedure, companies can choose to obtain marketing authorisation via the mutual recognition procedure or a decentralised procedure. The mutual recognition procedure is used when a product is already authorised in one EU member state via

the pure national 210 days procedure, and the company intends to extend its marketing authorisation to other EU member states. When using this procedure, the company selects an EU member state who has already granted the marketing authorisation to act as the so-called reference member state (RMS). All other EU member states included in the procedure are so-called concerned member states (CMS). It is the volunteer decision of the company how many and which EU member states they want to include in the procedure. This flexibility is a big advantage of the procedure.

The reference member state's competent authority forwards the assessment report, normally updated, to the concerned member states' agencies and then may take up to 90 days for their assessment. CMS can ask questions, but no further clock-stop is possible within this 90 days. In case no agreement is reached during the MRP, a referral has to be started in order to clarify the open issues.

If the product is not authorised in any of the member states, the company may select the decentralised procedure. Here the applicant selects a reference member state and concerned member state 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 harmonisation.

In 2013 a total of 207 MRP and 1052 DCP were handled within the EU member states. The figures for 2014 are 249 MRP and 797 DCP, so numbers of procedures for MRP are decreasing, and numbers for DCP are increasing [15]. 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.

3.2.3 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 granted 193 (2012), 124 (2013) and 60 in 2014. The numbers are decreasing [16]. 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 authorisations 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 generic applications, and this as well is in the interest of public health, because without generics the health system won't be affordable.

3.3 Regulatory Life Cycle Management of Medicinal Products

Each medicinal product has to be launched within 3 year after receiving the marketing authorisation which has been granted; otherwise, the marketing authorisation is cancelled. Further each marketing authorisation has to be renewed once after 5 years.

Once on the market, a medicinal product undergoes, on average, about three regulatory life cycle changes per year, so-called variations. Two of these are usually minor, such as a change of the market authorisation holder's address or, say, minor changes in the quality documentation, 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, or a major change in the manufacturing process.

In the following we will focus on products containing NAS, which in the EU are mainly authorised through the centralised 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 completely. Second, we have only information on efficacy but not effectiveness.

The regulatory life cycle for centrally authorised products is described on the EMA's homepage in the section on European public assessment reports [17].

3.3.1 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 (premarketing) clinical trials. Efficacy data are typically considered when regulators make their first-time benefit–risk assessment and are the basis of marketing authorisation. By contrast, 'Effectiveness is the extent to which an intervention does more good than harm when provided under the usual circumstances of healthcare practice' [18]. The distinction is relevant as it addresses the well-described efficacy–effectiveness gap, implying that treatment with a medicinal product usually yields better results in the controlled environment of clinical trials than under the conditions of usual care [19]. 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 maximise benefits while minimising risks.

Moreover, there is only scarce information on relative effectiveness at the time of marketing authorisation. Relative effectiveness (called comparative effectiveness in the current debate in the USA) 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' [20] and information on post-marketing relative effectiveness is increasingly demanded by patients and healthcare decision-makers [21].

Note that relative effectiveness may mean more than comparing two medicinal products. In some therapeutic situations, there may be drug and nondrug 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 [22]. 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.

3.3.2 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 authorisation [23].

According to the EU/726/2004, Art 57(2) [24], a safety database has to be built up, including all marketing authorisations available on the EU market, independent by which authorisation procedure authorised.

Art. 57(1)l: 'creating a database on medicinal products, to be accessible to the general public, and ensuring that it is updated, and managed independently of pharmaceutical companies; the database shall facilitate the search for information already authorised for package leaflets; it shall include a section on medicinal products authorised for the treatment of children; the information provided to the public shall be worded in an appropriate and comprehensible manner; (2). The database provided for in paragraph 1(l) shall include the summaries of product characteristics, the patient or user package leaflet and the information shown on the labelling. The database shall be developed in stages, priority being given to medicinal products authorised under this Regulation and…'

In case of a pharmacovigilance issue with a medicinal product containing a particular active substance, all in the EU-authorised medicinal products containing this active substance can be identified and assessed within a single procedure.

Since the new Pharmacovigilance Regulation EC/1235/2010, which came into effect in July 2012 and was the biggest change to the regulation of human medicines in the European Union (EU) since 1995, more emphasis is put on individual reporting [25].

For statistical reasons less frequent adverse drug reactions can only be detected after market authorisation, 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 underreporting rate higher than 90 % [26]. Some adverse events may remain unreported if left to healthcare providers only. Therefore, the new pharmacovigilance legislation EU/1235/2010 has extended the concept to include 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 [27], 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, and this is one of the main tasks of the PRAC

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 authorised products: the PRAC advises the CHMP as well as the Coordination Group for MRP and DCP – human 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 (SmPC) 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 3.3 we describe, as an example, the work and regulatory actions by the Austrian agency related to and triggered by pharmacovigilance issues [10].

Table 3.3 Regulatory actions by the Austrian agency related to and triggered by pharmacovigilance activities

	2012	2013	2014
Case reports originating from Austria	5.490	7.414	7.964
Change of SmPC and PIL	1.614	1.071	2.038
Quality defects	309	323	273
Recalls of medicinal products/batches	32	31	49
Public letters to healthcare providers	17	28	32

3.3.3 Risk Management Plans (RMPs)

Recognising the largely reactive and spontaneous nature of conventional pharmacovigilance, recent EU pharmacovigilance regulation introduced the concept of risk management strategy [28]. This has resulted in a requirement for industry to submit, under defined conditions and at the time of application, for a marketing authorisation: '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 an RMP, 'a set of pharmacovigilance activities and interventions designed to identify, characterise, prevent or minimise 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 minimisation activities and, where applicable, a risk minimisation 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 randomised controlled studies, pragmatic clinical trials, registries and various types of observational studies [29]. Risk minimisation 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 medicine regulation. The future challenge will be to communicate to all stakeholders the knowledge gained from the RMP activities to the benefit of public health.

3.3.4 When Should a Medicinal Product Be Authorised?

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. The case as such happened several years ago but still is a good and valid example as since that time this has not happened again.

This product's efficacy was moderate, but it was intended for patients with a disabling, though nonfatal, 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 authorisation. These ranged from listing additional adverse effects in the summary of the product characteristics to issuing special warnings and, finally, suspension of the marketing authorisation in February 2009: the modest effect in a usually nonfatal 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

authorisation, this risk potential was not known. To detect the risk of PML before marketing, authorisation 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 authorised 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 [30].

Psoriasis vulgaris is a chronic, inflammatory skin disorder that affects 0.5 % up to 3 % of the world's population. It is a T-cell-mediated immune disorder in which CD4 þ and CD8 þ memory T cells stimulate the hyper proliferation 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 humanised monoclonal immunoglobulin G1(IgG1) antibody with immunmodulatory properties. It binds specifically to the CD11, a subunit of LFA-1 (lymphocyte function-associated antigen-1, a leukocyte cell surface protein), and inhibits the binding of LFA-1 to ICAM-1, ICAM-2 and ICAM-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 upregulated 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 authorisation, 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 authorisation was suspended in the EU, and the company withdrew voluntarily Raptiva's market authorisation from the US 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 patient's life, usually not being life-threatening, and there were increasing safety issues. Being a selective immunosuppressant, the risk of opportunistic infections is increased [18], 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 authorised for the treatment of relapsing remitting multiple sclerosis in defined patients [31], and rituximab (Mabthera) is authorised for the treatment of patients with chronic lymphocytic leukaemia, non-Hodgkin's lymphoma and rheumatoid arthritis [32].

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Current Topics in Drug Reimbursement

4

Anna Bucsics, Robert Sauermann,
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Abstract

Making pharmaceutical products available for patients (i.e. reimbursement) is an important part of healthcare delivery. Most payers rely on a system of evaluating new drugs to see if they are cost effective and affordable, in order to ensure that healthcare delivery systems remain sustainable. An Austrian case study illustrated the considerable discrepancies between the views of the payers and those of industry on the innovativeness and the added benefit of new products for which reimbursement is being sought.

Within the European Union, efforts have been made to foster cooperation among member states. This cooperation encompasses joint assessments of new pharmaceuticals as well as dialogue between assessors of health technology and the pharmaceutical industry. These dialogues help to inform industry about the evidentiary needs for health technology assessment of pharmaceuticals, so that clinical trial results are not only useful for obtaining marketing authorisation but also for determining the added benefit for patients. Clinical pharmacologists can make unique and significant contributions to these efforts.

In addition to describing the projects aiming to streamline the process of making new medicines available for patients with inadequate treatment options, a proposal is outlined for a European Institute of Health (modelled on the NIH in the USA), which can foster innovation and also conduct clinical trials which are in the public interest, but do not have the backing of the pharmaceutical industry.

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4.1 Introduction

Reimbursing pharmaceuticals is considered, in most developed countries, an important part of delivering healthcare, be it by the state, in so-called "Beveridge" systems; by independent, non-profit statutory institutions, in so-called "Bismarck" systems [1, 2]; or by private health insurance providers. Notably, the Medicare Prescription Drug Improvement and Modernization Act, which was passed in 2003, also made reimbursing pharmaceuticals an important part of healthcare delivery in the USA; and the importance of reimbursing pharmaceuticals was enhanced by the Affordable Care Act ("ObamaCare") [3].

The reimbursement of pharmaceuticals deals with a fascinating array of ethical, social, economic and scientific questions, e.g.:

- How should the price of a new medicine be determined and by whom?
- What should be taken into account to determine the value of new medicines?
- Which drugs should be excluded from reimbursement – so-called lifestyle drugs [4]? Contraceptives [5, 6]?
- Which is the most equitable type of copayment?
- Whether or not to take the economic contribution of the local pharmaceutical industry into account with regard to reimbursement decisions?

These are just to name a few. Sir Michael Rawlins described these types of questions as pharmacopolitics [7].

This chapter will focus on that part of the reimbursement process which is the domain of clinical pharmacology, namely, the scientific evaluation of a new pharmaceutical for reimbursement or its re-evaluation. The chapter aims to inform readers about how such decisions are made and to sensitise them to the consequences that their work might entail. Hopefully, it will emphasise – to clinical pharmacologists as well as to decision-makers – how important and useful clinical pharmacologists are in this field.

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 wish to discourage the use of ineffective drugs, even if they are cheap.

Reviews and reports of the individual reimbursement systems in Europe and worldwide are, by their nature, ephemeral because reimbursement systems change frequently, reflecting political, demographic, economic as well as scientific changes. This is why the WHO Collaborating Centre for Pharmaceutical Pricing and Reimbursement Policies maintains overviews of reimbursement systems ("Pharma Profiles"), which are supposed to be updated by the individual participating countries [8]. A standardised process for assessment often involves an application by a pharmaceutical company as is the case in Austria [4]. Other institutions, such as NICE, are requested to assess certain drugs on the basis of need for guidance.

Institutions are fond of bemoaning the inadequacy of the data at their disposal for forming a sound basis for reimbursement decision making. Evaluation for reimbursement is usually conducted soon after marketing authorisation, so assessment

Table 4.1 Comparison of the questions asked by regulators and reimbursers when assessing pharmaceuticals

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

and appraisal for reimbursement are often based on a subset of the data generated for marketing authorisation. 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 4.1a) is a source of frustration for both industry and payers. 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" [9]. Another shortcoming is the lack of clinical trials with an active comparator [10]. Efforts to remedy this are ongoing ("early dialogue", "adaptive pathways"; both are described below).

4.2 Marketing Authorisation Versus Reimbursement

Representatives of pharmaceutical companies are fond of saying that after a drug has received marketing authorisation, 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 the list price or asking price for all pharmaceuticals without further scrutiny of their effectiveness and/or price. This scrutiny is, however, different from the marketing authorisation process (see Table 4.1a). 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.

Generally, marketing authorisation is considered a necessary, albeit not a sufficient, precondition for reimbursement, because paying for (i.e. buying) drugs which are not authorised in a specific indication or not authorised at all would make the granting of a marketing authorisation somewhat pointless. This is an ideal point of view, because exceptions are made in real life. 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 of non-authorised products); another more common exception is use of a drug in a non-authorised indication.

To illustrate how controversial this issue can be, let us consider the case where the marketing authorisation holder (MAH) is not willing to apply for marketing authorisations in a certain indication, even though there are data to show that it is efficacious. The "poster child" example for this is the anti-angiogenic monoclonal antibody bevacizumab for the treatment of "wet" age-related macular degeneration and macular oedema of other causes. Bevacizumab is not licenced for this indication; nevertheless it is effective, [11, 12, 14], widely used and reimbursed [13], e.g. by Medicare [14], and recommended by the WHO in its essential medicines list

[15]. Bevacizumab costs a fraction of the price of ranibizumab (or aflibercept), which is licenced for these indications [16]. This situation has led national institutions to question whether the marketing authorisation holder is making fair use of the current licencing system [17, 18].

Another example is the case of alemtuzumab, originally licenced as MabCampath® for patients with B-cell chronic lymphocytic leukaemia (BCLL) [19]. After learning that alemtuzumab can be used (at lower doses) to treat multiple sclerosis, the marketing authorisation holder took MabCampath® off the market – for commercial reasons. This has led to two adverse effects, namely, (1) that the cost of alemtuzumab (currently licenced as Lemtrada® in the EU and USA) for treating multiple sclerosis is higher than it could have been and (2) doctors using alemtuzumab to treat BCLL have to go through a burdensome administrative procedure to obtain this medicine for their patients [20].

The fact that commercial reasons can prevent a drug being licenced for an indication in which it could be useful and important from a public health point of view is arguably a shortcoming of the current system of licencing drugs for marketing authorisation.

However, there are usually several drugs available for a given indication, necessitating an evaluation of a new medicine in comparison to available alternatives. This is even the case for some orphan diseases (seven drugs licenced by the EMA for pulmonary hypertension, as can be seen from the list of European Public Assessment Reports [21]). So, it is more common to compare new drugs to already licenced alternatives. Drug committees are an accepted 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 [22] or IQWIG [23] or HAS [24]. Other examples of such committees are the Pharmaceutical Benefits Advisory Committee of Australia [25] and the Canadian Agency for Drugs and Technologies in Health [26].

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 it is better, how much better. This will ultimately lead to a decision on whether or not to reimburse the medicinal product, 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 [10]. How to assign monetary values to the benefits themselves – 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.

4.3 Relative Effectiveness and Health Technology Assessment in Europe: The Role of EUnetHTA

The concept of "relative effectiveness" (RE) was developed by the High-Level Pharmaceutical Forum [27]. This project was initiated by the European Commission in 2005 as a multi-stakeholder forum to address the challenges regarding

pharmaceuticals. Based on the proposals by Brian Haynes, RE 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 healthcare practice [28].

The Forum's Working Group on Relative Effectiveness saw a need for strengthening the exchange and cooperation among national agencies involved in assessing pharmaceuticals for reimbursement. The approach chosen by the European Commission was to strengthen the role of EUnetHTA (the European Health Technology Assessment Network, www.eunethta.eu). The assessment of RE for reimbursement was thus seen as something akin to, or part of, health technology assessment (HTA).

4.3.1 HTA: Definition

EUnetHTA defines HTA as "…a multidisciplinary process that summarises information about the medical, social, economic and ethical issues related to the use of a health technology in a systematic, transparent, unbiased, robust manner" [29]. This information is mostly used to inform decisions at the policy level, ensuring that such decisions are science based and give due consideration to the needs of the individual patient in need of treatment as well as those of society. Since the decisions are often about whether to adopt a new health technology, an HTA can have a major impact on the provider of the technology, such as a pharmaceutical company, and robust methodologies are needed to ensure that the decisions (and their HTA-based rationale) will stand up to the most rigorous scrutiny, be it by doctors wanting to use the new technology, budget reviewers questioning its necessity, the press or in court, if challenged by the provider standing to lose revenue.

EUnetHTA was given the task, in two Joint Actions, of developing methodologies for cooperation among European HTA institutions for assessing relative effectiveness jointly and in a way that would be useful for institutions in several member states [30]. If an evaluation which has already been performed could be reused, duplication could be avoided, thereby saving resources for both institutions and pharmaceutical companies. This work is based on the Core Model™, a generic instrument which was already developed by EUnetHTA for sharing HTAs. The model consists of a hierarchically organised list of questions about the technology ("ontology"), methodological guidelines and a standardised reporting structure. The ontology lists nine domains at the highest level (see Table 4.2), which are further subdivided into topics and then further into issues [31].

4.3.2 Relative Effectiveness Within the Context of HTA

One hurdle in developing the Core Model™-based methodology for relative effectiveness assessment was how to integrate the concept of "relative effectiveness" into the Core Model™, as neither the reporting template nor the structure of the

Table 4.2 Domains of EUnetHTA's Core Model™

1. Health problem and current use of the technology
2. Description and technical characteristics of technology
3. Clinical effectiveness
4. Safety
5. Costs and economic evaluation
6. Ethical analysis
7. Organisational aspects
8. Social aspects
9. Legal aspects

ontology foresaw RE as part of the "core". Currently, RE is reflected in a summary table showing the most relevant data on benefits and harm vs. the most relevant comparators (see, e.g. Canagliflozin for the Treatment of Diabetes Mellitus [32]).

4.3.3 Rapid Assessment

Another major hurdle was the timeline: While a "full-blown" HTA addresses all the domains of Table 4.2 and can take a year or more to complete, this is neither compatible with EU law (which allows for 6 months at the most for a reimbursement decision, including price negotiations) [33] nor is it something that patients are likely to accept, if they perceive an urgent need of the newest innovative drug. Moreover, while non-drug HTAs are usually based on a systematic review of the evidence, this is not the foremost basis for the assessment of new pharmaceuticals for reimbursement; these assessments are usually based on a dossier submitted by the company applying for reimbursement (although an additional systematic review may be performed).

To solve this problem, a checklist for the last four domains (see table 4.2) was introduced, so that these are only addressed extensively if the new drug poses problems in these domains. Moreover, the economic domain was initially not part of the joint assessment, as the factors contributing to economic assessment were deemed to be too different from country to country.

While this approach has led to numerous successes at the scientific/methodological level, including several pilot joint assessments of medicinal products, there is a lot of work to be done in order to translate such assessments into something which is acceptable to all the participating nations when it comes to decision making [34].

A major concern involves the appraisal process which follows the assessment and which ultimately involves value judgments based on local values, as well as the national legal frameworks. Of course, currently in the European Union, local values vary widely, depending on the cultural, demographic, infrastructural and 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 they cannot afford, because of positive assessments made by less constrained countries.

4.4 Austrian Case Study: Vive La Différence?

Applications for inclusion in the Austrian "Code of Reimbursement" (EKO, positive list drugs of reimbursed for ambulatory care) must be made online (www.sozialversicherung.at).

The submission is then evaluated, and a recommendation made by the Drug Evaluation Committee (representatives of stakeholders and academics). Based on this recommendation, a positive or negative decision on whether to list the drug or not is taken by the Main Association of Austrian Social Security Institutions (HVB).

Applicants must specify the "degree of innovation" for their product on a scale of one (no innovation, e.g. for generics) to eight (first-ever treatment of a disease). The claimed patient benefit must also be specified on a scale of one (no additional benefit, e.g. for generics) to six (major benefit for the majority of patients who can be treated with the drug in question). If the claim of major benefit (for the majority of patients suitable for treatment with the drug in question or only a subgroup) is accepted, the price can be higher than it would be with a lower degree of benefit (in the latter case, the bonus, based on the price of the comparators, is limited to 10 %). Claims of major benefit must be accompanied by a pharmacoeconomic evaluation showing that the product is cost effective, meaning that reimbursement is based on sound reasons and can be justified.

Not all such claims, be they for major therapeutic benefit or for "first-ever" innovation, are accepted during the evaluation of the application. In some cases, the applicant has a more enthusiastic view of the product, or the benefit is seen as more important by the applicant than by the evaluators. We wanted to examine how often these claims were accepted during the evaluation of the application. The rejection of a claim does not necessarily lead to the rejection of the application as such.

The application database at HVB was queried for all applications for inclusion of a new product between 2005 and 2015. We eliminated duplicates (resubmissions) but included as distinct submissions those for different strengths or formulations of a new active substance.

Applications with a claimed degree of innovation of "first-time pharmaceutical treatment of a disease which was hitherto treated with non-pharmacological methods" or "first-time treatment of a disease" were defined as "first ever".

Applications with a claim of "major benefit" were defined as those providing a major added benefit, compared to the alternatives defined during the processing of the application. This benefit can apply to the majority of the patients suitable for treatment or only a subgroup.

The total number of distinct applications (including those for generics) was 3158 (see Table 4.3). Of these, only 24 had a "first-ever" claim. Six of these claims were actually accepted.

We identified 269 applications for which a major benefit was claimed, which was markedly higher than claims for "first-ever" innovation. However, only 19 of these were accepted. In seven cases, the applicant claimed a major benefit for the majority of patients, but the benefit was accepted only for a subgroup.

Table 4.3 Analysis of the 3158 applications with regard to claims of "first-ever" innovation status or "major benefit" by applicants and their acceptance during appraisal in Austria

	Application with claims of "first-ever" innovation or major added therapeutic benefit		Claims accepted by HVB	
Applications since 2005	Number of applications	% of total applications	Number	% of claims
Degree of innovation claimed by applicant				
First-time pharmacological treatment of a disease which was hitherto treated with non-pharmacological methods	17	0.54 %	5	29 %
First-time treatment of a disease	7	0.22 %	1	14 %
Sum of "first-ever" claims	24	0.76 %	6	25 %
Degree of therapeutic benefit claimed by applicant: major added benefit, compared to the alternatives defined during the processing of the application				
For all patients suitable for treatment with the new drug	82	2.6 %	3	4 %
For a subgroup of patients	187	5.92 %	9	5 %
Major added therapeutic benefit claimed for the majority of patients, but accepted for a subgroup only			7	4 %
Sum of "major benefit" claims	269	8.52 %	19	7 %

4.4.1 Interpretation

Our results are not directly comparable with similar analyses, such as that of decisions of the Joint Federal Committee, which is responsible for appraising new drugs prior to price negotiations in Germany [35]. The German rating system differs markedly from the one used by the HVB, and our analysis of the whole set of Austrian applications focuses on a small subset, namely, those with claims of "first-ever innovation" or major benefit. However, Fischer and Stargardt also observed a low level of agreement between the manufacturers' ratings and those of the Federal Joint Committee.

The low percentage of applications claiming a major advance is largely due to the fact that HVB assesses all submissions, including generics (with an abbreviated procedure). The latter accounts for the majority of all submissions. Of course, the number of breakthrough innovations or products with proven major clinical benefit constitutes only a small percentage of products authorised. An additional explanation is that not all products suitable for ambulatory use were submitted for inclusion into the EKO. Notably, many modern oncology products, such as imatinib and sunitinib, as well as several anti-HIV products are not included – this means that their reimbursement needs prior approval for individual patients. The reluctance of MAH to submit these products may be related to the fact that they can demand higher

prices if the products are not listed: A prerequisite for listing is that the price of the product does not exceed the EU average.

"First-ever" drugs are rare, and the designation as such does not directly affect pricing, which is based on the therapeutic advantage. Still, the high percentage of disputed cases is somewhat surprising. One reason for disagreements is whether to consider existing drugs used "off label" as alternatives. Also, applicants tend to exclude unlisted products or extemporaneous preparations of active ingredients from their considerations, while assessors pragmatically take note of all these options.

The claim of major therapeutic benefit is "softer" than that of a "first-ever" therapy, so it is unsurprising that these claims were disputed in a higher percentage of cases. Unlike the German system [36, 37], there is currently no further official definition of what a major added benefit is in this context. Moreover, assessors are much more reluctant to assign a "major benefit" than applicants. This is not only by way of being less enthusiastic than developers, who have invested much time and money in their product; assessors are also concerned that assigning the highest levels of benefit (instead of acknowledging an incremental improvement) could dilute this valuation and want to leave headroom for adequately rewarding products which offer an undisputed major benefit.

Due to all these reasons, it is unrealistic to assume that the divergences can be wholly reconciled. So "la différence" in the perception of applicants vs. payers is alive and well. Reducing it would be helpful though, saving the need for preparing arguments and counterarguments on both sides.

One way to do this could be to publish further clarifications on what can constitute "major added benefit".

4.5 Thinking About Reimbursement During Drug Development

Another approach to reducing controversies about new products targets pre-marketing authorisation dialogues between MAH, HTA assessors and/or payers. These can be about defining treatment priorities, designing clinical trials to meet the evidentiary requirements of HTA and payers as well as those of regulatory bodies. The dialogue is also about designing reimbursement agreements which ensure sustainability of healthcare systems while rewarding those products which can demonstrate that the claims made during reimbursement application also apply to the "real-world population".

There are several initiatives at the European level involving exchanges between applicants for market in authorisation and reimbursement on the one side and regulators, HTA agencies and payers on the other side.

- The largest is the one initiated by EMA, currently designated as "adaptive pathways". Its aim is "improving timely access for patients to new medicines" [38, 39]. EMA considers three factors important for a product to be successful along this pathway: (1) an iterative development plan, which starts either with an indication for a small population and subsequently expands this or starts with an authori-

sation based on surrogate endpoints and subsequently provides further evidence of patient benefit; (2) engagement with stakeholders who are important after initial marketing authorisation to make sure the product's development plan takes consideration of their requirement; and (3) a plan for collecting further data after marketing authorisation. See also chapter (3). This endeavour is accompanied and supported by an IMI (Innovative Medicines Initiative [40]) project called "ADAPT SMART" [41], which will collect IMI projects investigating pertinent tools and methodologies, and engage in a dialogue with all relevant stakeholders to prove and develop workable concepts for the adaptive pathways to patients (MAPPs).

- Another is SEED, which stands for "Shaping European Early Dialogues for health technologies" [42], which is an international project financed by the European Commission. The objective of these early dialogues is to reduce the risk of generating a data package which does not support the future MAH's reimbursement application [43].
- For orphan medicinal products, the EU Process on Corporate and Social Responsibility in the Field of Pharmaceuticals developed the Mechanism of Coordinated Access on Pharmaceuticals (MoCA) initiative [44, 45]. MoCA is currently an informal, voluntary and nonbinding process of discussions among developers of orphan medicinal products, patient groups and payers. These discussions should not only be about the development phase of products but also about finding consensus between the MAH and payers about what the added benefit of the product is for patients. Such a consensus should form the basis for price negotiations [46], thus simplifying the assessment process at the national level. Another foreseen objective of discussions within the MoCA framework is agreement on post-marketing data collection on the effectiveness and cost-effectiveness of the product in question, ideally defining steps to be taken (e.g. changes in price or reimbursement status) if the product works better – or worse – than expected.

4.6 Tying It All Together: Electronic Health Records, Registries and Outcome-Based Pricing

Common to EMA's adaptive licensing pathway and to MoCA, as well as to other projects such as Italy's approach of establishing registries and managed entry agreements [47], is the effort to collect "real-world" data after marketing authorisation. Besides being part of the regulatory pathway, these data are meant to inform performance-based payment schemes [48, 49].

4.6.1 Managed Entry Agreements

Managed entry of new pharmaceuticals is growing in importance to ensure the sustainability of financing new medicines [50] and is an essential part of the adaptive pathway concept. It is needed to ensure that the newly licenced drug is not used "off

label" (which would make the adaptive pathway pointless) and that data are collected post-marketing. Managed entry agreements are defined as "formal arrangements between payers and manufacturers with the aim of sharing the financial risk due to uncertainty surrounding the introduction of new technologies" [51].

Performance-based payment schemes are a subset such agreements. Other schemes are purely financial, e.g. discounts, rebates or price-volume agreements. Performance-based agreements aim to tie the expenditure for a particular drug to its performance. This can be measured in each individual patient treated, in a sample or collectively [52]. Although these agreements are conceptually attractive, they have been criticised for being difficult to implement and not being very effective [53, 54].

Classic randomised controlled trials (RCTs) are particularly challenging as a basis for prospective performance-based schemes, as they are costly and randomisation is difficult to implement if a drug is already licenced on the basis of unmet need in the indication to be tested. Proposed alternatives are registries and/or administrative (reimbursement) data. Both approaches have a plethora of problems: interoperability and ownership of registries, data privacy issues, channelling and confounding of data in administrative databases, just to name a few. Additionally, such approaches require considerable resources; due to the economies of scale, this is a larger hurdle for small countries. Hopefully, electronic health records will aid in solving some of these problems. While sensible and sensitive regulation and legislation can help deal with issues of privacy, interoperability and data ownership, it remains to be seen if and how nonrandomised data will be acceptable to HTA agencies and payers – or to MAH for that matter, if their products are destined to be discounted or delisted on the basis of such data.

4.7 Summary and Conclusions

Pricing and reimbursement of pharmaceuticals have become "hot topics" recently, due to the introduction of extremely expensive "specialty medicines", not only for orphan [55, 56] and oncological indications [57, 58] but also for more common ones [59]. Analyses of the benefit and the certainty of evidence of recently approved cancer medications show little correlation between these and the costs for the new medicines [60]. Clearly, novel solutions are needed to address the problem of providing the right medicine to the right patient at the right price [61], and clinical pharmacologists can and should provide their expertise to ensure that these solutions are scientifically sound, fair and equitable:

- Critical appraisal of the clinical trial data submitted in applications for reimbursement is key to informing economic evaluations. Clinical pharmacologists are ideally suited for this task, as they have the necessary technical and methodological expertise and a broader view than specialists in individual diseases or systems.
- Learnings from these appraisals are essential for development of the methodology of relative effectiveness assessment, hopefully leading to harmonisation of procedures.

- Clinical pharmacologists also have the appropriate training to discuss drug development and study design with companies, not only for regulatory purposes but also for HTA and, ultimately, reimbursement.
- Expertise in epidemiology with clinical pharmacology is also ideal for analysis of reimbursement data in drug utilisation research. The latter is gaining importance in the context of adaptive licencing. Such expertise is needed to ensure that the design of post-marketing data collection protocols is scientifically sound and minimises bias. Perhaps most importantly, expertise is needed to help design viable performance-based manages entry agreements.

Clinical pharmacologists can participate as academics who are members of drug committees, as industry employees advising health economics and outcomes research (HEOR) departments, in regulatory bodies [62], but their contribution to HTA and reimbursement decision making is becoming more and more needed. While agency work may not be as financially attractive as work in industry, it can be at least as multi-faceted as academic research, due to the interdisciplinary nature of HTA and as rewarding as any of the other areas of work, because of the opportunity to directly contribute to shaping healthcare systems and policy.

Although engagement in pharmacopolitics can help remedy the situation, it cannot compensate for suboptimal research infrastructure. We therefore suggest the establishment of a "European Institute of Health". Besides fostering innovation, such an institute could conduct post-marketing studies which are in the public interest.

Convincing countries that such an institution is worth financing is, of course, a challenge. On 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 of multiple products, such as ALLHAT [63]). Doing this at the European level instead of a national level only 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.

Disclaimer The contents presented here reflect the personal opinion of the authors. They are not necessarily identical with those of the of the Lower Austria District Health Insurance Fund, the Department of Pharmaceutical Affairs of the Federation of Austrian Social Security Institutions, its Advisory Committees, or its management.

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Part II

Clinical Trials

Ethics in Clinical Research

5

Ernst Singer and Christiane Druml

Abstract

Physicians engaged in clinical research face the ethical question of how to combine the delivery of individual care for the patient with the rigorous demands of science. The first documents in Europe adressing the need of introducing standards in clinical research ethics were the 1900 Regulation of the Prussian ministry of Education and the 1931 Reich Circular "Regulations on New Therapy and Human Experimentation". These pre-war documents contained already important ethical principles in clinical research such as informed consent, voluntary participation and the concept of vulnerable patient groups. However, they were only national documents. The development of more generally accepted guidelines started not until after World War II as a result of the inhuman Nazi experiments. Thus the "Nuremberg Code" was formulated in 1947, and in 1964 the World Medical Association issued the Declaration of Helsinki, one of the most important documents in the history of research ethics. The Declaration has undergone several revisions, one of paramount importance in 1975 when the concept of oversight by an "independent committee" was introduced, thus giving birth to independent Ethics Committees (IEC) worldwide.

Today the function of IEC is multifaceted. Over the last decades they have grown from small groups of peers voluntarily reviewing protocols to institutions implemented under various laws, performing specialized tasks requiring a high level of professionalism. This development over time along with the various strategies employed to effectively handle the increasing number of tasks is described using the IEC of the Medical University of Vienna as an example.

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5.1 Development of Worldwide 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, and 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 nineteenth-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 prostitutes, some of whom were minors, by injecting them with an infected serum without their knowledge. The experiment did not 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 prewar 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 worldwide 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 Brandt[1] [2] 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).

In the same year, the Nuremberg Code was written by the time the World Medical Association (WMA) was founded. WMA today has a membership of over 80 national medical associations and represents about nine 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 USA in the wake of the probably most famous unethical postwar 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

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

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 the 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 1970s "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
- Justice: ensuring reasonable, nonexploitative, 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 worldwide 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 as 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.

5.2 Research Ethics Committees Today: Function and Composition

The function of Ethics Committees today is multifaceted. 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 the 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 time frame. 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.

5.3 Research Ethics Committees: Issues of Debate

5.3.1 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. 5.1) shows the number of applications at the Ethics Committee over the course of the last 10 years.

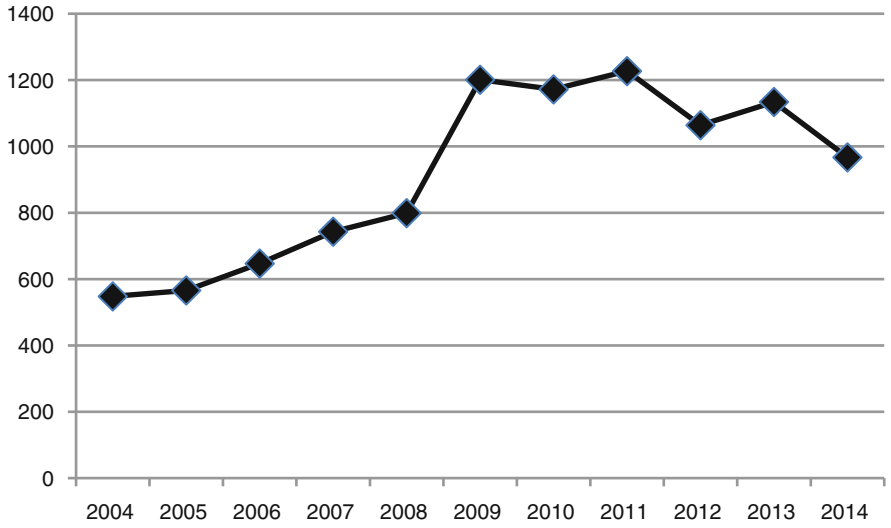


Fig. 5.1 Number of clinical studies at the Medical University of Vienna in the years 2004–2014

For instance, 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.

A further step to a more efficient handling of workload was done in 2012. Due to the more and more evident capacity and performance problems of the available software systems and the limited archive space, it was decided to implement a global software solution that would render paper documentation unnecessary, satisfy all requirements of an EC office, and meet the necessary regulatory standards: ECS[2] ("Ethics Committee System") constitutes an externally validated, exclusively

[2] ECS – an open-source software solution satisfying all requirements of an EC office. The Vienna experience.

Ernst Singer, Ethics Committee Medical University Vienna, Borschkegasse 8b, A-1090 Vienna, Austria P20, Poster presented at the DIA 26th Euromeeting Vienna March 2014

web-based, easily expendable open-source (https://github.com/ethikkom/ecs) software solution. Its main features are online data input, reading, searching, annotating of all uploaded documents, generation of pdf/A documents from data input, flexible and expandable workflow, and safe long-term data storage in encrypted form. The system generates session agenda and session protocols; the votes are digitally signed and automatically sent to the concerned parties. The post-vote workflow allows upload of amendments, notifications, and all safety-related reporting. Reminder functions support the investigators (e.g., renewal of vote after a year).

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, and 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. 5.2) 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.

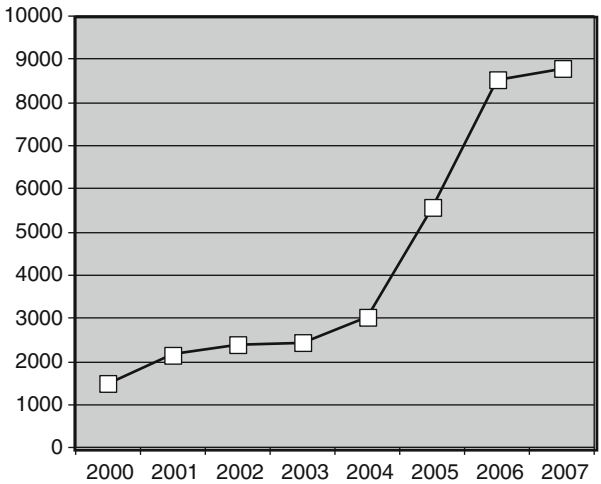


Fig. 5.2 Number of reports and notifications received by the Ethics Committee of the Medical University of Vienna from 2000 to 2007

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 [6] (new quotation, 8, see references).

In fact, the European Commission financed in its 7th Framework Programme a 1-year project to measure and analyze 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 [7].

A major change was consequently introduced in 2014 with the new Clinical Trials Regulation [(CTR) EU No 536/2014, entered into force on 16 June 2014, applicable no earlier than 28 May 2016]. Its most prominent feature is a streamlined application/assessment procedure. It avoids the hitherto necessary multiple submission of largely identical information in a multinational trial and replaces it by the submission of one application dossier to all the Member States concerned through a single submission portal. This portal also serves for all other types of clinical trials with medicinal products (i.e., also multicenter national trials and single-center trials). The involvement of the Ethics Committees in the assessment procedure will be within the overall timelines defined by the Regulation. A single assessment report, delivered to the sponsor via the portal, will be the result of the assessment of the competent authorities and the Ethics Committee opinion.

5.4 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 toward 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 abovementioned 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 ([8]; Fig. 5.3) reported 22 examples of research inconsistent with ethics and the Nuremberg Code, but published in the current medical literature. In the year of Beecher's publication, the Public Health Service (PHS) issued a new policy requiring institutional review for:

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*)

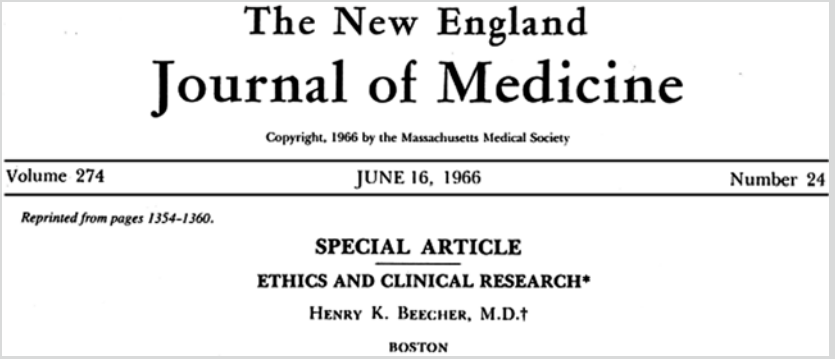
The sulfonamides were for many years the only antibacterial drugs effective in shortening the duration of streptococcal pharyngitis and in reducing its suppurative complications. The investigators in the study took to determine if the occurrence of the serious nonsuppurative complications, rheumatic 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 a plastic 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 2 g versus 6 g were tested. Toxic bone marrow depression occurred in 2 of 20 in the 2 g group and in 18 of 21 in the 6 g 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.



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SPECIAL ARTICLE

ETHICS AND CLINICAL RESEARCH*

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BOSTON

Fig. 5.3 Headline of the Beecher Article in 1966

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Good Clinical Practice (GCP) and Scientific Misconduct

6

Brigitte Bloechl-Daum

6.1 Good Clinical Practice

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 Harmonisation (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.

6.1.1 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

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has evolved through a series of regulation and policy formulations. These are some of the major milestones [2] in the evolution of GCP.

6.1.1.1 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.

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].

6.1.1.2 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 Congress of the United States 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.

6.1.1.3 The Sulphanilamide Disaster and the 'Food, Drug and Cosmetic Act', 1938

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

In September 1937, 240 gallons were shipped across the United States. 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 authorised the US Food and Drug Administration (FDA) to demand evidence of safety for new drugs [7].

6.1.1.4 Second World War Crimes

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.

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) sulphonamide experiments).

6.1.1.5 Nuremberg Trial and Nuremberg Code

In 1946, the military trials (Nazi Doctors' Trial) in Nuremberg, Germany, were performed with guilty verdicts for 15 out of 23 defendants, seven received death sentences. In their final judgement, 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 recognised 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.

6.1.1.6 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 (1914–2015) 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. Two hundred thousand American patients received thalidomide, and by the mid-1960s, 10,000 birth defects had occurred worldwide.

In the United States, the Kefauver–Harris Amendments of 1962 were inspired by the worldwide 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].

6.1.1.7 Declaration of Helsinki [8]

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'. The Declaration of Helsinki is the basis for Good Clinical Practices used today.

6.1.2 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 [9]. 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 [10]. Further developments led to the Council for International Organizations of Medical Sciences (CIOMS) and World Health Organization (WHO) guidelines on GCP in 1993, which were followed by the GCP–International

Conference of Harmonisation (ICH) guideline in 1996 [11]. 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.

Requirements for the conduct of clinical trials in the European Union (EU), including GCP and good manufacturing practice (GMP) and GCP or GMP inspections, are implemented in:

- The 'Clinical Trial Directive' (Directive 2001/20/EC) [12] (http://ec.europa.eu/health/files/eudralex/vol-1/dir_2001_20/dir_2001_20_en.pdf).
- The 'GCP Directive' (Directive 2005/28/EC) [13]. So the adherence to ICH–GCP is now a legal requirement within the European Union.

Further information can also be found on the website of the European Medicines Agency [14].

Australia has adopted a very similar version of the Note for Guidance on Good Clinical Practice [15].

6.1.2.1 International Conference of Harmonisation (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 United States. 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.

ICH Parties

- European Commission – European Union (EU)

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

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.

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.

- 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, the FDA is responsible for the approval of all drug products used in the United States.

Pharmaceutical Research and Manufacturers of America (PhRMA)

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

- 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.

- 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.

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 organisation (NGO) representing national industry associations and companies from both developed and developing countries.

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 harmonisation 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.

6.1.3 ICH Topics [17]

The ICH topics are divided into four categories, and ICH topic codes are assigned according to these categories.

- Quality Guidelines

Harmonisation achievements in the quality area include pivotal milestones such as the conduct of stability studies, defining relevant thresholds for impurities testing and a more flexible approach to pharmaceutical quality based on Good Manufacturing Practice (GMP) risk management.

- Safety Guidelines

ICH has produced a comprehensive set of safety guidelines to uncover potential risks like carcinogenicity, genotoxicity and reprotoxicity. A recent breakthrough has been a non-clinical testing strategy for assessing the QT interval prolongation liability: the single most important cause of drug withdrawals in recent years.

- Multidisciplinary Guidelines

Those are the cross-cutting topics which do not fit uniquely into one of the quality, safety and efficacy categories. It includes the ICH Medical Terminology (MedDRA), the Common Technical Document (CTD) and the development of Electronic Standards for the Transfer of Regulatory Information (ESTRI).

- Efficacy Guidelines [18]

The work carried out by ICH under the Efficacy heading is concerned with the design, conduct, safety and reporting of clinical trials. It also covers novel types of medicines derived from biotechnological processes and the use of pharmacogenetics/genomics techniques to produce better targeted medicines.

6.1.3.1 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:

- 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).
- 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.
- The rights, safety and well-being of the trial Subjects are the most important considerations and should prevail over interests of science and society.
- The available non-clinical and clinical information on an investigational product should be adequate to support the proposed clinical trial.
- Clinical trials should be scientifically sound and described in a clear, detailed protocol.
- 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.
- 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.
- Each individual involved in conducting a trial should be qualified by education, training and experience to perform his or her respective task(s).
- Freely given informed consent should be obtained from every Subject prior to clinical trial participation.
- All clinical trial information should be recorded, handled and stored in a way that allows its accurate reporting, interpretation and verification.
- 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).
- 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.
- Systems with procedures that assure the quality of every aspect of the trial should be implemented.

The three ‘main players’ of ICH–GCP are Ethics Committee, Investigator and Sponsor.

6.1.3.2 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 Chap. 5.

6.1.3.3 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' [19].

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.

The Investigator is responsible for supervising any individual or party to whom the Investigator delegates study tasks conducted at the trial site and should ensure this party is qualified to perform those study tasks. He should implement procedures to ensure the integrity of the study tasks performed and any data generated [20].

- 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 inclusion and exclusion criteria, to see who would really be eligible for the trial.

- 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' [21]. However, the well-being of the individual research Subjects takes precedence over any research questions [16].

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
- 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.

- 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, etc)

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

- Randomisation procedures and unblinding

Correct handling according to the protocol of the abovementioned procedures is also the sole responsibility of the Investigator, and 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 [22].

- 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 [16].

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.

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 [23], 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' [24].

'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' [25].

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 ethically acceptable amount of 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].

However, the FDA states [26] that the amount and schedule of all payments should be presented to the IRB at the time of initial review. The IRB should review both the amount of payment and the proposed method and timing of disbursement to assure that neither are coercive nor present undue influence.

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 [27]. 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 [28]. The amount of money expected by healthy volunteers is based on each Subject's perception of study burden and associated risk [29]. 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' [30]. 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.

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.
- Randomisation ('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 versus general benefit of participation.
- Alternative treatment options (risks and benefits).
- Concomitant medication and lifestyle modifications (diet, sport, handling of machinery).
- Insurance (insurance company, policy number, contact address, insurance exclusions) and protocol violations by Subjects (e.g. concealed medical information or treatments).
- Compensation and payment of Subjects.
- Confidentiality: Monitor, Auditor and 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 authorising 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 number).
- 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.

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.
- 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.

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).

Source Data

The Investigator should maintain adequate and accurate source documents and trial records that include all pertinent observations on each of the site's trial Subjects. Source data should be attributable, legible, contemporaneous, original, accurate and complete. Changes to source data should be traceable, should not obscure the original entry and should be explained if necessary [20].

Case Record Form (CRF)

To standardise the documentation of all trial-related procedures and results, it is mandatory to use appropriate CRFs. A CRF is a printed, optical or electronic document designed to record all of the protocol-required information to be reported to the Sponsor on each trial Subject.

- One per Subject.
- Contains data and other information about each included Subject – according to protocol.
- Serves as a means of analysis and standardisation of obtained data – source data.
- Design of a CRF (minimal requirements).
- Identification under the rules of data protection laws.
- Date, place and study identification.
- Demographic and ethnic data.
- Characteristics (smoker, diet, etc).
- Diagnosis and indication for study drug.
- Fits entry criteria.
- Mode of treatment (single dose, daily dose, duration, etc).
- 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 ~~130/78~~ 130/87 *Corr*: *NN Error dd*:*mm*:*yyyy*

- Certified copy [20]
- Instead of the original source document, a paper copy or an electronic copy of the original record can be used if this has been verified (e.g. by a dated signature or has been generated through a validated process to produce an exact copy having all of the same attributes and information as the original).

Archiving

All trial-related documents need to be archived, the regulations vary from country to country, and a minimum of 2 years is required following market approval in the European Union 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.

Progress Reports

At least once a year, the Investigator has to submit a progress report to the Ethics Committee.

Safety Reporting

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

Serious Adverse Event

All serious adverse events (SAEs) should be reported immediately to the Sponsor [15] 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 hospitalisation
Results in significant disability
Results in a congenial abnormality/birth defect

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 [31]. 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 or otherwise 15 days, to the IRB/IEC and the authorities.

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
- 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) [15].

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' [15].

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'.

6.1.3.4 Sponsor

Per definition the Sponsor is 'an individual, company, institution, or organisation 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
Utilise 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)

Quality Assurance and Quality Control

Implementing and maintaining quality assurance (QA) and quality control (QC) systems for clinical trials are 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; 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.

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.

6.1.4 Discussion

6.1.4.1 Is ICH–GCP Just a 'Bronze Standard'? [20]

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 and informal consensus [32], 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 [33], ICH-E6 has not been updated since 1996, and no timetable for revision is specified; however, an integrated addendum is in development.

6.1.4.2 The E6(R2) Integrated Addendum [34]

Since the development of the ICH–GCP guideline in 1996, the scale, complexity and cost of clinical trials have increased. This guideline is now in the process of being amended to encourage improved and more efficient approaches to clinical trials. Especially the standards regarding electronic records and essential documents efficiency have been updated.

The E6(R2) Integrated Addendum has reached step 2 of the ICH process in June 2015; changes were integrated directly into several sections of the parental guideline [20]

6.1.4.3 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 [35]. The EU Clinical Trials Directive must also take some blame. Set up in 2004 to improve the quality and safety of trials and to harmonise and simplify application processes across Europe, it has been heavily criticised by academics [36].

McMahon et al. [37] 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' [38].

6.1.5 Summary

Despite some 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.

6.2 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 [39].

6.2.1 What Is Research Misconduct?

The Joint Consensus Conference on Misconduct in Biomedical Research [40] 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 [35]:

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

Effective June 16, 2005, the US Public Health Service, which administers its integrity programme through the Office of Research Integrity (ORI), defined research misconduct as [41]:

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

6.2.2 Forms of Research Misconduct

- Honest mistakes
- Gift authorship, guest authorship, ghostwriting and exclusion of rightful authors
- Plagiarism: appropriation of another person's ideas, processes, results or words without giving appropriate credit
- Undeclared post hoc subgroup 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

6.2.3 How Common Is Research Misconduct or Fraud?

Usually professionals and the public focus on headline-grabbing cases of scientific misconduct, but researchers should no longer ignore a wider range of questionable behaviour that threatens the integrity of science. In a survey published in nature [42], 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 [37] $n = 3247$
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 %	Unauthorised 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

In systematic review and meta-analysis on survey data on fabrication and falsification of research data, the authors concluded that it is likely that if on average 2 % of scientists admit to have falsified research at least once and up to 34 % admit other questionable research practices, the actual frequencies of misconduct could be higher than this [43].

6.2.4 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 [44]. 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 17 December 2000, the Washington Post [39, 45] 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 lb. 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 and 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 meningococcal 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 [40, 46]. 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' [47]. The story in the Washington Post 40 described the slow death of a 10-year-old girl known only as Subject 6587–0069 [48]. 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 expose 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 [42].

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' [41].

In August 2011 the British Medical Journal announced that Pfizer had paid $175,000 (£107,000; €120,000) to each of four families whose children died during a study of an experimental antibiotic in Kano, Nigeria [49]

Conclusion

The globalisation 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 [50]; thus, the increasing globalisation of clinical research trials calls for more effective ethical and legal rules to protect both research Subjects and scientific integrity [44].

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

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Phase I Studies and First-In-Human Trials

7

Ulla Derhaschnig and Bernd Jilma

7.1 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 stepwise procedure in which information gained from early, typically smaller, studies is used to plan and perform larger studies with more detailed objectives.

7.2 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 nontherapeutic 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 can

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

  - In single ascending dose (SAD) studies, 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 crossover design (see Chap. 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.

7.3 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-human 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
- 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 preclinical 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 bypass 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 preclinical 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-human trial and extensively explained in the study protocol. And in doubt, higher risk should be assumed [2].

7.4 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 preclinical studies relevant to the safety of human exposure, for instance, in the form of a confidential database.

7.5 Choice of Subjects, Study Population

In general there is no anticipated benefit to a patient or volunteer subject in a first-in-human 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-human 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.

7.6 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

nonclinical 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 and 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-human 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].

7.7 Route and Rate of Administration

The route and rate of administration should be based on preclinical 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-human 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 pharmaco- and toxicokinetics and pharmaco-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 nonclinical 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 and also processes 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].

7.8 Clinical Environment

First-in-human 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 prearranged 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 nonexpert 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.

7.9 Specificities of First-in-Man Trials with Monoclonal Antibodies

It is often the purpose of a first-in-human trial (particularly anticancer drugs) to define a maximum tolerated dose (MTD), as defined by the dose where less than 1/3 of patients experience intolerable adverse effects. However, this is often not possible with monoclonal antibodies because their high target specificity decreases the risk for off-target effects. A recent comprehensive review of first-in-human trials

provided interesting insight into recent designs of such trials with monoclonal antibodies [9]. Only 1/3 of trials reported the reasons for the starting dose. Preclinical toxicity data (48 %) and/or pharmacokinetic/pharmacodynamic were used to justify the starting dose, a MABEL approach in only 11 %. More than half of the trials used a 3 + 3 design for dose escalation; dose escalation based on a data monitoring committee was only used in 7 % of the trials. The median number of dose levels was 5 but ranged from 2 to 13. The highest planned doses exceeded the starting dose on average 27-fold (median; range two- to 3333-fold). No dose-limiting toxicity was observed in 57 % of the trials. In cases where a MTD could be defined, this was the highest planned dose in all cases, which was fourfold to 1000-fold higher than the starting dose.

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 [10].

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 leukaemia 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 and had varying degrees 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 1 h later.

All subjects were initially empirically treated in the independent clinical trial unit with hydrocortisone, chlorpheniramine, acetaminophen, ondansetron, 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 monocytopaenia and neurologic symptoms in terms of multi-organ failure. Four subjects received continuous positive airway pressure, two underwent mechanical ventilation, and all six needed renal support by means of continuous venovenous haemodiafiltration 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 6 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) [10].

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

Firstly, for this type of IMP the ability of nonclinical 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 [11] 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 CD28 antibodies [12]. However, according to the investigator's brochure, 100 % homology between the CD28 binding site in humans and monkeys exist and no sequence comparison was included [13]. It had been known for almost two decades before that targeting T cells with OKT3 induces a severe cytokine release reaction. 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 antihuman CD28 antibody induced rapid depletion of peripheral T cells in mice with a humanized immune system [12, 14]. 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 [12]. Additionally, some other points of concern about the

preclinical tests were raised [13]. 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 [13], while primates are typically less sensitive (compare Chap. 19). 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 8 days. Thus, the full removal from the body would take about a month [13]. In this view, a 10 min 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 [15]. 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 [13].

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 [8].

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 [16, 17]. In the investigator's brochure, little guidance is given to doctors on how side effects can be controlled and treated [13].

Finally, interviews with the victims yielded various motives for participating in this study, from altruism to monetary reward [18]. However, a study revealed that informed consent forms may not have informed participants adequately for consent [19].

Lessons have been learned from this trial and expert groups were formed to improve guidelines for phase I trials [2, 20]. 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 [21]. 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 [21]. 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 [22].

However, specific reactions and 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.

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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

Clinical Trials: Interventional Studies

8

Michael Wolzt and Stefan Aschauer

8.1 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.

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8.2 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 example of an observational study is the Framingham Heart Study which started in 1948. In this study data from 5209 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. 8.1). 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.

8.2.1 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.

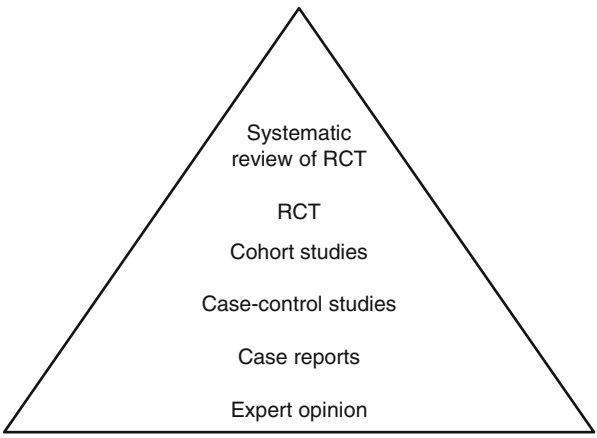

Fig. 8.1 Randomized clinical trials (RCT)

8.2.2 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 predefined 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.

8.3 Randomization

8.3.1 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 World 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 assignment of the participants to treatments, 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.

8.3.2 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, and 4, and treatment B to the numbers 5, 6, 7, 8, and 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.

8.3.3 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 numbers 1–6. With the use of a random number list, a block randomization can be achieved.

8.3.4 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, sex, tumor status, study centre, etc. To achieve balanced groups, it is advisable to use permuted blocks. Simple randomization would easily produce numerical imbalance in the subgroups. This is an example of a study population in which age, gender, and glucose tolerance are prognostic variables.

	Age (year)	Sex	Glucose tolerance
1.	20–35	Male	Normal
2.	36–50	Female	Prediabetes
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").

8.3.5 Pseudo-randomization

Some treatment allocations are often incorrectly regarded as randomization methods. Randomizations according to 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.

8.3.6 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].

8.4 Blinding

8.4.1 Human Behavior 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, and 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 belief of a patient in getting a new promising treatment or a readily available treatment already changes his attitude toward 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 the 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 into 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.

8.5 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.

8.5.1 Parallel Group Design

One of the most common methods in clinical investigation is the parallel group design (Fig. 8.2) 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 design, in which one subject receives either the interventional or the control treatment. At the end the outcome is compared.

Study subject selection and treatments do not necessarily remain stable over the course of the trial. In order to provide flexibility and optimized dosing, selection of target populations and interventions may be refined following an ongoing interim review of safety and efficacy data. This enrichment of a responder population may lead to earlier market access of medicines and devices and has become known as "adaptive clinical trials" by the authorities [8]. However, a continuous change in population characteristics and intervention during an ongoing study may be more appropriate to the development of human medicines and devices in therapeutic studies that aim at early and conditional marketing approval.

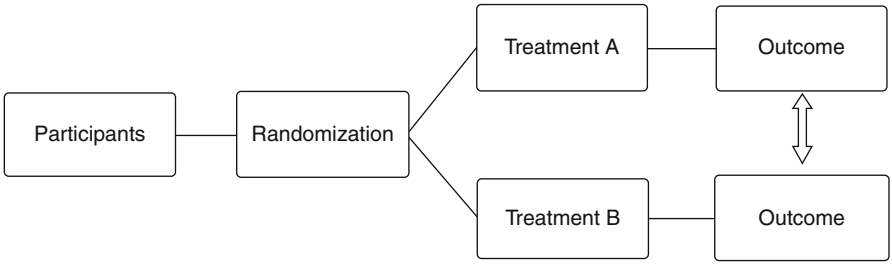

Fig. 8.2 Parallel group design

8.5.2 Crossover Design

The crossover 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 crossover 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 crossover trial has some advantages. The fact that every subject serves as own control reduces interindividual 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 crossover studies is the carryover effect, which means that the effect of the first period still persists in the second period, might influence the outcome. To prevent carryover, a long washout period should be established. Another limitation is that crossover 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 [9].

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 versus not A and B versus 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 investigated the effect of aspirin on the risk of myocardial infarction and the effect of beta carotene on the risk of cancer. The participants were assigned to one of four possible combinations (Fig. 8.3). 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 2×2×2 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.

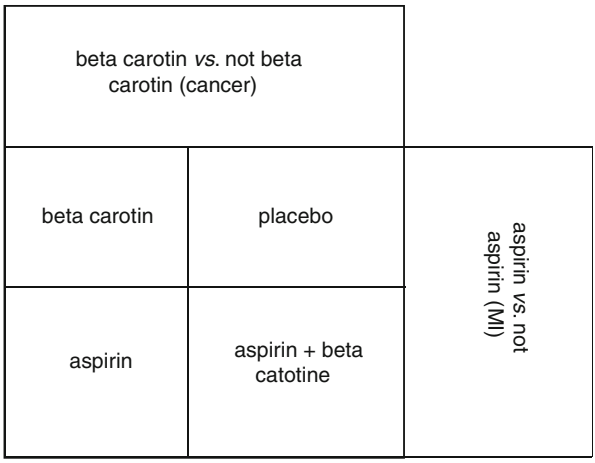

Fig. 8.3 Factorial design

8.6 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 and measurable, and should reflect validated aspects of the disease process [10]. Full remissions of all disease symptoms, a disease relapse, and mortality are regarded as hard study endpoints, whereas pain and 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 experts [11]. 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 [12]. 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 [13]. However, an effect on a composite does not necessarily mean that all individual endpoints are affected or influenced in a consistent way.

8.7 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 cut-offs. 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 antiarrhythmic drugs in patients with myocardial infarction and ventricular ectopy/non-sustained ventricular tachycardia. Responders to antiarrhythmic 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 antiarrhythmic 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 versus 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 antiarrhythmic 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.

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Observational Studies

9

Harald Herkner and Christoph Male

Abstract

When drugs are on the market observational studies are essential tools to further investigate their benefits and harm. Several observational study design types are available. These study design types share a number of common methodological principles, and they all will always include some degree of random error, bias, and confounding. In this chapter we will illustrate design principles, practical applicability, limitations, and discuss critical appraisal of observational studies.

9.1 Introduction

Epidemiologists have used observational studies for a long time to explore the effects of infectious and non-infectious exposures on health outcomes. Outstanding people who performed milestone epidemiological research include Ignaz Semmelweis (1818–1865), William Farr (1807–1883), John Snow (1813–1858), 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 randomised trial on the benefits of streptomycin for pulmonary tuberculosis in 1948 [1], interventional studies seemed to gradually replace 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].

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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]. In recognition of the relevance of drug safety, the Pharmacovigilance Risk Assessment Committee (PRAC) was established at the European Medicines Agency in 2012. This additional committee is concerned with all aspects of drug safety after marketing authorisation. Understandably observational studies are an important evidence base for the resultant regulatory actions.

Nonetheless investigations on adverse drug reactions were still conceived as descriptive research. An illustration of how to properly use analytical observational studies to investigate adverse drug reactions was published only in 1978 [6], and established epidemiological methods were increasingly utilised in pharmacology. With the availability of large databases, the methods of observational research are still being advanced [7]. Big data analyses, which mean the exploration of very large datasets, are becoming more popular since appropriate data storage and processor capacities were becoming available. Big data advances the observational study methodology by relaxing the sampling assumptions because very large samples or sometimes even the totality of data can be analysed. On the other hand, such analyses can only identify correlations but cannot prove causal associations [70], which is a fundamental limitation of this approach for the assessment of drug safety.

There is no doubt that randomised controlled trials are the gold standard method to study the effectiveness and efficacy of pharmaceuticals. There are, however, also some limitations of the randomised trials that may be outweighed by observational studies. Randomised trials are usually powered to detect short-term efficacy and are often not designed to detect rare but still important side effects [8]. Results from suitable trial cohorts cannot necessarily be generalised to the actual daily life practice in more vulnerable co-morbid populations. In particular effectiveness of interventions in elderly people, children, or pregnant women is 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 harms. Is there sufficient equipoise for starting a randomised trial, implying that we withhold a drug that is marketed for decades to a study population, despite many people believe in its benefits? In the drug regulatory context, such studies are referred to as post-authorisation safety studies (PASS) or post-authorisation efficacy studies (PAES). Recently also applications in pharmacoeconomics are reported [9]. Contemporary applications of observational studies in drug research are presented in Table 9.1.

Accordingly, observational research is an important complementary tool to randomised experimental 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 [10] and sufficient training in critical appraisal for those who apply the results are therefore required.

Table 9.1 Application 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 randomise)
Patterns of drug utilisation
Pharmacoeconomics

As a consequence of using epidemiological 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 (www.pharmacoepi.org), 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.

9.2 Methodological Principles

Key elements of any epidemiological (analytical) study are the following:

- *Study population* (P)
- The risk factor or *exposure* (E)
- *Controls* (C), i.e. those without the risk factor or exposure
- The endpoints of interest or *outcomes* (O)

- (which may be remembered by the acronym PECO), and the The *type of study design* (Gordis 2014). These elements compose the study objective and need to be precisely defined at planning stage to choose the appropriate study methods in order to minimise errors (bias, confounding) during conduct and misinterpretations when analysing 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 [12].

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).

9.2.1 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 [11].

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 [13]. 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 [14]. 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 criterion 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 [12].

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 utilisation and drug benefit and safety in all subsets of the population (Strom 2013).

9.2.2 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 lifestyle data to control for confounding.

Data sources used for pharmacoepidemiologic studies are automated databases, pharmacy records, or physician records [22, Strom 2013]. Automated databases are the most valuable data source because they are large and 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 claim 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. Datasets 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 USA). *Medical record databases* are specifically generated for research purposes and make use of the electronic documentation of diagnostic and therapeutic records both on in-hospital and outpatient care. Prominent examples are the *General Practice Research Database*, *GPRD* in the UK [16] and the PHARMO system of record linkage in the Netherlands [17]. Medical record databases usually have more valid disease and outcome data. However, their completeness may be an issue. One general limitation of automated databases is the limited information on potential confounders such as clinical factors and, particularly, lifestyle 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 [18, 19]. The latter approaches are very resource intensive, and data obtained from patients is the least reliable [20]. Sometimes drug registries as held by pharmaceutical companies are used for pharmacoepidemiologic studies. Such registries include detailed information on individuals who were exposed to a specific medicinal product, but usually these registries do not contain information on non-exposed individuals, who would normally serve as the controls. Therefore, ‘drug registries’ are considered less valuable compared to the above described ‘disease registries’ or ‘population registries’ in drug safety research.

9.2.2.1 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 lifestyle factors [7, Strom 2013].

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, dose 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, lifestyle 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.

9.2.2.2 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 [22, Strom 2013].

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, and 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.

9.2.3 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, and 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 minimally 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 boundary were used when data were categorised (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.).

9.2.4 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 two groups, the ratio of the two risk of the outcome is an obvious solution and well known as the *risk ratio* ($RR = risk_{exposed}/risk_{controls}$). 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, an 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 ($RD = risk_{exposed} - risk_{controls}$). 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 $PAF = \left(risk_{total\,population} - risk_{controls}\right) / risk_{total\,population}$. Alternatively, if we have adjusted relative risks available, we can incorporate the prevalence of the exposure (p_{exp}) into a useful equation:

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

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

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

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.

Moreover, in drug safety studies, the event rates are mostly low overall, but sometimes with zero events in the control groups. This results in a division by zero, which is a complicated situation shared by all ratio measures. Several methods are available to circumvent this issue, but ultimately this is another case for presenting absolute risk differences.

9.2.5 Interpreting an Effect: Bias, Confounding, and Sampling Error

Whether such an effect reflects a true causal 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.

9.2.5.1 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; consequently 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.

9.2.5.2 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 [21]. Other calibration techniques, e.g. by using external data, are available [7]. If correlated observations like repeated measurements are incorporated in the analyses, more complex techniques like random effects models, mixed models, or generalised 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 [23, 24]. The major source of uncertainty, however, comes from unmeasured confounders, which results in *residual confounding* – a shortcoming that can only be mastered by appropriate randomisation and is therefore inherent in non-randomised observational research.

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

9.2.5.3 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. 1 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 the chapter "Epidemiology and Biostatistics" (Chap. 14).

9.3 Overview of Study Design Types

Figure 9.1 shows a systematic overview of study design types. Study types are differentiated based on certain design principles: studies using individual patient data versus aggregate data, descriptive versus analytical studies, and, within the latter, observational versus experimental studies [25]. The following section will briefly discuss study design types used in pharmacoepidemiology with a focus on observational studies.

9.3.1 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 has become the primary method of collecting post-marketing information on drug safety [26]. Spontaneous

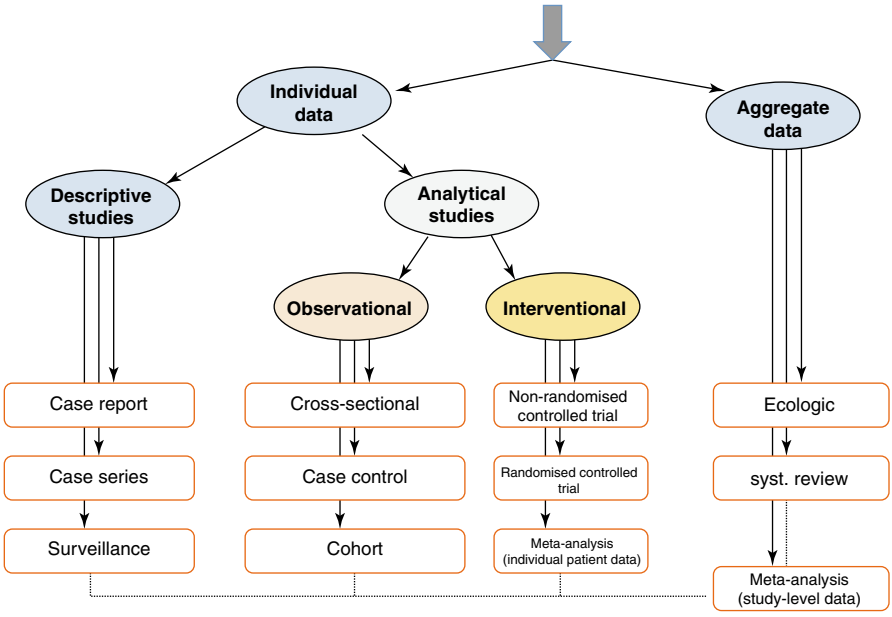

Fig. 9.1 Systematic overview of study design types

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.

Suspicions 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 databases 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 [27]. 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 [28].

Typical descriptive studies are *surveillance studies* that describe the distribution of characteristics in a defined population [29]. 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 utilisation 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 utilisation may be compared between subgroups based on demographic characteristics, e.g. age, morbidity, and SES. 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, countries).

Time trends or comparison between populations is 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 utilisation 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 them 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*.

9.3.2 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 (randomised controlled trial) are discussed in the chapter "Clinical Trials: Interventional Studies" (Chap. 8).

The *STROBE guidelines* (*ST*rengthening the *R*eporting of *OB*servational Studies in *E*pidemiology) have been published to set standards for reporting of observational studies [12, 15]. 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 a matter of debate [30].

9.3.2.1 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 [25]. 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 PE since drug exposure is a time-dependent variable and drug effects occur over time.

9.3.2.2 Cohort Study: Principles and Practical Example

Cohorts are defined as groups of persons sharing certain demographic and clinical characteristics [31]. 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 efficacy, 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 [15]. 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 defining study objective, the study population, and collection of data in detail since each of these items has 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) [32], pharmacy-based drug surveillance studies [34], 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 and 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, and General Practitioners' Oral Contraceptive Study, etc. [33].

Cohort studies usually have good external validity. Because of their observational character and more pragmatic follow-up, they have less selection in recruitment and retaining 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 [10, 35]. 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, 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 [36].

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 standardisation 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 [37]. An example was that statins were believed to reduce the risk of Alzheimer's disease based on an observational study [38] which was disputed by a randomised trial [39]. 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 [40].

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-randomised 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 [41]. 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.

Example 1: Cohort Study

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 [42]. 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 estimated 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 categorised 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, 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, and hypertension, 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; and (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 the 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 lifestyle, 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 the Netherlands published at the same time came to quite similar conclusions [43]. 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.

9.3.2.3 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 of the outcome, defined as 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 [44]. At that time increasing attention was paid to risk factors for malignancies [45]. 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 [46]. Accordingly, to detect only one case, an average group of more than 2,100 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) [47]. 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 [48]. Accordingly, the case-control design is frequently used in infectious disease outbreak research. Sample size of cohort studies depends very much 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, 49]. Applications of the case-control design are also reported in pharmacoeconomics [9].

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.

Example 2: Case-Control Study

Juurlink and co-workers have used the case-control design to investigate the drug interaction between proton pump inhibitors and clopidogrel [50]. 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 [51, 52]. On the other hand, there is some evidence suggesting a drug interaction between clopidogrel and proton pump inhibitors. Clopidogrel is a prodrug, 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 [53]. For a quick and meaningful investigation of this important health problem, the researchers opted for the case-control design.

The Cases

Cases were patients who had myocardial re-infarction within 90 days after hospital discharge for a first myocardial 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 worse 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 all existing 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 reliable in this region [54, 55]. 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 % [56]. 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 [57, 58]. 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, 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 socio-economic status has a well-established association with many health outcomes [59–61]. However, in Canada the coverage is known to be high, so this issue should not be too problematic for this example [22].

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 taken 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 approach 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 socio-economic 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 reasonable 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 for studies with a complete sampling frame of the source population (population-based studies), like our study example, 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 [62].

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 in equal fashion for cases and controls from one reliable source. This is not always a simple task. For example, if hospitalised cases are compared to non-hospitalised controls, a chart review will only be possible for the cases, and controls might need to 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 of risk factor to 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.

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 socio-economic status is hard to measure correctly, 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 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 [63] 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.

Analysis and Results

In contrary to cohort studies, we compare cases to controls here, as usually reflected in a ‘characteristics of participants’ table. 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 comorbidities 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-control studies. Multivariable logistic regression provides very flexible models to directly estimate odds ratios, to simultaneously adjust for confounders, and to allow for dependence in matched designs. Confidence intervals around the odds ratios are used to describe the degree of uncertainty due to sampling error (Table 9.2).

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

Table 9.2 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 $n=734$	Controls $n=2057$	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 Juurlink et al. [50]
*Adjusted for several confounders using multivariable regression analysis.

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 [64]. 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.

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 erroneous 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.

9.3.2.4 Case-Crossover Studies

In a case-crossover study, each patient acts as his or her own control [65, 66]. 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 patients experience an outcome event, they will be asked whether they 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.

9.3.3 Meta-analysis of Observational Studies

Meta-analysis of randomised research is well developed, and up-to-date methods are available [67]. 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 randomised studies for a long time, because observational research itself is very heterogeneous and is inevitably limited by confounding, and sources of bias are much more complex than in randomised studies. Nonetheless, recent advances in observational study meta-analysis methodology lead the Cochrane Collaboration to incorporate also non-randomised studies into their systematic reviews. Methodological issues of non-randomised 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/). Assessing the risk of bias included in observational study meta-analyses is a very important exercise but methodologically challenging. The ACROBAT-NRSI ('A Cochrane Risk Of Bias Assessment Tool for Non-Randomized Studies') is a good example of a useful instrument [68]. Several instruments have been developed to assess specifically the quality of adverse event reporting [69]. Bayesian methods are now increasingly used because they allow for more flexible meta-analytical modelling. An example of the usefulness of Bayesian meta-analysis is the combination of evidence on adverse effects from randomised and observational evidence. If carefully performed, this method can be used to integrate the advantage of both randomised (internal validity) and observational (external validity) studies, by giving less weight to observational studies, which often have higher precision but may contain confounding [71]. Network meta-analysis may handle direct and indirect comparisons simultaneously and is therefore essential when comparative effectiveness is assessed. Prospective meta-analyses including individual patient data meta-analysis are other emerging methods in the field.

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Part III

Tools in Clinical Pharmacology

Tools in Clinical Pharmacology: Imaging Techniques

10

Martin Bauer and Oliver Langer

10.1 Introduction

Biomedical imaging has been changing the way medicine is practised ever since. Imaging has the ability to show functional and biochemical changes that can help not only the understanding of disease mechanisms but also the response of the body to treatment [1]. Imaging techniques used in clinical pharmacology can be categorised as either functional or anatomical modalities. Functional modalities are capable of visualising 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 ultrasound imaging, X-ray, X-ray computed tomography (CT) and magnetic resonance imaging (MRI).

In addition, there is the possibility to combine different imaging modalities such as PET/CT, SPECT/CT and PET/MR in order to match functional to anatomical information.

In clinical studies, imaging end points might be closer to the cause of disease rather than non-specific physiological measures, such as vital signs or biomarkers (distinctive biological or biologically derived indicators). Such end points allow accurate quantification of disease effects or some associated correlate, and so

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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 [2, 3]. In the discipline of clinical pharmacology, imaging modalities can provide important information in the following areas:

- 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 and 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. The total time to bring a candidate drug from the start of human testing to market is nearly 9 years (this excludes the preclinical, animal testing phase, as well as discovery and research) [4]. Furthermore, it costs a company about US$1.4 billion in spending on research to develop a new drug [5]. Attrition is a major issue, e.g. in anticancer drug development, up to 95 % of drugs tested in Phase I trials are not reaching a marketing authorisation [6]. Furthermore, 40 % of exits from Phase I trials are caused by inappropriate pharmacokinetics of the test compound [7]. From an economical point of view, shortening the process of drug discovery and development would be a major contribution in reducing this substantial cost. Regulatory authorities acted in order to advance exploratory investigational new drug studies in humans. The European Medicines Agency (EMA) requires only reduced preclinical safety studies to support human clinical trials with a single dose of a pharmacological compound using microdose techniques [8]. According to this guideline, a microdose is defined as not more than a total dose of 100 μg that can be administered as a single dose or divided doses in any subject. A second microdose approach is one that involves <5 administrations of a maximum of 100 μg per administration (a total of 500 μg per subject) [8].

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 unlabeled 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 [9].

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" [10]. 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.

10.2 Positron Emission Tomography (PET)

For PET imaging, the so-called radiotracers are used, i.e. molecules labelled with short-lived positron-emitting radioisotopes, such as oxygen-15 (^{15}O, $t_{1/2}$, 2 min), nitrogen-13 (^{13}N, $t_{1/2}$, 10 min), carbon-11 (^{11}C, $t_{1/2}$, 20 min), gallium-68 (^{68}Ga, $t_{1/2,}$ 68 min) and fluorine-18 (^{18}F ($t_{1/2,}$ 110 min). For radiolabelling of large molecules (e.g. antibodies), which possess very slow in vivo kinetics, the use of longer-lived PET radionuclides, such as iodine-124 (^{124}I, $t_{1/2}$, 100.2 h), copper-64 (^{64}Cu, $t_{1/2}$, 12.7 h) or zirconium-89 (^{89}Zr, $t_{1/2}$, 78.4 h), is required. 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. 10.1. 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 localisation and quantification of the radiolabelled compound in the living organism. The sensitivity of PET for the detection of mass is very high (10^{-11}–10^{-12} mol/l), which allows administration of very small (micrograms), non-pharmacological drug quantities. The spatial resolution of PET depends on the size of the single detector component, varying between 2 and 8 mm^3 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 the radiotracer is injected intravenously and that arterial blood sampling is commonly employed for parent drug and metabolite analysis. The use of ^{11}C 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 ^{18}F-tracer of 400 MBq given intravenously corresponds to a total effective dose of about 5 mSv; the same amount of ^{11}C-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 (1–5 mSv/year).

As mentioned above, regulatory authorities have proposed a reduced preclinical safety testing package, when microdose quantities of drugs are administered to

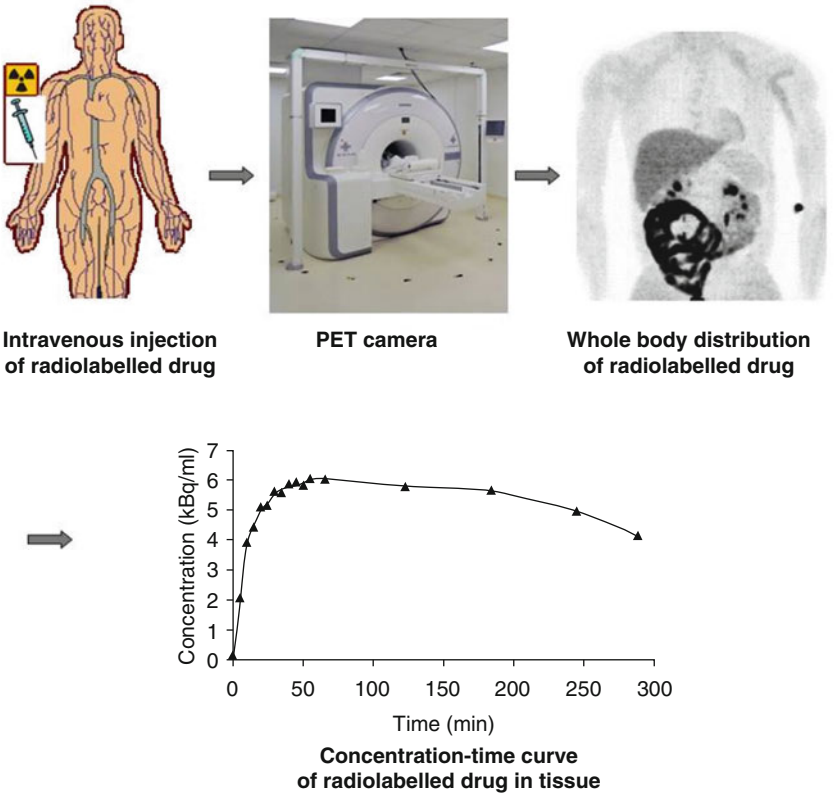

Fig. 10.1 The use of PET for pharmacokinetic measurements. Radiolabelled drug is intravenously injected, and its distribution and pharmacokinetics measured non-invasively by PET imaging. Analysis of serial PET images taken over time provides concentration-time curves of the drug of interest (e.g. in tissue targeted for therapeutic treatment). The shown example image and curve represent the radiolabelled antibiotic agent [^{18}F]ciprofloxacin (see Ref. [11])

humans [8, 12]. The current definitive international guideline was released in 2009 [13]. This endables and simplifies the use of PET studies with radiolabelled drug candidates ("PET microdosing") in first in human trials [14].

One limitation of PET is the low spatial resolution compared with anatomical imaging modalities such as CT and MRI. Moreover, PET gives limited anatomical information, which may make it difficult to identify and delineate certain tissues and organs in PET images. However, this can be mitigated by PET/CT or PET/MR combinations. Besides providing additional anatomical information, PET/MR now opens up the possibility to simultaneously acquire different functional readouts in one imaging session. Another limitation is that the short radioactive half-lives of PET radioisotopes commonly used for labelling of small drug molecules (^{11}C, ^{18}F) 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 for which human metabolism was difficult to predict by means of animal or in vitro models (warfarin, ZK253, diazepam, midazolam and erythromycin) [15]. Of the five 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 failed to provide detectable plasma levels for the oral microdose as being acid labile [15]. A later project included research on pharmacokinetics between a microdose and therapeutic dose for clarithromycin, sumatriptan, propafenone, paracetamol/acetaminophen, and phenobarbital in human volunteers. For all five drugs, an oral microdose predicted reasonably well the PK, including the shape of the plasma profile, following an oral therapeutic dose. An important finding of this study is that any deviation from linearity following the oral therapeutic doses occurs during the absorption process [16].

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. 10.1) [17]. In some instances, this approach has proven very valuable to predict response to treatment with the corresponding unlabelled drug, in particular for anticancer drugs [18]. Moreover, an emerging area of research is to use PET with radiolabelled drugs to study transporter-mediated drug-drug interactions in vivo in different organs (e.g. the brain, liver, kidneys, etc.) [19, 20].

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 allow for studying the displacement of the PET tracer from its pharmacological target by the drug in vivo. An example for this approach is given in Fig. 10.2. 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 [14, 22]. It has also been useful for assessing treatment response, particularly in oncology, by using metabolism tracers such as 2-[^{18}F]fluoro-2-deoxy-D-glucose or proliferation markers such as [^{18}F]fluorothymidine.

PET can also be combined with other modalities than imaging modalities in clinical pharmacology such as clinical microdialysis to assess intracellular drug

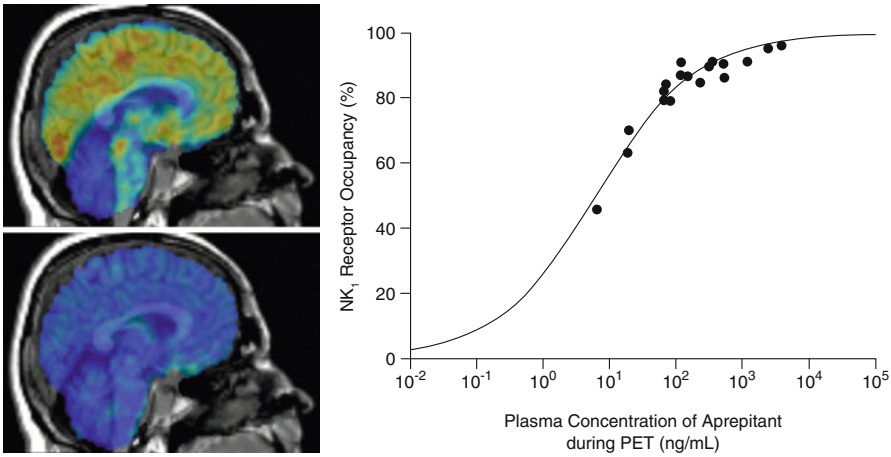

Fig. 10.2 The use of PET for receptor occupancy studies (Reprinted with permission from Ref. [21]). Different doses of unlabelled drug (aprepitant) are given, and binding of a validated PET tracer ([^{18}F]SPA-RQ) to drug target receptor in brain (neurokinin 1 receptor) is measured with PET. *Upper panel* (*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*)

pharmacokinetics in vivo. This is of considerable interest as many drugs possess an intracellular site of action. 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 [23].

10.3 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. technetium-99 m, ^{99m}Tc, $t_{1/2}$, 6.1 h; iodine-123, ^{123}I, $t_{1/2}$, 13.3 h; or indium-111, ^{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 localisation and distribution of the labelled compound are recorded. Advantages of SPECT over PET include lower costs, the potential for imaging with different radioisotopes simultaneously, the commercial availability of many molecular probes already in clinical use and the longer 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, drug penetration in gynaecology, myocardial perfusion and activity in cardiology, for perfusion and ventilation measurements in pulmonology and transporter occupancy in psychiatry and neurology [24–27].

10.4 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 visualise 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 localisation 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 haemoglobin and deoxyhaemoglobin in breast tumours shows promise as a biomarker for effective neoadjuvant chemotherapy in cancer patients as well as in detecting drug-induced vascular injury [28–31]. Furthermore, treatment response can be effectively monitored. The treatment effect of anticancer drugs measured as the optical metabolic imaging index showed responsive decrease and was further reduced when effective therapies were combined [32].

10.5 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 magnetisation 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, to assess the integrity of the blood-brain barrier or for differentiation of tumour and scar tissue. For improved visualisation 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 [33]. 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 [34]. MRS has found applications for measuring drug tissue levels in vivo [35, 36] and as fMRI in neuropsychiatry for drug trials, for example, in mood disorders and addiction [37].

10.6 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 heard contraction function in cardiology and in neurology for assessment of brain infarctions [38, 39].

10.7 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) [40], detection of inflammation [41]

and oncology (tumour size and extent, tumour perfusion) [42, 43]. Furthermore, placement inside or outside of the target tissue (e.g. muscle tissue, subcutaneous adipose tissue) of devices such as microdialysis probes can be detected [44].

10.8 Example for 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) [45]. To evaluate the plasma concentration-occupancy relationship, aprepitant dosed orally at 10, 30, 100 or 300 mg or 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. 10.2). High (≥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 at approximately 10 ng/ml and approximately 100 ng/ml, respectively. The presented study included 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. 10.2). 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 [14].

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 [21]. 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 [46].

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Pharmacokinetics II: ^{14}C-Labelled Microdosing in Assessing Drug Pharmacokinetics at Phase 0

11

Graham Lappin

Abstract

Microdosing came onto the scene with the first publication of data in 2003 (Lappin and Garner, Nat Rev Drug Discov 2(3):233–240, 2003). Since this time the number of compounds where the pharmacokinetics observed at a microdose compared to a therapeutic dose has grown steadily. Based upon the most up-to-date review at the time of writing (2013, reference Lappin et al., Expert Opin Drug Metab Toxicol 9(7):817–834, 2013), there are 35 compounds where microdose and therapeutic dose pharmacokinetics can be compared (oral, intravenous, human and animal). Of these, 79 % showed scalable pharmacokinetics between a microdose and a therapeutic dose when administered orally and 100 % when administered intravenously (scalable is defined as the pharmacokinetics being within a factor of 2). Where pharmacokinetic non-linearity is seen, a growing understanding of the mechanisms involved is 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, obtaining an early indication of the drug's metabolism in humans.

11.1 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 (a review on the history of radiotracers can be found in reference [3]). One of the

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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:

> 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 5,760 years. Calvin, exploiting this newly discovered isotope, pulsed $^{14}CO_2$ into illuminated algal suspensions, which were then solvent extracted. Over periods of time, the extracts were analysed 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 $^{14}CO_2$ 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 (^{1}H) and tritium (^{3}H), the KIE can be significant as ^{3}H is three times as heavy as hydrogen; consequently, the C–^{3}H bond has a lower zero-point energy than the C–^{1}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 [4]. 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.

11.2 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 C is radioactive, there have traditionally been restrictions on administration to humans, thereby limiting the possibilities for the study design [5]. 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 sieverts, 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 [6]. 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.

11.3 Accelerator Mass Spectrometry

AMS was first developed for archaeological radiocarbon dating in the mid-1970s [7]. 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 is therefore 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 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. 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 [8], 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 (2 μCi) without compound-specific approval.

11.3.1 The Emergence of Microdosing

In the 1990s, a technique known as microdosing emerged [1] where both the mass of drug and amount of radioactivity administered to humans were kept very low (≤100 μg and typically 200–1,000 nCi). Because of the low levels of radioactivity used in these studies, the analytical method of choice at the time was AMS. Subsequently, a number of microdose studies have been performed using LC-M/MS [9], but the method of analysis is not the focus of this chapter rather than the application of microdosing itself. 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 [10]. The term ‘microdose’ has however been confused with similar but distinctly different applications in pharmacokinetics, and this has led to some unfortunate misinterpretations of data. The definition of a microdose, in respect to an early stage (phase 0) study, has therefore been extended to a dose limited to 1/100th of the predicted pharmacologic dose or 100 μg whichever is the smaller, irrespective of the route of administration (see reference [2] for a more detailed discussion and examples of misinterpreted data).

The low dose administered in a microdose study is 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 phase 0 study would typically consist of four to eight 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 [11]. 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 [12]. 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 an 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, 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 only a few examples of metabolic profiling in the literature from microdose studies [13].

11.3.2 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 exhibit 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 crossover 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. 11.1 showing data from a microdose study with sumatriptan. ^{14}C-sumatriptan was administered to six volunteers as an oral dose on one dosing occasion and an intravenous dose on another dosing occasion. The dose was 100 μg, 200 nCi for both dose routes (the pharmacokinetics was reported in reference [14]). Plasma samples collected over time were analysed for unchanged parent drug and for the total ^{14}C concentration.

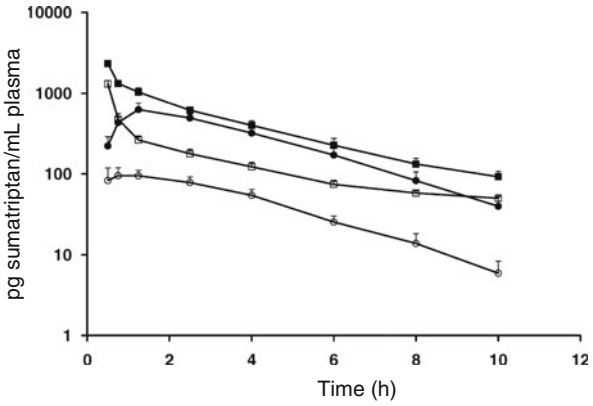

Fig. 11.1 Log-linear plot of plasma concentration vs. time following a single oral or single intravenous dose of 100 μg of ^{14}C-sumatriptan to human volunteers. Key, total ^{14}C of oral dose (●) unchanged parent drug for oral dose; (○) total ^{14}C for intravenous dose (■) and unchanged parent drug for intravenous dose (□). Error *bars* are + standard deviation, $n=6$

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 μg ^{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 was 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, AUC^{parent}/AUC^{total} was 0.17, thus showing only 17 % of circulating ^{14}C was parent drug.

11.4 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 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 (e.g. 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 35 drugs, approximately 27 have scaled between a microdose and a therapeutic dose for oral administration and 100 % for those given intravenously. Detailed comparisons have been made in a number of reviews on microdosing, the latest, at the time of writing, being reference [2], and so no further detailed discussion will be made here, other than to consider conditions under which non-linearity might appear.

Sumatriptan was given as an example above (also see Fig. 11.1). 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 is 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, 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-dose (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 scales very well from a microdose [15]. 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 [15]. Although a microdose study has been conducted with a therapeutic protein [16], many antibody-based drugs exhibit target-mediated disposition, and therefore microdosing is likely to have limited application in terms of pharmacokinetic predictions. 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 humans. Renal elimination of such compounds is severely impaired in the dog compared to the rat and humans [17]. 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 humans 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.

11.5 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. Metabolic profile data, however, have been obtained, and an example is shown in Fig. 11.2 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 [13]. Plasma samples collected at 24 h from dosing were pooled by subject and extracted and analysed by HPLC and AMS. The profiles presented in Fig. 11.2 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.

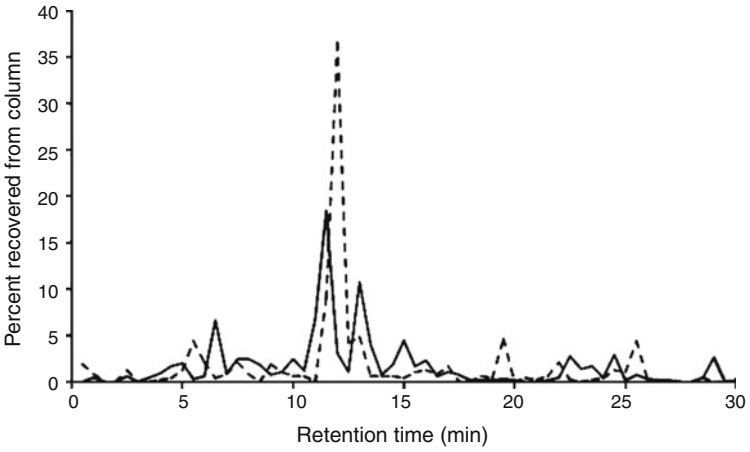

Fig. 11.2 Chromatogram showing the metabolic profiles of IDX989 (- – -) and IDX899 (—) in plasma 12 h from oral administration of 100 ug, 100 nCi of each candidate drug to four healthy male volunteers. The retention time of IDX989 was approximately 27 min and IDX899 26.5 min (From Ref. [13])

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 obtaining data from the target species, namely, humans.

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Current Concepts of Pharmacogenetics, Pharmacogenomics, and the "Druggable" Genome

12

Wolfgang M. Schmidt and Robert M. Mader

Abstract

The "post-genome" era we live in holds the great promise that our steadily growing knowledge on the genetics of interindividual drug response variability will be translated into clinical practice. According to the current intriguing concepts of pharmacogenetics and pharmacogenomics, genetic information of individuals can 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 "precision" medicine is expanding rapidly, and outstanding examples already exist of how the outcome of a genomic test dictates specific therapies. Major challenges, however, still lie ahead until genomic medicine will find its place in 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.

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12.1 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 [1, 2]. The human genome consists of 23 chromosomes containing a total of 3.54×10^9 base pairs of sequence information that includes ~20300 protein-encoding genes (Genome Reference Consortium, December 2013; http://www.ensembl.org/). The total mRNA content of a human cell, the transcriptome, however is estimated to consist of nearly 200000 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 fivefold lower than originally anticipated, the higher number of transcripts mainly results from alternative splicing events during mRNA maturation. Owing to a multitude of posttranslational 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 [3].

Except for monozygotic twins, humans differ on average in every 100th to 1000th 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 errors introduced during DNA replication. 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 biallelic rather than polymorphic. The human genome is scattered with many millions of such SNPs (also named single-nucleotide variants or SNVs), which constitute a dynamic molecular basis for interindividual 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 ~100 million human SNPs have been identified, ~10 million of which have been validated as common SNPs with a minor allele frequency higher than 5 % [4]. 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 [5–7]. The number is steadily growing and has been subject to substantial reconsideration after completion of the "1000 Genomes Project" (which provides the complete whole-genome sequence of more than 2500 individuals; http://www.1000genomes.org), especially with respect to more complex sources of variation such as inversions, "indels" (i.e., deletions with concomitant insertions), and copy number variations (CNV) [8]. With the advent of next-generation sequencing technologies [9], even larger datasets capturing a tremendously wide spectrum of human genetic variation within coding regions were created and are available to the public (i.e., the "Exome Sequencing project" (ESP), comprising the exome sequences of 6500 individuals, or the "Exome Aggregation Consortium" data (ExAc, January 2015 release, encompassing data from more than

60000 unrelated individuals). The "Precision Medicine Initiative" will even set a new standard in this context by producing datasets comprising the genetic information of a million or more Americans [10].

While the majority of SNPs are located in intergenic regions, SNPs affecting gene sequences fall into 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 and (ii) coding-region SNPs affecting exon sequences with the potential to alter the amino acid sequence or the correct length of the encoded gene product. To date, more than 235000 validated non-synonymous coding SNPs that tag haplotypes are known (HapMap release 27; http://hapmap.ncbi.nlm.nih.gov/) [11], but it is estimated that literally each of the ~500000 exons encoded by the human genome harbors at least two rare coding variants.

12.2 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) [12, 13]. 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 [14–17]. 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 [18]. 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. The PharmacoGenomic Mutation Database (PGMD™) represents the probably most comprehensive database resource hosting all published genetic variants that have been shown to affect drug response in patients (http://www.biobase-international.com/product/pgmd) [19]. 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 a discipline that uses genome technology to study the relationship between drug effects and all relevant genes. Advanced technology such as GeneChip arrays or "RNASeq" (deep sequencing of RNA by next-generation sequencing technology) [20, 21] 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 tumor cells, suitable to predict response to an anticancer 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 [22]. Figure 12.1 shows an illustration of different applications of the underlying concept, and Table 12.1 summarizes important examples, which are elaborated within this chapter. To provide a clinical example, the selection of patients with melanoma was successfully performed based on the *BRAF* status, because dabrafenib was specifically synthesized to inhibit the V600E mutation of the *BRAF* gene: The proper patient selection was one of the first steps ahead in the treatment of a life-threatening type of cancer [23].

12.3 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 [24, 25]. Only some members of this family, such as CYP1A1, CYP2E1, or CYP3A4 (a key enzyme involved in drug metabolism), are

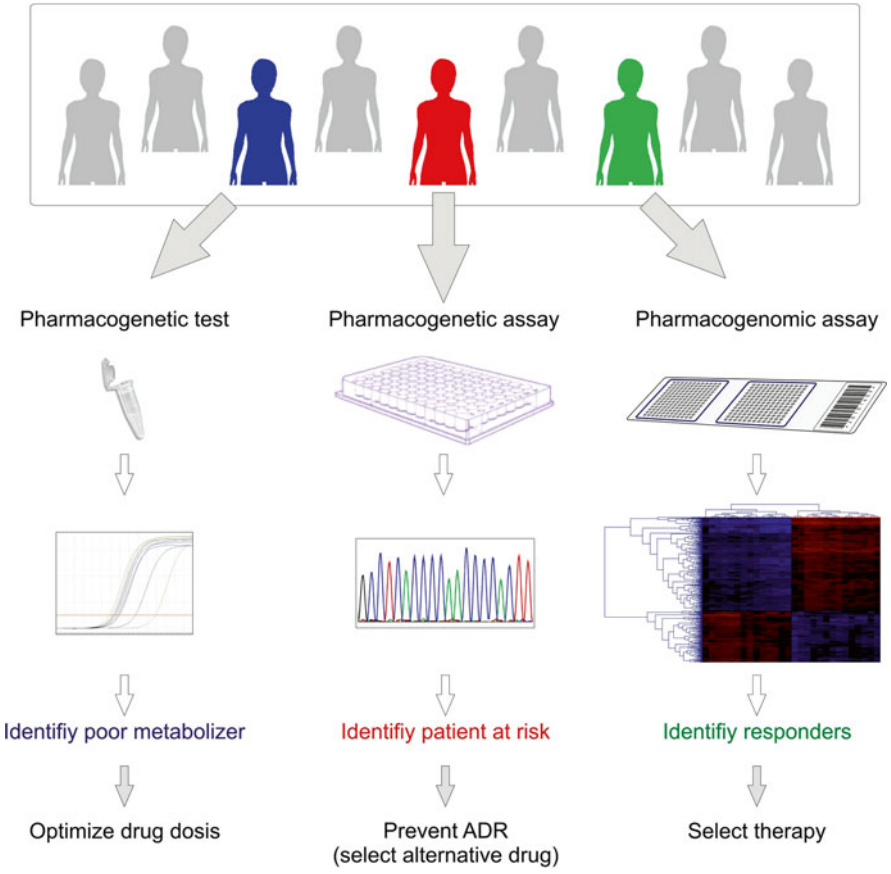

Fig. 12.1 Applications of the "Genomic Medicine" concept. Pharmacogenetics- and pharmacogenomics-based assays employ information from the individual's genome to guide individualized treatment decisions and risk predictions. The illustration summarizes three possible applications, showing how genetic 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 or next-generation sequencing ("RNASeq"), whose readout allows, e.g., to relate gene expression changes in tumor 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 12.1 Selected examples of pharmacogenomic assays with clinical relevance

Genomic marker/class	Molecular phenotype	Clinical phenotype	Clinical utility	Example
Gene polymorphism				
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 nonresponders	*ABCB1* (antiepileptics)
Organic anion transporter	Reduced function	Reduced drug uptake	Identify nonresponders/risk of ADR	*SLCO1B1* (statins)
Metabolism (phase II reaction)	Reduced function	Reduced elimination	Identify patients at risk of ADR	*UGT1A1* (irinotecan)
	Deficiency	Severe toxicity	Identify patients at risk of severe toxicity	*TPMT* (6-mercaptopurine)
Drug-related pathway	Specific gain of function	Hypersensitivity	Identify patients at risk of ADR	*HLA-B*5701* (abacavir)
Gene duplication				
Metabolism (phase I reaction)	Hyperfunctional	Ultrarapid metabolizer	Increase drug dose	*CYP2D6* (antidepressants)
			Lower dose of prodrugs	*CYP2D6* (codeine)
SNP profile				
Drug target/receptor	Variable	Therapy response	Individualize drug therapy	*AGT*, *APOB*, *ADRA2A*
Protein biomarker				
Somatic cancer phenotype	Overexpression	Drug response	Identify patient subgroup for treatment	HER2/neu (trastuzumab)

Gene mutation				
Somatic cancer mutation	Proapoptotic	Increased drug response	Identify patient subgroup for treatment	*EGFR* and *KRAS* (cetuximab, gefitinib, and erlotinib) *BRAF* V600E (vemurafenib, dabrafenib)
Germline mutation	Deficiency	Inherited disease	Select patients for therapy	*DMD* nonsense mutation (Translarna™)
Gene expression signature				
Somatic cancer phenotype	Altered expression profile	Response to therapy	Prediction of prognosis/ therapy response	MammaPrint™, OncoType DX®, EndoPredict®
		Tumor subgroup	Classification	Lymphomas
Genome-scale sequencing				
NGS of tumor and control genomes	Somatic cancer mutations	Treatment prioritization and clinical trial finding	Clinical treatment decision support in cancer patients	TreatmentMAP™

Abbreviations used: ADR adverse drug reaction; *NGS* next-generation sequencing

relatively highly conserved. In contrast, to date more than 60 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 [26]. Another example of a clinically relevant polymorphism affects the *CYP2C19* gene and occurs predominantly in Asian populations. This variant renders omeprazole therapy and eradication of *Helicobacter pylori* much more effective in Japanese as compared to Caucasians [27]. 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 [28]. More recently, also gene promoter methylation or microRNA-mediated regulation of the expression of some CYP genes has been described [25, 29].

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 tumors. 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 [30, 31]. 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 overexpression of *ABCC6* in 5-fluorouracil resistance of colon cancer [32].

It is not unlikely that similar to the polymorphic CYP gene family considerable genetic variation also exists in genes encoding organic anion transporters [33, 34]. For instance, a common genetic variant of the *SLCO1B1* gene encoding organic anion-transporting polypeptide 1B1 has been shown to reduce the hepatic uptake of many statins, increasing the risk of statin-induced myopathy [35–38]. In addition, the same gene has been shown to harbor two SNPs that are important determinants of pharmacokinetics and clinical effects of methotrexate [39, 40].

12.4 Pharmacogenetic Testing for Optimizing Drug Dose

Assessment of allelic variation of the *CYP2D6* and *CYP2C9* genes is generally regarded as 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 [24, 25], CYP2C9 metabolizes 10–20 % of commonly prescribed drugs [41]. 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 hyperfunctional 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 in clinical studies by increased warfarin plasma levels, decreased clearance, and increased frequency of bleeding [42]. Pharmacogenetic testing may therefore represent 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 protein1 [43]. The clinical pharmacology advisory panel to the US 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), which had been developed and launched by Roche Diagnostics (Indianapolis, IN), has been available to assess the major allelic variants in the *CYP2D6* and *CYP2C19* genes (AmpliChip CYP450 test). Although the product had been approved as in vitro diagnostic device (CE-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 231 genes in one assay. By employing this highly advanced system, an additional variant associated with the abovementioned warfarin dosing variability was discovered [44]. 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 [45]. Recently, next-generation sequencing approaches have been adapted to comprehensively screen a large number of genes

in a single and cost-effective test, such as, e.g., PGxOne™ clinical pharmacogenomics (Admera Health, South Plainfield, NJ).

A most promising methodological step ahead was the analysis of circulating DNA to predict therapy response to the multikinase inhibitor regorafenib in patients with metastatic colorectal cancer [46]. So far, a tissue biopsy was necessary to diagnose mutations in the malignant lesion as in the case of *KRAS* mutations impairing cetuximab activity. With this noninvasive approach, we are entering the era of "liquid biopsy," where all cancer-associated mutations are either detected as circulating DNA [46] or isolated from exosomes, which seem to be a representative blood compartment to detect genetic cancer aberrations [47].

12.5 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 [48–50]. It was estimated that adverse drug reactions account for 10 % of all hospital admissions and constitute a leading cause of death [51]. 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 [52]. Intolerance to 6-mercaptopurine due to thiopurine methyltransferase (TPMT) deficiency, a standard drug used in the 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 [53]. 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 glucuronyl transferase 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 [54]. The common European UGT1A1*28 allele was further significantly associated with grade IV neutropenia in a prospective trial [55]. Refining research on this issue has demonstrated that specific SNPs in *UGT1A* other than UGT1A1*28 influence irinotecan toxicity. Most interestingly, one of these alleles was protective, whereas the other had unfavorable effects on the dosage effect [56]. Recently, this knowledge has been successfully applied to patients with gastrointestinal or lung cancer to individually dose irinotecan according to the *UGT1A1* genotype [57].

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 [58]. However, the existence of many different rare *DPYD* alleles related to compromised 5-fluorouracil metabolism severely complicates genetic testing. For instance, a deep intronic variant has been described in intron 10 of *DPYD* (c.1129-5923C>G). The resulting cryptic splice donor site inserts 44 bases in the mRNA of DPD and introduces a premature stop codon resulting in enhanced 5-fluorouracil toxicity due to reduced DPD expression [59]. 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 has not taken action to recommend genetic *DPYD* testing for 5-fluorouracil until 2011. In contrast, pharmacogenetic testing of *TPMT* and *UGT1A1* had been supported earlier, leading to appropriate changes to the labels for 6-mercaptopurine and for irinotecan in 2004 and 2005, respectively.

12.6 Genetics of Pharmacodynamics

A significant number of patients, with estimates ranging from 30 to 60 %, treated with various drugs, do not respond to treatment [15, 24]. The presence of non-responsiveness is usually detected clinically and the reasons for the lack of drug effects often remain "idiopathic." During the development, over a quarter of drugs that enter clinical development fails because they are ineffective. Numerous pharmacogenetic studies hold great promise to change this situation in the future. A recent comprehensive survey of genetic evidence predicting drug mechanisms allowed the conclusion that selecting genetically supported targets could ultimately double the success rate in clinical development (from phase I to approval) [60].

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 α2A-adrenoreceptor gene (*ADRA2A*) was associated with response to the β1-adrenoreceptor blocker atenolol [61]. Another SNP within the *APOB* gene was also associated with the blood pressure response to irbesartan but not to atenolol [62]. 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 [63]. Genotyping of the *HMGCR* gene could help in selecting patients suitable for additional or alternative therapeutic strategies in cholesterol reduction [64]. 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 [65].

12.7 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 nonresponders 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 [66]. 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 [67, 68]. This

will also force the 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 [69]. 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 (~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 [70].

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 ataluren (now called Translarna and formerly known as PTC124) [71], 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 are disrupted by mutations, such as the antisense oligonucleotide drisapersen (also known as PRO051) [72] or the morpholino AVI-4658 (now called eteplirsen) [73]. These therapies aim at healing a genetic lesion rather than the disease itself [74]. Therefore, ataluren therapy could, e.g., be applied 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 the future [75]. The conditional approval in late 2014 by the EMA and FDA of ataluren for the treatment of Duchenne muscular dystrophy [76–78] represents a milestone of personalized medicine in the context of rare diseases but also demonstrates that "personalized" means even smaller numbers of patients: only 10–15 % of Duchenne muscular dystrophy patients will be eligible for Translarna therapy.

Another great potential for genomic testing lies in the 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 tumors, such as the distinction between Burkitt's lymphoma and diffuse large B-cell lymphoma [79].

In addition, brilliant genomic approaches that go beyond disease classification have been developed for the prediction of prognosis and response to cancer therapy [22, 80]. 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 [81, 82]. 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 [83]. 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®) [84–86] or prognosis in early-stage non-small cell lung cancer [87].

Gene expression signatures have also been developed to predict resistance to four common drugs used to treat acute lymphoblastic leukemia in children [88, 89]. 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 [32]. 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 [90]. The interrogation of the transcriptome of peripheral blood mononuclear cells or even plasma-based miRNAs represents a further possibility to measure dynamic gene expression data, e.g., to address inflammation-related states [91–93]. 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 [29].

Owing to next-generation sequencing technologies, like 454 (Roche), SOLiD (Life Technologies), or Illumina (formerly Solexa), 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 [94] likely will generate additional useful knowledge to be applied in genomic medicine [95, 96]. It is also foreseeable that in the near future “metabolomics” might complement genomics- and proteomics-based strategies on the way to individualized drug therapy [97].

12.8 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 [98]. The druggable genome is composed of the subset of the ~20000 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 30000 – 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 [99]. 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 [100]. 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 [101]. 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 [102].

Although there are many issues still unsolved, in the treatment of cancer, we have already started to apply this knowledge to the benefit of patients. In an exciting pilot study, Von Hoff and coworkers demonstrated how molecular tumor profiling is suitable to select and address druggable mutations and molecular targets [103]. At the Comprehensive Cancer Center of the Medical University of Vienna, we have extended this approach in the "EXACT" trial to treat cancer beyond anatomic boundaries. In this ongoing prospective trial, real-time biopsies are taken from patients without further standard therapy and analyzed by genomic profiling with ultrahigh multiplex PCR. Druggable aberrations are then addressed by a treatment algorithm including off-label use of anticancer medicines independent of their indication. This leads to an individualized approach, where every enrolled subject serves as his own control for outcome, when, e.g., progression-free survival from the last line of therapy is taken as baseline parameter for therapeutic success. This concept may be easily completed by other diagnostic procedures including imaging and, as done already, by ex vivo testing of tumor sensitivity to an array of targeted and non-targeted drugs.

12.9 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 represents milestones in pharmacogenetics. The current path of regulatory authorities such as the FDA is highly promising [68]: 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 ~40 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" or the Exome Sequencing Project (ESP), set the scene for the vision even of personal genome sequencing for clinical purpose. Additionally, rapidly evolving DNA technology platforms, high-throughput screening systems, and advanced bioinformatics will allow the tailoring of therapeutic agents targeted for specific subgroups of the population. This necessitates a specific infrastructure suitable to fulfill the scientific as well as the regulatory requirements to serve as diagnostic basis for individualized patient therapy. 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 [22, 50, 104]. 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 [67]. 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 remain 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 "pharmacoepigenetics" 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 [29]. 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 nongenetic 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 relevance [105–107]. 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 three HLA genes was identified to be highly associated with hypersensitivity to abacavir [108, 109]. 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 [105, 110]. Clinical studies revealed that withholding 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 % versus 7.8 % in the control group consisting of patients without prospective screening) [111]. 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 [112]. Screening for *HLA-B*5701* is now mandatory according to guidelines of the European AIDS Clinical Society (EACS).

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Pharmacokinetics I: PK-PD Approach, the Case of Antibiotic Drug Development

13

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Abstract

The therapeutic efficacy of antibiotics is very dependent not only on the drug itself but also on whether there is enough drug concentration at the site of infection for a sufficient amount of time that would stop the development of bacterial resistance and on the susceptibility and severity of the infection. Therapeutic success of therapy is also dependent on the physiological characteristics of the host, including disease state, age, comorbidity, and other factors that are highly variable in the clinical setting. Understanding how these factors or covariates affect antibiotic disposition and their pharmacological effects as well as the relationship between drug concentration in the body at the site of infection and the drug effect on the bacterial pathogen is part of the general concepts of pharmacokinetics (PK) and pharmacodynamics (PD). By using PK-PD principles and linking specific PK exposure parameters to microbiological outcomes, clinicians have designed better dosing strategies for specific classes of antibiotics. By evaluating how covariates affect the drug disposition in specific patient population, dosing regimens can be designed for that population to achieve an optimal therapeutic goal. We examined PK-PD principles that characterize antibiotic activities, experimental designs to characterize pharmacodynamic properties of antimicrobial agents, modeling and simulation approach for translation from *in vitro* time-kill and animal infection models to human efficacy, and dosing strategies in special populations including critically ill, renal-impaired, obese, geriatric, and pediatric patients, as well as Bayesian approach to individualize dosing

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regimens based on the sampled drug concentration in a therapeutic drug-monitoring setting. The model-based approach can also streamline the drug development process and support decision-making with greater confidence. These decisions include but not limited to planning clinical trials and developing optimal dosing strategies, and these crucial steps in the drug development process can be costly if the wrong decisions are made. From the perspective of clinical practice, the modeling and simulation approach can provide a more precise medicine to the patients and improve the healthcare outcome. By utilizing all the information available, from *in vitro* studies, animal models, clinical trials, and patient characteristics, the goal is to maximize the benefits to the patients through evidence-based medicine and practice.

13.1 Pharmacokinetics and Pharmacodynamics Concepts in Antimicrobials

Establishing pharmacokinetic-pharmacodynamic (PK-PD) relationship of a new drug candidate is very crucial for the drug development process. Because of its importance, many pharmaceutical companies have a translational group within their clinical pharmacology department to bridge the PK-PD information from preclinical studies to design first-in-human trials. In this chapter, we shall use antimicrobial drug development for our discussion given that the PK-PD approach in this therapeutic area is very well developed and these concepts are broadly applicable to other therapeutic areas.

13.1.1 Principles in Antimicrobial Pharmacokinetic and Pharmacodynamic

When a drug is given to a patient, there are processes that govern its absorption, distribution, metabolism (or biotransformation), and elimination, which are collectively known as ADME and belong to a larger concept known as pharmacokinetics. Pharmacokinetics, commonly referred to as "what the body does to the drug," evaluates the kinetic behavior and the concentration-time profile of the drug, resulting in important information such as total body clearance, volume of distribution, bioavailability, and protein binding. The information is predictive of the time-course of drug kinetics in both physiological and pathological conditions and can be used to project other doses, dosing regimens, as well as steady-state conditions.

When a sufficient drug concentration is present at the site of action for a duration long enough to elicit an interaction with its target resulting in a pharmacological response, the process is called pharmacodynamic, which is also known as "what the drug does to the body." The bacterial pathogen is the target site of action for antibiotics. Unlike a biochemical marker that is produced by the body in response to the drug, the challenge with antimicrobial therapy is that the time-course of bacterial pathogen response is very difficult to measure in the host. Instead, an *in vitro*

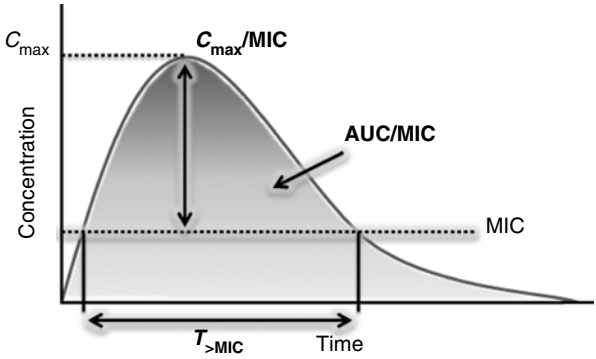

Fig. 13.1 Pharmacokinetic-pharmacodynamic indices as targets for achieving antimicrobial efficacy (Image is adapted from Asin-Prieto et al. [5] and used with permission)

parameter that provides information of the susceptibility of the pathogen toward a specific or combination of antimicrobial agent(s) is determined, called minimum inhibitory concentration (MIC). The MIC is the lowest antibiotic concentration that inhibits the visible growth of the microorganism after a 16–20-h incubation period. The quantitative relationship between a pharmacokinetic parameter and the microbiological outcome is known as pharmacodynamic index. The three common MIC-based PD indices (Fig. 13.1) for *in vivo* prediction of antimicrobial efficacy are the time at which the drug concentration is above MIC over the 24-h interval ($T>$MIC), the peak drug concentration over MIC (C_{max}/MIC), and the 24-h area under the concentration-time curve over MIC (AUC/MIC) ratios. The italicized prefix *f* often seen associated with these indices refers to the free drug concentration.

If the relationship is time dependent as characterized by $fT>$MIC, the strategy is to maintain the free drug concentrations above the MIC for an extended period of time. For example, the target PD index for ceftazidime is to maintain its concentration above MIC for 60 % of the dosing interval [1]. If the efficacy is concentration dependent, the aim is to attain sufficient peak drug concentrations above MIC. For gentamicin, which belongs to the aminoglycoside class of antibiotics, the target fC_{max}/MIC ratio is between 8 and 10 [2]. The third type is concentration independent with prolonged persistent effects, which is best characterized by the 24-h *f*AUC/MIC ratio. Vancomycin, an example of antimicrobial belonging to this group, is dosed to achieve a target *f*AUC/MIC ratio $\geq$400 h for treating *Staphylococcus aureus* pneumonia [3, 4].

Complementing the MIC-based approach, the time-course-based approach can be developed from time-kill kinetic studies that trace the time-course of bacterial response to both constant and dynamic drug concentrations. The time-course approach gives more detail of how the bacterial density changes or reacts to an antimicrobial challenge over time. The information from static time-kill studies are used for model development to link the free drug concentration to the changes in bacterial density; the dynamic time-kill curves are then used to validate the model or to simulate *in vivo* microbiological outcome by mimicking human pharmacokinetics for humanized dosing regimens. The effects of various dosing regimens, drug half-lives, and even starting inoculum sizes can be simulated in the *in vitro* setting to evaluate the best dosing strategy going into the clinic.

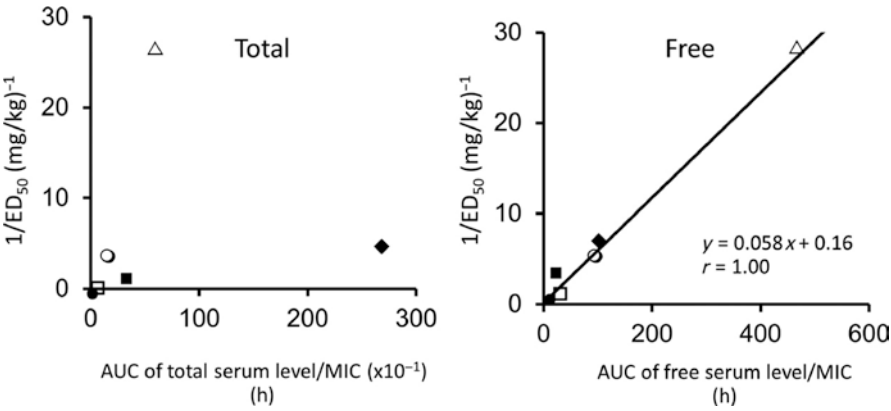

Fig. 13.2 Correlation between therapeutic potency (ED_{50}) of several cephem antibiotics against intraperitoneal infected *K. pneumoniae* mice model against AUC/MIC of total drug concentration (*left*) and unbound drug concentration (*right*) (Image adapted from Tawara et al. [7] and used with permission)

13.1.2 Free Unbound Drug Concentrations

It is generally accepted that only the free, unbound antibiotic concentrations in the interstitial fluid at the target site can exert its antimicrobial effect. Hence, the free drug concentration is often used in predicting therapeutic efficacy rather than the total plasma concentrations. Given that most infections occur not in the plasma but in tissue sites, the ability of antibiotics to reach the target sites is key in determining clinical outcome. It is noted that tissues are not homogeneous compartments and the drug distribution from the plasma into the tissues depends on the physicochemical properties of the drug.

Once in the plasma, a portion of the drug binds to plasma proteins or blood cells. The other unbound portion, also called free fraction, in the plasma can move freely into the tissues. A similar scenario occurs in the tissue wherein some drug molecules could bind to the tissue proteins or cells, whereas the remaining portion stays unbound in the tissue fluid. The difference between the total plasma concentrations and the free tissue concentrations can be quite significant especially when protein binding of the antibiotic is high [6]. For this reason, the total plasma concentration is not an ideal measure for the purpose of dose finding of an antibiotic candidate. Rather the free unbound drug concentration at the infection site should be used, if available. The result from a study that investigated the therapeutic efficacy of a series of cephem antibiotics in mice that were infected with *K. pneumoniae* intraperitoneally indicated a close relationship between efficacies measured by ED_{50} with the AUC/MIC of the free drug concentration (Fig. 13.2) [7]. The unbound drug exposure is clearly a better predictor of microbiological outcome than the total drug exposure.

It is also important to consider that the *in vitro* MIC values are determined in free antibiotic concentrations and protein binding of the antibiotic is often not taken into

consideration. To fully evaluate antimicrobial activity, free drug concentration at the target tissue would be the appropriate measure for evaluation. Although MIC is an established pharmacodynamic parameter that is routinely determined in microbiological studies, it is not an ideal measurement of antimicrobial activity. One has to consider that antimicrobial activity is a dynamic process and MIC is only a threshold value [8]. Thus, MIC can only provide an approximate information of antibacterial effect of the antibiotic. An alternative pharmacodynamic approach is the bacterial time-kill kinetics, which can offer more detailed information about the antimicrobial activity as a function of both time and antibiotic concentration.

13.1.3 Model-Based Drug Development

The model-based drug development approach is particularly important for the development of antimicrobial agents, as one can see that the determination of which of the PD indices best characterizes the antimicrobial activity of the drug and the translational value of PK-PD models based on *in vitro* time-kill kinetics are all model-based approaches. Using the MIC-based PD indices, the goal is to find a dose or dosing regimen where the index is attained or surpassed. Assuming that the PD index and the target value are already known, one would then use the human PK model from phase I ascending dose studies to determine the dose, dosing interval, and infusion duration, through simulations of human pharmacokinetics, for the design of the phase II and III trials.

The population PK model has both deterministic and stochastic components [9]. The deterministic model often takes the form of ordinary differential equation (i.e., compartmental models). The stochastic component describes the variability between individuals and also determines the sources of intersubject variability using covariate models. The second level of variability is intraindividual variability which can be characterized by a residual error model and inter-occasion variability. For antimicrobial therapy, the covariate model is very important as many antibiotics are dosed by body weight, renal function, etc. This is the case because body weight, creatinine clearance, and other variables were shown to be important and influential covariates or predictors of drug disposition from the data collected from human trials.

Once the population PK model is developed for humans and the PD index is determined from *in vitro* or animal studies, simulations in virtual patients can be done to evaluate which dosing regimen(s) will achieve the desired PD index. As with most phase I studies, the pharmacokinetic data used for model development usually come from healthy volunteers. Therefore, in the design of the proof-of-concept phase II study, the preliminary assumption would be that the pharmacokinetic in the phase II patients is similar to that in the phase I, although this is not always the case. Thus, the process of model development and refinement is an ongoing process in drug development, because the pharmacokinetic data from different patient populations come at different stages and the population PK model will need to be updated with more information from these populations as more information becomes available.

With the MIC-based approach, the concept of probability of target attainment (PTA) and cumulative fraction of response can be determined from simulations.

The PTA is the evaluation of the percentage of the simulated individual's profiles that are over the threshold PD index. About 10,000 concentration-time profiles are simulated, based on the interindividual variability information from the population PK model. The distribution of PD index from the simulated profiles becomes the basis for estimating the likelihood of achieving a specific target value. Assuming a simple case scenario where the drug's PD index is based on *f*AUC/MIC, the AUC is related to clearance for a one-compartment intravenous model. Given that both the mean and variance for the population clearance are known, the AUC distribution can be derived by simulating 10,000 clearance values of virtual individuals and dividing the dose by the clearance values, as $\mathrm{CL} = \mathrm{Dose} / \mathrm{AUC}$ or $\mathrm{AUC} = \mathrm{Dose} / \mathrm{CL}$. The derived AUC values from the virtual population would then be multiplied by the fraction of the drug unbound and then divided by the MIC value. Using a statistical program, PTA is computed by ranking the simulated individual *f*AUC/MIC values and estimating the percentiles that these values are greater than the target PD index [10, 11]. The plot of PTA has MIC on the *x*-axis and the probability on the *y*-axis. In many cases, the 90 % probability is considered an acceptable criterion [10].

An alternative prediction of microbiological outcome using Monte Carlo simulation is the cumulative fraction of response (CFR), which is the expected population PTA given a population of microorganisms for a specific dosing regimen [5]. CFR is one further step after PTA is already obtained, such that $\mathrm{CFR}(\%) = \sum_{i=1}^{n} \mathrm{PTA}_i \times F_i$, where PTA at specific MIC denoted by the subscript *i* is multiplied by the frequency (*F*) of bacterial isolates with the specific MIC. The summation of this product over the range of MIC values is CFR. Because PTA is reported as a percentage, CFR also has percent for its unit. The expected probability of success for a dosing regimen is based on the population distribution of the MIC values rather than a single MIC value. This approach is more applicable for community medicine than for drug development because the pathogen susceptibility may not be available when the patient is diagnosed with an infection. Instead, the range of MIC values from the specific healthcare facility could be used. With this situation, it becomes important that the selection of the range of MIC values is representative of the locality, as pathogen susceptibility may be different between countries, areas, and hospitals, as well as over time.

Another extension of the PTA concept is the classification of specific treatment regimen against a microorganism population. The microorganism's antibiotic phenotype can be classified based on a quantitative microbiological susceptibility evaluation that is associated with the likelihood of therapeutic success. Clinical breakpoints are established on whether the patients are likely or unlikely to respond to an antimicrobial treatment by categorizing microorganism susceptibility by a defined phenotypic test [12]. Breakpoints are determined either from statistical approach, for example, decision tree models or logistic regression, to define a PD index value that differentiates treatment success from failure or by probabilistic approach using PTA. The microorganisms with MIC value that result in PD index with values lower than the target are categorized as resistant, whereas those with values higher than target are susceptible. The target value that separates the two susceptibility phenotypes is the clinical MIC breakpoint. It is important to keep in

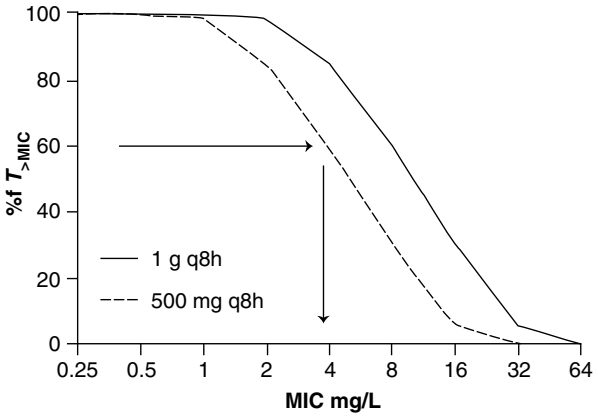

Fig. 13.3 The percentage of time that the free ceftazidime concentration is above MIC (*%fT*>MIC) for two dosing regimens of ceftazidime (1 g q8h vs. 500 mg 18 h) against MIC to illustrate that clinical breakpoint is dependent on the dosing regimen. *Arrows* indicate that the pharmacodynamic target corresponding to 60 % *fT*>MIC is 4 and 8 mg/L for 500 mg q8h and 1 g q8h, respectively (Image is adapted from Mouton et al. [12] and used with permission)

mind that different dosing regimens could shift the clinical breakpoint. An example is the relationship between *fT*>MIC and MIC of ceftazidime for two different dosing regimens that resulted in distinct curves (Fig. 13.3) [12, 13]. As previously discussed, the target for PD index of ceftazidime is 60 % *fT*>MIC, and the dosing regimen of 500 mg t.i.d. and 1 g t.i.d. would result in breakpoints of 4 and 8 mg/L MIC, respectively.

With the modeling approach using time-course data from time-kill studies, one can also simulate the bacterial response to a dosing regimen. Prior to being able to simulate bacterial response to changing drug concentrations, a semi-mechanistic model, which is an empirical model that captures the broad general characteristics of bacterial growth, drug killing effects, and bacterial regrowth in response to several static drug concentrations, is developed from the static time-kill data. The dynamic time-kill data is then used to validate the model. The most common type of semi-mechanistic models in the literature is based on the logistic growth model. As Sy and Derendorf have provided an extensive discussion of the various types of semi-mechanistic models, the details of the logistic model can be found in their article [14] and will not be discussed in detail here.

The semi-mechanistic model developed from the *in vitro* time-kill curves and incorporating the population PK model of the antimicrobials can be used to simulate the *in vivo* antibacterial activity in changing free drug concentrations that mimic human pharmacokinetics. An example illustrates how the semi-mechanistic model and the PK model of gentamicin in end-stage renal disease (ESRD) patients undergoing hemodialysis are used to evaluate two dosing regimens of gentamicin, assuming the typical patient's body weight of 70 kg: (1) 120 mg immediately after hemodialysis and (2) 240 mg given 1 h before hemodialysis [15]. Because hemodialysis has a tendency to remove majority of the administered drug in ESRD patients, the current guideline for dosing gentamicin is to administer half of the full dose

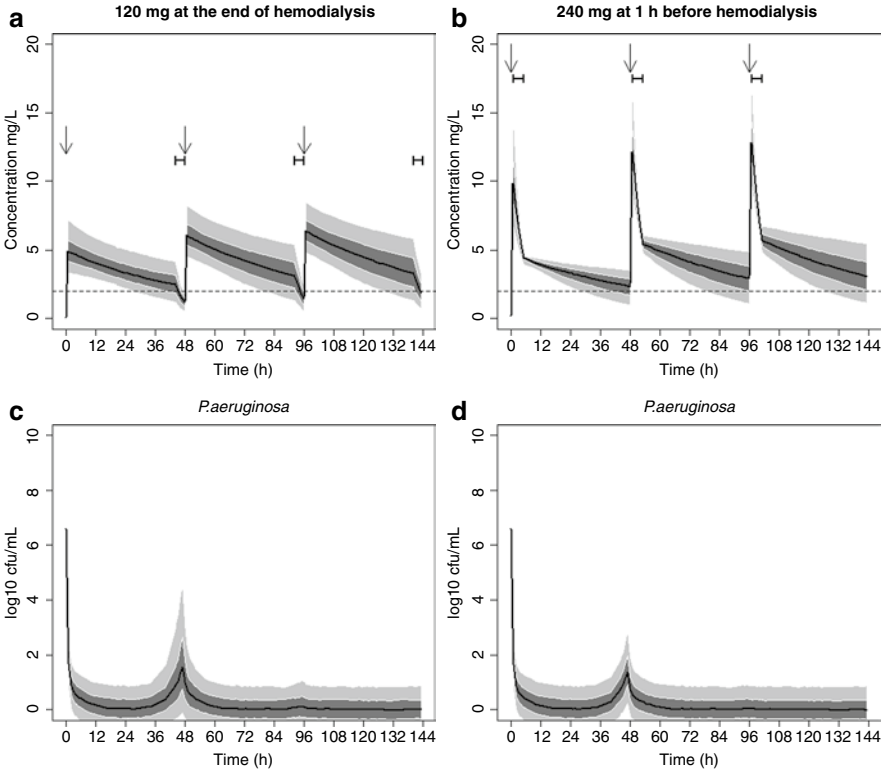

Fig. 13.4 Prediction of antibacterial activity of gentamicin against P. aeruginosa under two dosing regimens. (**a**) Concentration-time profile with 120 mg at the end of hemodialysis. (**b**) Concentration-time profile with 240 mg 1 h before hemodialysis. (**c**) Bacterial response-time profile with 120 mg at the end of hemodialysis. (**d**) Bacterial response-time profile with 240 mg 1 h before hemodialysis. Dashed lines represent the safety threshold (2 mg/L). *Arrows* represent dosing time. I bars represent the time interval of hemodialysis (Image is adapted from Zhuang et al. [15] and used with permission)

immediately after hemodialysis to avoid drug loss during dialysis and to mitigate toxicity associated with slower drug clearance in these patients. With this dosing regimen, however, the required fC_{max}/MIC ratio of 8–10 for gentamicin may not be achieved. Thus, a predialysis dosing using the full dose was recommended to hit this target.

Figure 13.4 shows the simulated PK profiles and the corresponding predicted antimicrobial activities. The simulated bacterial densities in response to the two dosing regimens indicate that the half dose of gentamicin at the end of hemodialysis resulted in similar antibacterial activity as the proposed dosing wherein full dose of gentamicin was administered an hour before the procedure. This example is a case wherein the PD index may not give sufficient information about how the dialysis procedure is affecting the time-course of antibiotic action, while a PK-PD model simulation can potentially give the whole picture of the time-course of bacterial response to specific dosing regimens.

13.2 Designing Antimicrobial Experiments

13.2.1 Microdialysis for Determination of Time-Course of Free Drug Concentrations

Originally developed in the 1960s to measure neurotransmitters in the brain [17], microdialysis has since been adopted in drug development to evaluate free drug concentrations in the tissue. Microdialysis is a tool to measure target site concentrations of antibiotics or other drugs, metabolites, and endogenous substances in different tissues and organs in both *in vitro* and *in vivo* setting. As a practical and data-rich *in vivo* method, this procedure is a useful tool for investigating the PK-PD relationship of drug candidates. The pharmacological action and total plasma concentrations, even when corrected for plasma protein binding, are often not as tightly related as the relationship between drug action and the free drug concentration at the interstitial fluid. Microdialysis has been used to assess free drug concentrations in interstitial spaces. This procedure has become part of drug development process for evaluating antimicrobial agents [18].

One of the necessary components to quantitatively determine the relationship between drug concentrations at the site of action and a drug response is the ability to measure the drug compound in the biophase or tissue that is closer to the actual site. Microdialysis has brought new dimension in pharmacokinetic-pharmacodynamic research that will allow a better understanding of exposure-response relationships, in this case, exposure at the site of action. Its ability to directly access the extracellular fluid of the tissues is valuable in evaluating drug concentrations of anti-infectives at the effect site, where most infections are located in the interstitial fluid. Microdialysis, however, is not for measuring intracellular concentration, which could be disadvantageous for drugs where its site of action is inside the cell. Due to low volume of the collected sample with this approach, a very sensitive assay is required.

Once the microdialysis probe is implanted into a tissue or an organ, it is continuously flushed with a physiological solution, for example, lactated Ringer's or saline solution at a constant flow rate of 0.1–5 μL/min. The probe's semipermeable membrane allows the uptake of drug substance in the interstitial fluid by passive diffusion. The protein-free drug concentration is collected from the probe, given that only low molecular weight substance is diffusible through the membrane. Sampling by microdialysis 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 microdialysis probe. A schematic representation of the microdialysis process is shown in Fig. 13.5. An equilibrium period is established before samples are collected. The subsequent concentrations are computed based on relative recovery as determined for the specific drug of interest by a particular method, for example, retrodialysis and no net flux. The method assumes that recovery is constant over time. Recovery could be influenced by factors such as the membrane area of the probe, molecular weight cutoff, flow rate, perfusate, and

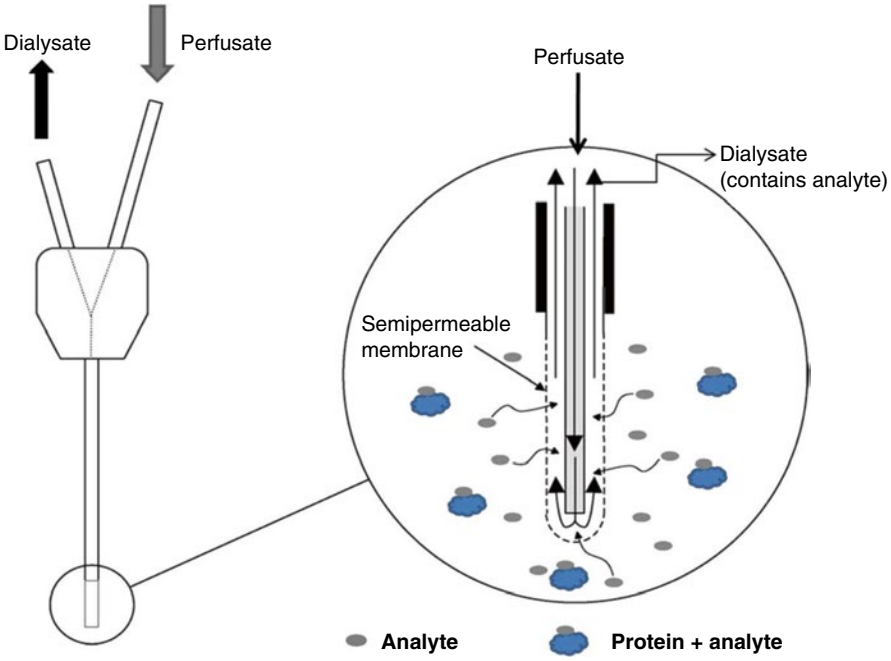

Fig. 13.5 A representation of microdialysis probe inserted in tissue of interest showing passive diffusion of protein-free analyte into the probe

temperature [19]. Given these challenges, the feasibility experiments for a particular drug or compound of interest are often performed *in vitro* prior to implementing it in clinical trials.

A regulatory guidance has emphasized the value of obtaining drug concentrations at the site of action in human tissue, thus supporting the use of this information in drug development [19]. The clinical application of microdialysis to investigate antimicrobial therapy has increased over the past few years. This procedure was used in both single and multiple doses of clarithromycin in humans to evaluate free drug concentrations in the subcutaneous adipose tissue and skeletal muscle [20]. This study showed higher concentrations in the muscle than in the adipose tissue, but the free drug concentrations in both tissues were lower than that in the plasma. Consequently, the pharmacodynamic index characterized by *f*AUC/MIC for the macrolide class of antibiotics was twofold lower when using the free drug concentrations in the tissues than in the plasma. Thus, using free drug concentrations in the plasma could potentially overstate the clinical antimicrobial activity [20]. There are cases wherein the free drug concentrations in the interstitial fluid of the skeletal muscle exceeding that of the plasma free drug concentration, as in the case for cefuroxime [21].

The utility of cefazolin as prophylactic agent against infection during cardiac surgery was investigated by measuring the free drug concentration in subcutaneous adipose and muscle tissues after a 4-g dose via infusion [22]. The study showed

that the soft tissue interstitial cefazolin concentration was greater than MIC of the pathogens often occurring in the wounds for more than 5 h after the initial infusion, which provides sufficient antimicrobial coverage during and immediately after the surgery [22].

The free tissue drug concentrations of antimicrobials are useful for evaluating whether a specific dosing regimen could provide sufficient antimicrobial activities and microdialysis is a useful procedure to provide pharmacokinetic information on the time-course of free drug concentrations in the tissue of interest.

13.2.2 Defining *In Vitro* Antimicrobial Action

The distinction between bacteriostatic and bactericidal agents was developed primarily from *in vitro* studies. The definition for these two terms, bacteriostatic and bactericidal, seems straightforward: bacteriostatic refers to agents that inhibits the growth of bacteria but does not eradicate the bacteria, and bactericidal means that the agent kills the bacteria. In fact, the classification for an agent could be specific for particular laboratory conditions and bacterial strains.

MIC is based on the definition that the concentration or amount of antimicrobial agent inhibits visible growth of microorganism. This parameter does not quantify how much of the bacterial population was eradicated. A separate definition for minimum bactericidal concentration (MBC) was developed, referring to the lowest concentration of the antimicrobial agent that results in a ≥99.9 % decrease in the initial inoculum or a 3-$\log_{10}$ reduction in bacterial density in unit of colony forming unit (CFU)/mL. Subcultures of samples obtained from clear tubes or wells from microtiter testing are plated on a drug-free solid agar medium and reincubated for another 18–24-h period to determine the MBC. Time-kill curves can also be used to determine whether an agent is bacteriostatic or bactericidal, even though this method is more extensively used to study the kinetics of bacterial killing *in vitro*.

A minimum of 3-$\log_{10}$ kill in the viable bacterial density in this incubation window is a generally accepted definition of bactericidal effect [23]. A critical component is a starting inoculum of approximately 10^6 CFU/mL, as is often done for MIC determination. Inoculum sizes are known to affect microbiological outcome, as higher bacterial load is known to be more difficult to eradicate, especially for β-lactams [24]. Another factor affecting MIC and MBC determination is subpopulation of resistant bacteria.

Translating bacteriostatic and bactericidal categorization to clinical practice is not well defined and can be misleading regarding antimicrobial therapy. Evidence is scarce to support MBC evaluation in patient care [25], even though the *in vitro* evaluation of whether a new antimicrobial agent is bacteriostatic or bactericidal is fairly routine. Many bacteriostatic agents tend to have bactericidal activities at high enough concentrations. Macrolides are considered bacteriostatic, but erythromycin, azithromycin, and clarithromycin have bactericidal activities *in vitro* against *Streptococcus pyogenes* and *Streptococcus pneumoniae* [26–28]. Clindamycin may be bactericidal *in vitro* depending on the organism and growth conditions [29].

Linezolid is both bactericidal and bacteriostatic depending on the microorganism [30, 31]. In clinical practice, bacteriostatic agents such as tetracycline [32], chloramphenicol [33], linezolid [34, 35], and trimethoprim-sulfamethoxazole [36] that are capable of penetrating the cerebrospinal fluid efficiently have been used to treat gram-positive bacterial meningitis, which is a life-threatening infection, where one would expect that bactericidal agents would be more effective in eradicating infection as rapidly as possible. The presumed superiority of bactericidal agents over bacteriostatic ones in the treatment of gram-positive bacterial infections tends to be misguided and is only one of many factors that determine clinical outcome. A recent meta-analysis study concluded that the categorization of whether an antibiotic is bacteriostatic or bactericidal is unlikely to be relevant in clinical practice against abdominal infections, skin and soft tissue infections, and pneumonia [37].

13.2.3 *In Vitro* Time-Kill Kinetic Models

Another approach to evaluating *in vitro* antimicrobial efficacy is to utilize pharmacokinetic-pharmacodynamic models that are based on the time-kill curves. The reason why time-kill curves are so useful for PK-PD modeling purpose is that they have both the concentration and time components. The antibiotic concentration can either be held constant or be changing with time to mimic an *in vivo* profile. The kill curves have been used to evaluate humanized dosing and to assess the optimized dose going into the clinic by investigating the detailed information about the time-course of bacterial response [38].

In the static or constant concentration time-kill study, the study design is often based on twofold dilution starting from 0.25- to 16-fold and sometimes 32-fold MIC value. The duration of the study varies from 6 to 72 h and can be single or multiple doses. The static time-kill experiment is carried out in 70-mL vented Falcon flasks with an approximate volume of 20 mL of broth [16]. At each prespecified time points, a small sample of 20-μL volume is taken, diluted, and plated on agar plates. The bacteria colonies are counted after an 18- to 24-h incubation period at 37 °C. In some limited studies, the concentrations of the antibiotic in the media during the time-kill experiment are monitored to evaluate whether the rise in resistant bacteria is associated with drug degradation in the system [39, 40]. Several bacterial strains are evaluated in the static time-kill studies in order to provide a robust representation of bacteria encountered in the clinic.

When designing static time-kill experiments for combination therapy, a detailed design is used in order to observe the behavior at relevant concentrations of both drugs with 5^2 combinations of concentrations of two drugs plus a set of 5 concentrations for single agent of each drug. This is because the MIC will shift to a lower magnitude for one agent as the concentration of the other agent is increased. This design would cover 0.25- to 4-fold MIC of both agents. In the study of the β-lactam, ceftazidime, with a new aminoglycoside, vertilmicin, a 5^2 combination of concentrations of the two agents, was an optimized design to model the combination therapy to fully evaluate synergy [16].

An example of a badly designed time-kill kinetic study is also presented here. A single concentration of the second agent (doripenem) was evaluated in combination of 3 concentrations of the first agent (colistin) plus the 3 concentrations of colistin as monotherapy [41]. In this example, the concentration levels to evaluate the second agent are 0 and the single second agent concentration. The authors claimed that their modeling approach could "serve as a framework for combination modeling" when it is common knowledge that any two points can be fitted by any model. As a famous saying goes, the closest distance between two points is a straight line. Their complex model could have been better described by a linear shift of the kill curves to describe the effect of the second agent. Their model has low to nonexistent predictive value for clinical practice and dose optimization. Thus, when collecting information for the sake of developing a mathematical model for predictive purposes, one has to carefully evaluate whether the information is sufficient to support the type of model to fit to the data, as a model is only as good as the data used to the develop the model.

The dynamic time-kill curves, as opposed to static ones, are so called because the drug concentration in the medium is changing and is not constant throughout the duration of the study. The objective is to mimic *in vivo* conditions. Thus, the flow rates are set to follow the half-life of the drug in humans. The hollow-fiber model is probably the most commonly used dynamic model in the drug development setting. In this model, 150 polysulfone fibers are packed into a chamber, and their lumina are connected to a perfusion tube and a reservoir, as shown in Fig. 13.6 [42, 44, 45]. These capillaries of fibers, which give the name hollow-fiber model, operate as the central compartment that rinses the drug and the medium, while the

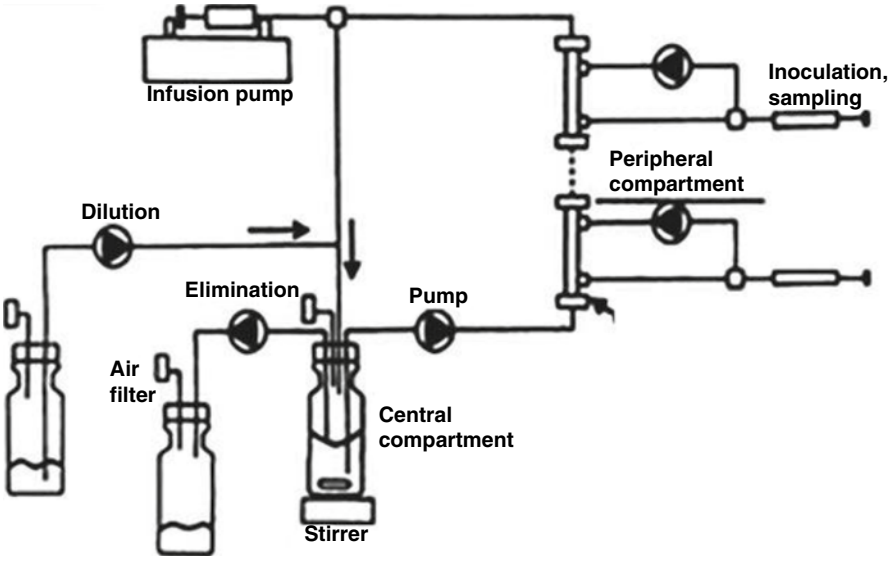

Fig. 13.6 Schematic representation of the hollow-fiber model for dynamic time-kill studies by Blaser et al. [42, 43] (Image is adapted from Michael et al. [45] and used with permission)

outer chamber outside the capillaries is where the bacteria resides and forms the peripheral compartment. The capillaries are continuously flushed with the medium by a pump. The connecting tube includes a stopcock where sampling and injection can occur. Both the medium and the drug diffuse from the inside of the capillaries into the chamber and back, while bacteria are kept outside the chamber. The drawback is the standing medium in the peripheral compartment which allows bacteria to adhere to the capillaries and other surfaces, resulting in diffusion blockage and inhomogeneous sample. The model was later modified by inserting a second or multiple bacteria compartments [42, 43]. Each of the peripheral compartments consists of a polycarbonate chamber where 150 capillaries of hollow polysulfone fibers are connected to the central compartment. A magnetic stirrer in the central compartment ensures a homogeneous distribution. The continuous dilution of the capillaries is achieved by using a pump where fresh medium is supplied from a reservoir and is pumped through the capillaries where the exchange of medium and drug takes place. The outgoing medium is discarded. The pump can be set to simulate drug kinetics. The utility of multiple peripheral compartments is to investigate several bacteria cultures simultaneously similar to how they appear *in vivo*. Even with these improvements, clusters of bacteria still adhere along the outside of the capillaries and within the pores of the capillary wall, which can potentially alter the flow rate. Later version of the model also includes a liquid flow meter, a computer control of the pump and fraction collector to make the process more accurate [46]. The hollow-fiber model has been used to study both monotherapy and combination therapies of humanized dosing, mimicking the drug kinetics in the human body over time [47, 48].

13.2.4 Animal Infection Models

There are some important differences between *in vitro* kinetic model and animal infection models. Animal models allow one to look at infections in specific tissues such as thigh or lung and to evaluate the drug distribution at the tissue site. Majority of the animal infection studies are carried out in rats or mice, which have much faster drug elimination than humans [24]. In order to simulate human dose and pharmacokinetics in the rodents, multiple decreasing doses of drug were given subcutaneously to mice [49]. For renal-eliminated drugs, 5–10-mg/kg uranyl nitrate given 3 days prior to treatment can induce a stable and reversible renal impairment enough to delay drug elimination [50, 51].

Many thigh infection models are carried out in neutropenic mice as different immunity levels of immunocompetent rodents can introduce large variability between *in vitro* and *in vivo* antimicrobial activities, which cannot be accounted for by the differences in protein binding [52, 53]. Many bacteria do not grow well in immunocompetent mice but would grow in neutropenic mice. Neutropenia can be induced by administering two injections of cyclophosphamide at 150 mg/kg 4 days and 100 mg/kg 1 day before infection [54]. Once the animal is rendered neutropenic, the starting inoculum ranges from 10^5 to 10^8 CFU per thigh and is carried out

by injecting 0.2 mL of the organism into the thigh 2 h prior to treatment. Once the treatment period is completed, the thigh is weighed and homogenized. The resulting CFU/g of the thigh is correlated with the pharmacokinetic parameters of the drug (i.e., $fT>\text{MIC}$, fC_{max}/MIC, $f\text{AUC}/\text{MIC}$) [55, 56]. Many microbiological endpoints and PD indices come from dose fractionation studies in rodents wherein several mouse thighs or lungs were injected with specific bacteria with predetermined MIC [55, 56]. The pharmacokinetic parameters can be obtained from microdialysis study. Studies using microdialysis in rats and humans have shown that drug in muscle interstitial fluid is very similar to the free drug concentration in the serum [57, 58].

The human simulated plasma exposure of ceftazidime-avibactam (2/0.5 g every 8 h as 2-h infusion) combination was evaluated in both neutropenic and immunocompetent mice using both thigh and lung infection models [59, 60]. Efficacies measured by 24-h change in $\log_{10}$ CFU/mL against the starting density were compared with ceftazidime alone. The $fT>\text{MIC}$ in the epithelial lining fluid (ELF) was also evaluated in the neutropenic lung infection model [60]. Reduction in bacterial density was observed for isolates with ceftazidime MIC of 32 μg/mL with corresponding ELF $fT>\text{MIC}\geq 12$ %. Efficacy was not attained in isolates with ceftazidime MIC of 64 μg/mL. This example illustrates how human drug PK in animal infection models is used in drug development to extrapolate efficacy to humans.

13.3 Special Populations

Prior to drug evaluation in patients, the pharmacokinetic information of the drug is often established from healthy volunteers first. It is known that patient population often has altered physiological state that could result in a different pharmacokinetics from that of healthy volunteers. In this section, we discuss a number of physiological and disease conditions that are known to alter the pharmacokinetics of antimicrobials.

13.3.1 Critically Ill Patients

Critically ill patients represent a large subpopulation of patients who require antimicrobial treatment, but their pathophysiological conditions pose a challenge to administering antimicrobial therapy using standard dosing protocols. The pharmacokinetics of many antimicrobials in critically ill patients is highly variable, and very often the serum creatinine levels are used to guide dosing regimens. The problem with using serum creatinine as an indicator of renal function is that patients that are under long-term care are often elderly patients with decreased muscle mass combined with undiagnosed acute kidney injury [61, 62]. Both conditions result in an artificially high estimated glomerular filtration rate, which may not reflect the true clearance of drugs that are eliminated by renal pathway [61, 62]. Consequently, plasma antimicrobial levels in the blood accumulate unnecessarily and further

damaging renal function. The physiological changes in the elderly patients and their effects on the pharmacokinetics of antimicrobials are discussed in greater details in Sect. 13.3.4 later in this chapter.

On the other end of the spectrum, there are critically ill patients who require hemodynamic support in the form of fluid resuscitations, particularly in sepsis and septic shock conditions when infection in the bloodstream resulted in hemodynamic decompensation [63]. In order to enhance tissue perfusion and to reverse anaerobic metabolism, volume repletion through fluid overload by administering 6–10 L of colloid solutions in the first 24 h produces significant improvement in cardiac function and systemic oxygen delivery in these patients [64, 65]. Fluid resuscitation would increase the volume of distribution of hydrophilic antibiotics, potentially altering plasma protein binding and further diluting the plasma drug concentration.

Other conditions including polytraumatism and severe burn are some illnesses that are presented at ICU and are also associated with systemic inflammatory response that increase the volume of distribution, partly due to capillary leaks [5]. These conditions could also lower the systemic drug concentrations.

The changes in pharmacokinetics of antibiotic in critically ill patients are influenced by both the drug physicochemical properties and the pathophysiology of the disease. The effect of physicochemical properties of the drug on the volume of distribution and clearance is dependent on whether the drug is hydrophilic or lipophilic [66]. Hydrophilic compounds, which tend to have low volume of distribution and low intracellular penetration and are cleared renally, will see an increase in its volume of distribution in the critically ill patients, resulting in a decrease in plasma drug concentration. Some examples include β-lactams, aminoglycosides, glycopeptides, linezolid, and colistin. Lipophilic drugs, which tend to have high volume of distribution and high intracellular penetration, are predominantly cleared via hepatic metabolism in the normal subjects. In the critically ill patients, the volume of distribution of lipophilic drugs is largely unchanged. Examples include fluoroquinolones, macrolides, and tetracyclines. In patients with sepsis, the leaky capillaries and sometimes combined with hypoalbuminemia would result in a higher unbound concentration and consequently larger volume of distribution. The standard management of hypotension with fluid overload could result in an increase in renal perfusion. Consequently, an increase in creatinine clearance is also associated with a greater elimination of hydrophilic antibiotics. Dose adjustments are often based on creatinine clearance measurements.

For β-lactams whose pharmacodynamic index is characterized by $fT > \text{MIC}$, there are indications that more frequent dosing and extended infusion are associated with better outcome [67], with concentrations maintained at 4–5 times MIC [68–70]. In a study of piperacillin-tazobactam in 194 critically ill patients with *Pseudomonas aeruginosa* infections, those receiving extended infusions with an Acute Physiology and Chronic Health Evaluation II score ≥17 had a significantly lower 14-day mortality rate than those receiving bolus injections (12.2 % vs. 31.6 %, $p = 0.04$) [71]. Aminoglycosides in critically ill patients tend to have increased volume of distribution, which has important impact on its efficacy due to decreased maximum concentrations and lower C_{max}/MIC ratios. Sickness severity is proportionally associated

with increase in volume of distribution [72]. The maximum weight-based dosing (7 mg/kg for tobramycin/gentamicin) is recommended, as this dose is shown to consistently achieve adequate C_{max}/MIC ratios [73]. The nomogram-based dosing of aminoglycosides is not recommended as it had been shown to perform poorly in hospital patients including critically ill patients [74]. Bayesian-based dosing strategy can be used and is preferred [73–75].

These examples show that ICU patients with altered pharmacokinetic or pharmacodynamic could affect the PD index and target attainment [76, 77]. Consequently, drug monitoring and individualization are recommendable in order to improve clinical outcome. Several studies have called for therapeutic drug monitoring of β-lactams in ICU patients [77–81].

Population PK modeling is particularly valuable when dose individualization is required. The distributions of specific pharmacokinetic parameters, once known, are used as prior information. Often two plasma concentrations for the patient are collected in the therapeutic monitoring program, and the pharmacokinetic parameters for the individual are determined. To individualize dose to a specific patient, a target goal is decided, for example, a target C_{max} if the MIC is known; the algorithm then develops a dosage regimen to best achieve that target goal for the patient. In this scenario, there are several key information required, as outlined by Vinks [82]: (1) population pharmacokinetic parameters, including mean values, variances and covariances, information on the statistical distribution, and covariate relationships, for example, creatinine clearance for glomerular filtration rate; (2) measurement of performance index related to the therapeutic goal, for example, one or more plasma concentrations from the patient as feedback information to update the system; and (3) availability of reliable software for adaptive control strategy, for example, maximum a posteriori Bayesian fitting algorithm and the ability to compute the subsequent optimal dosage regimen.

The Bayesian-based dosing strategy allows for a more precise individualized therapy, as opposed to the "reactionary" form of therapeutic drug monitoring that only compares whether the measurement is within the therapeutic range and if so no further evaluation is taken [82]. A study by van Lent-Evers et al. showed that a Bayesian-based dosing regimen resulted in better clinical outcomes including better survival and also superior cost-effectiveness [83].

13.3.2 Renal Impairment

Patients with end-stage renal disease have deteriorating chronic kidney disease wherein their renal function are <10 % of the normal capacity [84]. Repeated dialysis is required to prevent the accumulation of fluid, electrolytes, and toxins. With an aging population, the incidence of end-stage renal disease has also increased over the past two decades [85]. Infection is the second leading cause of mortality in end-stage renal disease patients [85]. Thus, antimicrobials are highly used in this patient population.

Dosing of antimicrobials in end-stage renal disease patients is still quite controversial as these patients tend to have altered drug clearance due to their

impaired renal function [86, 87]. Continuous renal replacement therapy (CRRT) can remove antibiotics with low protein-binding capacity, while antibiotics that enter and bind to tissues have increased volume of distribution, reducing the quantity of drug removed during CRRT. The rate at which drug is cleared by the CRRT also depends on the characteristics of CRRT [88]. For example, membrane pore size is proportional to the extent of drug removal, as membranes with larger pore size allow for larger-molecular-weight drugs to pass through. Increased flow rate of the dialysate can increase the transmembrane pressure and affect drug removal rate. Dialysate concentration can also affect drug clearance during hemofiltration.

Table 13.1 shows the pharmacokinetic and pharmacodynamic properties of a number of antimicrobials and the recommended target drug concentration that corresponds to the upper limit of the MIC range of susceptibility in critically ill adult patients receiving continuous renal replacement therapy. As previously discussed, the volume of distribution for aminoglycosides may be significantly larger in critically ill patients due to capillary leaks and other factors, resulting in lower plasma drug concentrations. The filters from CRRT do allow unbound aminoglycosides to pass through at a rate of 10–40 mL/min, which is equivalent to approximately 6–20 h half-life [88]. With the dosing interval often computed as approximately 3 half-lives, most CRRT patients require 24, 36, or 48 h dosing intervals [88]. For the gentamicin maintenance dosage, there are some questions surrounding whether gentamicin should be administered as half of the full dose immediately after hemodialysis which is the current FDA recommendation or as a full dose an hour before dialysis in the once-daily dosing scenario [2, 86, 87, 89]. The predialysis dosing argument is based on achieving a higher fC_{max}/MIC ratio [2].

For β-lactamase inhibitor combinations, piperacillin-tazobactam combination has more literature on dosing schedule for patients undergoing CRRT. Piperacillin is removed during CRRT, whereas tazobactam which undergoes hepatic metabolism tends to accumulate relative to piperacillin concentration [90–95]. The increased plasma tazobactam concentration also allows for more hepatic metabolism. Derendorf and Dalla Costa had previously developed an equation for the prediction of the resulting major metabolite based on creatinine clearance [95]. Since the concentrations of tazobactam and its major metabolite in humans are relatively safe and much lower than those observed in animal toxicity studies, prolonged dosing intervals in renal patients can be used to adjust for piperacillin and tazobactam concentrations in patients with renal impairment [95]. A 20-min infusion of 4/0.5 g piperacillin-tazobactam administered every 6 h provided sufficiently high probability of target attainment against MIC values ≤32 mg/L in patients with severe renal failure [94].

Renal impairment can result in a significantly increased half-life of vancomycin [96–98]. Due to the size of the molecule, vancomycin is poorly removed by intermittent hemodialysis but is removed by CRRT [96, 99–101]. Vancomycin is administered to patients with a loading dose of 15–20 mg/kg followed by a maintenance dose ranging from 500 mg q24h to 1500 mg q48h. The loading dose is warranted

Table 13.1 Pharmacokinetic and pharmacodynamic parameters of antibiotics in critically ill adult patients receiving continuous renal replacement therapy

Drug	Protein binding, %	Primary route of elimination[a]	Volume of distribution, L/kg	Half-life in normal renal function, h	Pharmacodynamic index	Target trough level, mg/L[b]
Ampicillin	28	Renal	0.29	1.2	*fT*>MIC	8
Aztreonam	56	Renal	0.2	1.7–2.9	*fT*>MIC	8
Cefepime	16	Renal	0.25	2.1	*fT*>MIC	8
Cefotaxime	27–38	Renal	0.15–0.55	1	*fT*>MIC	8
Ceftazidime	21	Hepatic	0.23	1.6	*fT*>MIC	8
Ceftriaxone	90	Renal	0.15	8	*fT*>MIC	8
Cilastatin	40	Renal	0.2	1	NA	NA
Ciprofloxacin	40	Renal	1.8	4.1	AUC/MIC	1
Clavulanate	30	Hepatic	0.3	1	*fT*>threshold	NA
Clindamycin	60–95	Hepatic	0.6–1.2	3	AUC/MIC	2
Colistin	55	Renal	0.34	2	C_{max}/MIC	4
Daptomycin	92	Renal	0.13	8	AUC/MIC	4
Imipenem	20	Renal	0.23	1	*fT*>MIC	4
Levofloxacin	24–38	Renal	1.09	7–8	AUC/MIC	2
Linezolid	31	Hepatic	0.6	4.8–5.4	AUC/MIC	4
Meropenem	2	Renal	0.25	1	*fT*>MIC	4
Moxifloxacin	50	Hepatic	1.7–2.7	12	*f*AUC/MIC	2
Piperacillin	16	Renal	0.18	1	*fT*>MIC	16
Tazobactam	20–23	Renal	0.18–0.33	1	*fT*>threshold	4
Ticarcillin	45–65	Renal	0.17	1.2	*fT*>MIC	16
Sulbactam	38	Renal	0.25–0.5	1	*fT*>threshold	1–4
Vancomycin	55	Renal	0.7	6	*f*AUC/MIC	10

Table adapted from Trotman et al. [88] with modifications

[a]Information for the parent compound

[b]Denotes highest MIC in the susceptible range for applicable pathogens, such as β-lactam MIC for *Pseudomonas aeruginosa*

due to its increased half-life in renal-insufficient patients that would require a longer time to reach steady state. The target troughs of 10–15 mg/L are indicated for infections in more difficult-to-reach sites, such as meningitis, endocarditis, and osteomyelitis [102], as well as pneumonia due to suboptimal drug penetration into the lung [103]. Due to toxicity related to vancomycin concentrations, troughs should not be above 20 mg/L. These examples show how pharmacokinetic-pharmacodynamic approach is utilized in managing antimicrobial efficacy and toxicity in patients undergoing CRRT.

13.3.3 Obesity

Obesity is on the rise globally with 1 in every 9 adults being obese in 2008 [104]. The morbid obesity with BMI $\geq$ 40 kg/m^2 has a prevalence of 4.4 % in adult male and 8.3 % in adult female in the United States, based on 2011–2012 surveys [105, 106]. Their physiological condition can potentially alter antimicrobial pharmacokinetics. Obesity is often linked to other diseases such as diabetes, hypercholesterolemia, cardiac failure, etc., which make dosing in obese patients much more complex. With increasing prevalence of obesity, obese patients are likely to be encountered in the hospital and clinic settings. Obesity-related changes in physiology could potentially alter pharmacokinetics and pharmacodynamics of antimicrobials. Increased volume of distribution and clearance of some but not all drugs have been associated with obesity, which makes it difficult to universally apply dosing by body weight [107]. Recent study has indicated that obesity is a risk associated with antibiotic treatment failure [108]. For many antimicrobial agents, additional pharmacokinetic evaluations are required to optimize dosing regimens in these patients.

Before we discuss how specific antibiotics are dosed in obese patients, there are some body weight definitions worth mentioning. Total body weight (TBW) refers to the actual weight of the individual. Ideal body weight (IBW), originally used by insurance companies to determine the body weight associated with lowest mortality, is based on specific formulas for adult male and female [109]. The commonly used formulas are 52 kg plus 1.9 kg for every inch over 5 ft for male and 49 kg plus 1.7 kg for every inch over 5 ft for female. The adjusted body weight (ABW) is the IBW and a proportion of the difference between TBW and IBW called the dosing weight correction factor (DWCF). The DWCF is a correction factor for drug distribution to the adipose tissue and varies between drugs.

A number of antibiotics have been investigated in the obese population. As many antibiotics are dosed by body weight, some antibiotics in obese patients are dosed by ABW, while others are dosed by TBW. For aminoglycosides, the current recommendation is to dose by IBW and creatinine clearance for a once-daily dosing in the obese patients, with appropriate monitoring after the first dose [2]. For gentamicin and tobramycin, dosing is 5–7 mg/kg of IBW with appropriate dose reduction in renal impairment and/or age [2, 110–112]. Others have suggested to use ABW for aminoglycoside dosing, with the suggested DWCF ranging from

0.38 to 0.58 [113]. Ortega et al. found that ABW is a better predictor of gentamicin volume of distribution [114]. Leader et al. recommended the use of ABW to estimate the initial gentamicin doses in this population [115]. Others have similar recommendations for ABW dosing for amikacin [110, 116], where the current recommendation is 20–28 mg/kg of IBW with appropriate dose reduction for renal impairment and/or age, and similar recommendation for tobramycin dosing by ABW [110, 111, 117].

For vancomycin, obese patients have higher clearance requiring much higher dose to attain the 15–20 mg/L trough concentrations. The current recommendation is 15–20 mg/kg TBW with the maintenance dose reduced appropriately based on renal function. Adjustment of dosing interval may be required to ensure that trough concentrations are within the appropriate range [118–126]. Leong et al. showed that using ABW in the Cockcroft-Gault equation and vancomycin clearance as 0.9 of creatinine clearance is a better predictor of vancomycin clearance in the obese population [123]. As discussed previously, vancomycin trough concentrations are associated with toxicity. Lodise et al. observed that obese patients (≥101 kg) on at least 4 g/day dose have higher risks of nephrotoxicity [124, 125].

Obese patients generally require higher doses of cephalosporins, and dosing by TBW is recommended. As cephalosporins are often used in surgery, the concerns with these hydrophilic agents are primarily with the low drug penetration to the tissues, resulting in low tissue concentrations in the obese patients. Lower tissue concentrations in obese patients given 2 g cefoxitin were observed, as compared to nonobese patients given 1 g [127]. Inadequate soft tissue penetration was previously observed in morbidly obese women given 1.5 cefuroxime [21]. Higher doses of cefepime (2 g q8h) was required for obese patients undergoing weight loss surgery in order to achieve $T > \text{MIC}$ of at least 60 % [128]. Some investigators recommended dosing of cefamandole by TBW for morbidly obese patients during perioperative procedure [129]. The rates of perioperative wound infection were lowered with higher doses of cefazolin in obese patients (5.6 % in 2 g vs. 16.5 % in 1 g) [130].

With carbapenems, the need for dose adjustment from the standard dosing regimen is not clear. Ertapenem was shown to differ between obese and morbidly obese individuals in achieving PTA for bacteria with MIC of ≤0.25 mg/L as compared to ≤0.5 mg/L for normal volunteers [131]. In contrast, Zakrison et al. showed that the cure rates in surgical patients with complicated intra-abdominal infections receiving 1 g/day ertapenem were identical in those <30 vs. ≥30 BMI (80 % vs. 81 %, respectively) [132]. Another group studied the meropenem pharmacokinetics in morbidly obese and critically ill patients and concluded that the standard doses are sufficient without the need for dose adjustment [133]. There are much needed studies to be done in optimizing other classes of antibiotics in the obese and morbidly obese population. While therapeutic drug monitoring of aminoglycosides and glycopeptides is readily available, such facility is not routinely available for other drugs such that much care should be taken particularly to single and loading doses where early treatment is important in reducing the probability of bacterial resistance.

13.3.4 Geriatric Populations

Dosing medications in the geriatric population, particularly antimicrobials, are challenging due to physiological changes associated with age. In addition, the elderly has a greater propensity for polypharmacy, which could lead to drug-drug interactions and drug-related adverse events. Care has to be taken when initiating antimicrobial therapy in the elderly. The usual "start low, go slow" approach to medication with geriatric population has to be balanced with appropriate aggressiveness in dosing antibiotics to achieve optimal pharmacodynamic sufficiency to counter the rise in resistance.

Due to physiological changes that occur with aging, the pharmacokinetics of drugs, including antimicrobials, tend to be different from that in normal adults. Renal elimination parameters, including glomerular filtration rate, renal blood flow, and creatinine clearance, are only a third to a half for a typical 90-year-old patient compared to a healthy 20-year-old individual [134, 135]. There is further deterioration in renal function with increasing age. The elderly patients tend to have decreased muscle mass combined with undiagnosed acute kidney injury, both of which would result in artificially high estimated creatinine clearance when using serum creatinine in the Cockcroft-Gault equation. The Modification of Diet in Renal Disease (MDRD) equation is said to be a better estimate of renal function, particularly in the elderly, as this equation also takes age into account [136].

Other physiological changes affecting drug distribution are the amount of lean tissues and body fat, total body water, and protein-binding capacity. With age, both intracellular and extracellular water decrease; the proportion of adipose tissue to total body weight increases; and cardiac output also decreases affecting hepatic blood flow and consequently hepatic clearance [137–139]. Altered plasma protein affects protein binding of antimicrobials, and the elderly risks displacement of antimicrobial from its protein-binding site by another drug in a polypharmacy setting [140].

Changes in drug absorption in the geriatric population are due to age-related increase in gastric pH from reduced gastric acid secretion. Acid-labile antibiotics such as erythromycins and penicillins could be more readily absorbed resulting in higher plasma drug concentrations [139]. In contrast, the bioavailability of ciprofloxacin is reduced by approximately 20 % in diabetic patients with gastroparesis [141], which is a condition that is more common with geriatric patients than the general adult population.

Drug-drug interaction from polypharmacy (≥5 medications) and antimicrobial-induced adverse events are important concerns in this population. Fatality among patients admitted to an internal medicine department related to ≥1 medication was approximately 18.2 % [142]. 10.6 % of emergency visits in patients ≥65 years of age were due to adverse drug events [143]. Among the elderly, polypharmacy rate is 39 %, based on a 2001 survey [144]. Table 13.2 lists the drug interactions with antimicrobials for drugs commonly prescribed to elderly patients. There are reports of fluoroquinolone-associated hypoglycemia and hyperglycemia [146, 147], as well

Table 13.2 Drug interactions among antibiotics prescribed to geriatric patients

Antimicrobial class/ agent(s)	Interacting agent(s)	Potential clinical effect
Aminoglycosides	Amphotericin B, cyclosporine, cisplatin, loop diuretics, tacrolimus, and vancomycin	Additive nephrotoxicity
Amoxicillin and ampicillin	Allopurinol	Rash
Fluoroquinolones	Pharmaceuticals containing aluminum, iron, manganese, or zinc; antacids; and sucralfate	Decreased absorption of fluoroquinolones
	Antiarrhythmics	Ventricular arrhythmia
Ciprofloxacin	Calcium supplements	Decreased absorption of ciprofloxacin
	Theophylline	Increased theophylline concentration
	Warfarin	Increased anticoagulant effect
Linezolid	Serotonergic agents (SSRIs, TCAs, and MAOIs)	Serotonin syndrome
Macrolides		
Azithromycin	Pharmaceuticals containing aluminum or magnesium	Decreased azithromycin absorption
Clarithromycin and erythromycin	Calcium channel blockers, HMG-CoA-reductase inhibitors, cyclosporine, digoxin, theophylline and warfarin	Increased concentration or effect of interacting drug; increased concentration of macrolide (calcium channel blockers)
Metronidazole	Warfarin	Increased anticoagulant effect
	Alcohol (including alcohol-containing pharmaceuticals)	Disulfiram-like reaction
Tetracyclines	Pharmaceuticals containing aluminum, calcium, iron, or magnesium; antacids, and bismuth subsalicylate	Decreased tetracycline absorption
Trimethoprim-sulfamethoxazole	Phenytoin	Increased phenytoin concentration
	Sulfonylureas	Hypoglycemia
	Warfarin	Increased anticoagulant effect

Table adapted from Faulkner et al. [145] and used with permission
HMG-CoA hydroxymethylglutaryl-coenzyme A, *MAOI* monoamine oxidase inhibitor, *SSRI* selective serotonin reuptake inhibitor, *TCA* tricyclic antidepressant

as rare torsades de pointes [148]. Elderly patients with undiagnosed or borderline diabetes or with renal dysfunction could potentially result in higher fluoroquinolones. Aminoglycosides-associated vestibular toxicity could predispose elderly patients to falls; concurrent ototoxic medications including vancomycin and loop diuretics are risk factors as well [149]. There is a significantly higher risk of ototoxicity among the elderly patients of ≥90 years (26 % vs. 3 %) receiving

aminoglycosides [150]. The geriatric population poses unique challenges when prescribing antimicrobial therapy including propensity for polypharmacy and risk of drug-drug interaction, as well as physiological changes that alter drug pharmacokinetics.

13.3.5 Pediatric Populations

From the context of scaling dose by body weight, pediatric patients are not simply "small adults" in the sense that a simple dose reduction of adult dose to pediatric is an oversimplification, not taking into account ontogeny, physiological and biochemical differences in neonates, infants, children, adolescents, and adults [151]. For children 2 years and older, allometric size adjustments of adult dose give reasonable and similar exposure in this population as that in adult. For children less than 2 years of age, extrapolation of adult doses would not be appropriate because mere accounting for size difference does not take into account maturation processes in enzymatic activities or ontology, which are important sources of pharmacokinetic variabilities in this cohort of pediatric population [152]. Some drug metabolism enzymes are present at birth, including CYP3A7 and uridine 5′-diphospho-glucuronosyltransferase or UGT, whereas other enzymes develop over time, for example, 2E1, 2D6, 3A4, and 2C9 [153]. Thus, dosing may be different in neonates, infants, children, and adolescents and would require dose adjustments.

The dose scaling by allometry applies empirical scaling from adult to pediatric based on body weight. Sy et al. recently provided a simple approach using polynomial equations to simulate age-matched body weight and body mass index from birth to 18 years, which can be applied to dose adjustment in children [154]. The extrapolation by body weight can utilize the mathematical power law expression: $Y = a \cdot (\text{Weight} / \text{Median})^{b}$, where Y is the parameter of interest, weight refers to body weight normalized by the median, a is the median weight-centered parameter value, and b is the allometric exponent. Body weight as a primary covariate is often referenced to a 70-kg person using the allometric exponent of 0.75 for clearance and 1 for volume [155]. The allometric exponent values can vary for different drugs.

There are several population PK studies of aminoglycosides in children of different age groups [156–158]. The pediatric dosing recommendations for gentamicin including neonatal doses ranged from 4 to 6 mg/kg [159, 160]. The clearance of gentamicin in neonates was shown to be threefold higher than adults when adjusted for body surface area and twofold higher mean volume of distribution [161]. The neonates have low incidence of gentamicin-related nephrotoxicity, and it is speculated that the immature renal proximal tubules protect the neonates from renal toxicity, as aminoglycosides are not reabsorbed by their developing proximal tubule [162]. The therapeutic failure of another aminoglycoside amikacin in the pediatric population was shown to be associated with its C_{max}/MIC ratio based on the analysis from a retrospective study that included 80 neonates with 26 of the 80 patients having 35 confirmed episodes of sepsis [163].

A study in Japanese pediatric patients has shown that body weight is a significant covariate of meropenem PK parameters and that simulation should be based on body weight [164]. Another population PK study of single dose of 10- and 20-mg/kg meropenem in 37 infants utilized simulation to evaluate the PD target attainment of $T > \text{MIC} \geq 60$ %, using the MIC distribution of relevant bacteria pathogens [165]. These examples show that knowledge of PK-PD principle is important in treating pediatric patients, given that pediatrics, particularly in the very young infants and neonates, have different pharmacokinetics.

Conclusion

PK and PD, as shown in the examples of antimicrobials, are important tools in various aspects of drug development as well as patient care. The antibiotic therapeutic area represents a field that has successfully implemented PK-PD principles and modeling and simulation approach to evaluate dose optimization and the effects of various covariates, including special populations such as critically ill, renal-impaired, obese, geriatric, and pediatric population, as well as applying these knowledge to fight emergence of drug resistance. As a result, therapies are improved and have become more individualized to specific patient conditions; and the risks of adverse effects are mitigated.

Even with all these advancements, there are still areas of potential improvements as many antimicrobials are still poorly characterized in these special populations, particularly in neonates and infants, obese and morbidly obese, and geriatric patients. These populations represent a substantial proportion of the general population that requires hospitalization, medical attention, and critical care and also is likely to be requiring antibiotic therapy. And as such, more studies are needed to fill the gap in knowledge of antimicrobial PK and PD to develop more effective and safer dosing regimens. But as many antibiotics have already lost their patent protection, drug makers do not have the financial incentives to better improve the way we administer these drugs. Thus, the responsibilities rest on the shoulder of clinicians and researchers.

There are many opportunities moving forward to utilize these state-of-the-art tools to further characterize dose optimization in various patient populations during drug development process. The principles and applications examined here provide a useful guide to streamline drug development process and design experiments and clinical studies not only of antimicrobials but also other therapeutic areas, albeit to varying degrees. Maximizing the benefits to patients can be done by incorporating all available information from *in vitro* studies, animal models, and clinical trials in an evidence-based treatment program.

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Epidemiology and Biostatistics

14

Gerhard Garhöfer and Leopold Schmetterer

Abstract

Epidemiology and biostatistics are the main tools to collect, analyze and interpret data in order to draw valid scientific conclusions on the causes and consequences of diseases. The following chapter will give the reader a brief overview on how epidemiological data are gained and analyzed. Furthermore, the chapter will briefly cover the importance of biostatistics in the interpretation of study data as well as possible pitfalls.

14.1 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. Further, epidemiological research provides a methodological basis for determination of factors involved in disease progression and risk.

14.2 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

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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 and 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.

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.

14.2.1 Prevalence

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

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

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 worldwide 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 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. 14.1, age. Obviously, the prevalence of diabetes is not equally distributed via all age groups, but increases with increasing with 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

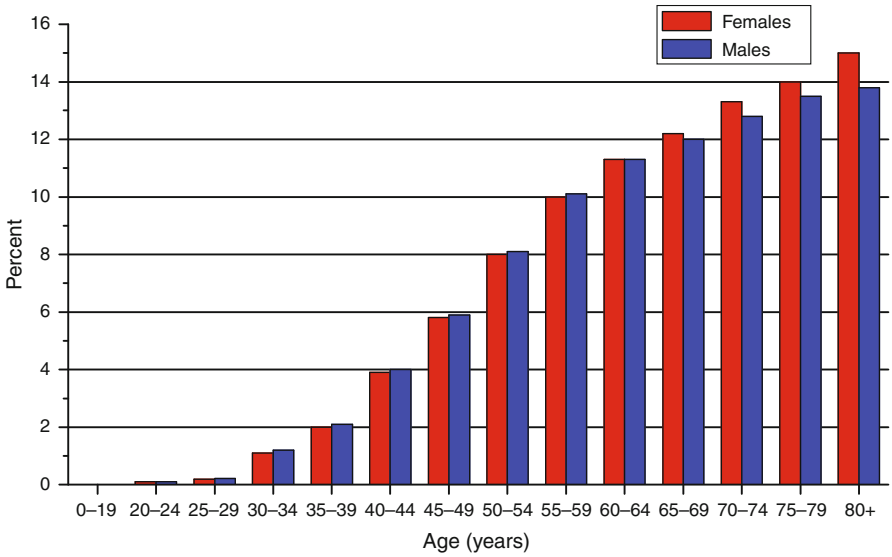

Fig. 14.1 Global diabetes prevalence by age and sex for 2000 (Modified from Ref. [1])

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.

14.2.2 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

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

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.

14.2.3 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 1,000. A disease-specific mortality indicates deaths caused by a certain disease and is often reported on the basis of 100,000 persons.

14.3 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. 14.2, every new case enters a prevalence pool and remains there until death or recovery.

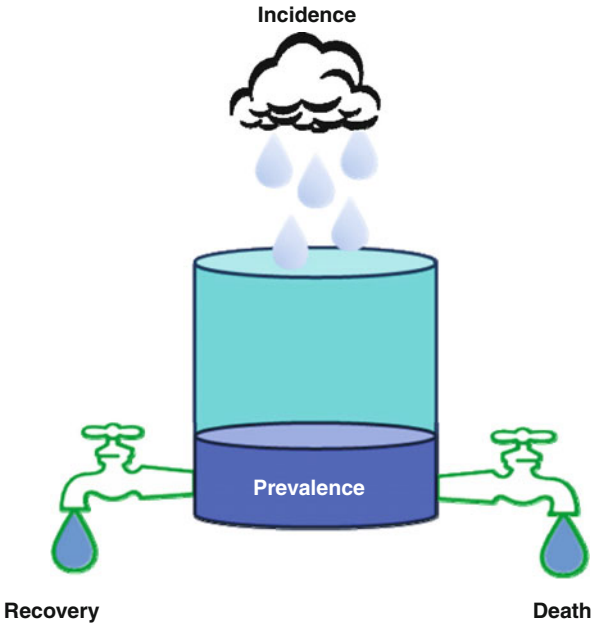

Fig. 14.2 Relationship between incidence and prevalence. Disease prevalence is dependent on the number of new occurring cases of the disease, but also on the number of patients who die or who recover

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

14.4 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, ten persons with lung disease being followed for 10 years. If two out of the ten persons die during this time span, then the 10-year survival is (10–8)/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 term survival time is used.

14.5 Censured 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 noncompliance. 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 censured data. Censured 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 abovementioned example of the ten 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 two subjects have survived, resulting in 10-year survival of 80 %. Alternatively, the two subjects could already be dead. This would result in a true survival of 60 %, leading to a severe 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 censured 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. 14.3.

14.6 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

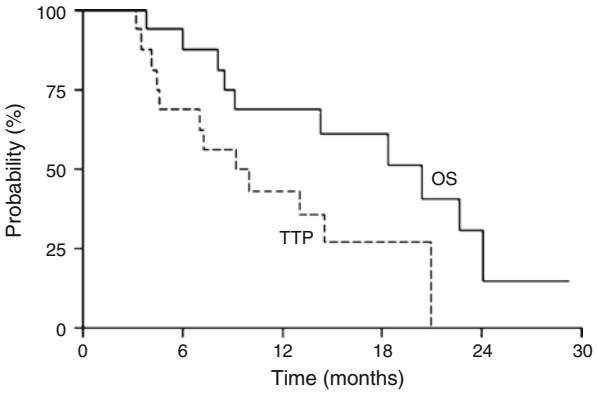

Fig. 14.3 Kaplan-Meier curves for the time to progression and overall survival in patients with pancreatic cancer. *TTP* time to progression, *OS* overall survival

deaths but also all deaths not related to the disease. As an example, severe diseases such as Marburg hemorrhagic fever show case fatality rates of 80 % and more [3].

14.7 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."

14.8 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 study designs, please refer to the chapter "Observational Studies."

14.9 Statistical Measures

14.9.1 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 observation does not only hold true for this kind of drug. All kind of biological experiments 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.

14.9.2 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.

14.9.3 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.

14.10 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, it is not feasible to perform clinical trials by including the entire population. The aim of performing a study under controlled conditions is, however, to infer results from the 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.

14.10.1 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 important. 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 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. Imagine 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 occasions, whereas it is not suitable for others. In contrast, other studies and hypothesis may require a more strict (or less strict) error definition.

14.10.2 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 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 safely 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 large number of tests.

This problem holds especially true for clinical studies that include post hoc stratification or subgroup 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 is to be explored afterward. This additional hypothesis that has not been defined before study starts may be presented in an explanatory manner or used hypothesis generating for a subsequent study specifically investigating this issue.

14.10.3 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 most of the studies more than one independent statistical test is used. This has to be considered when interpreting the results of such trails. 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 this correction method is that if we conduct n tests at a significance level of a_{sig}, we consider the results as statistically significant only if the p value is less than a_{sig}/n. For example, if we consider to perform five 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. However, some authors have claimed that the introduction of a study-wide error rate as it is done with the Bonferroni method is a rather conservative approach to interpret data. This is of importance in clinical trials because too conservative statistical approaches will result in unnecessarily increased sample sizes, which poses a significant ethical problem in interventional clinical studies.

14.10.4 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 direct caused by another. The relationship can then be expressed by a regression equation.

14.10.5 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 been 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, "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.

14.10.5.1 Association Strength

The stronger the association between possible cause and disease, the more likely 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.

14.10.5.2 Temporal Relationship

The temporal relationship is the only knockout 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.

14.10.5.3 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.

14.10.5.4 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.

14.10.5.5 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 sold cars in the western countries 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 understanding of diseases may not be correct or complete and may possibly be reconsidered based on findings of epidemiological studies.

14.10.5.6 Experiment

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

14.10.5.7 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 that 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 subgroups 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 glace – 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 that 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.

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Placebo Effects and Placebo Control in Clinical Trials

15

Johannes Pleiner-Duxneuner

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 color 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

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of immunobiochemical 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 favorable 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.

15.1 The Recent Debate About Research Ethics in Placebo-Controlled Trials (PCTs)

To harmonize attitudes toward ethical aspects of clinical research, a number of ethical codes have 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 4-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 newer versions of the DOH (Seoul 2008 and Fortalezy 2013) 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). In the latest version (Fortaleza 2013), the structure of the DOH was reorganized, and the importance of placebo-controlled trials is highlighted in paragraph 33. However, the content is almost unchanged. The main change is that the sentence "…the patient … who receive placebo, or no intervention will not be subject to additional risks of serious or irreversible harm" was amended by "as a result of not receiving the best proven intervention." Critics of this DOH revision state that the clear interpretation of this phrase still remains unclear, and it might preclude vital research that promises to improve the condition of the worst off [12].

15.2 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 [13]. 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*) [14]. 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 importantly, 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 [15].

15.3 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 the concept of assay sensitivity. Assay sensitivity establishes a trial's 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 [16]. 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).

15.4 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 [17].

Scenario 2: On the other hand, it is obvious that for diseases in which no proven therapy exists and 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 the 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 importantly, 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 [18]. 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. 15.1). An interesting case that illustrates the struggle of researchers and the scientific community over the ethical responsibility of researchers in PCTs is a publication on the use of "placebo surgery," which is presented in detail below.

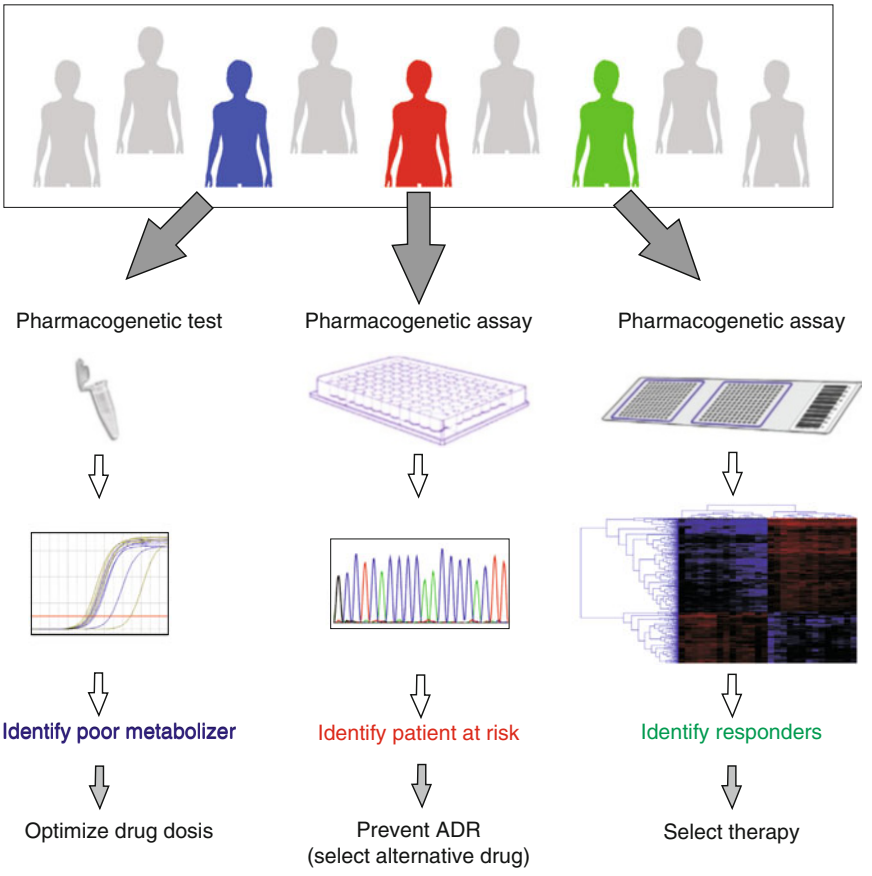

Fig. 15.1 When to use placebo. In most cases, the decision, whether a placebo-controlled trial is justified or not, is not as easy as in myocardial infarction (*white symbol*) or in otitis media (*black symbol*)

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

15.4.1 The Concept of Non-inferiority to Placebo

In a special situation, even a non-inferiority trial to placebo might be justified. The assessment of cardiovascular (CV) safety in patients with diabetes could be considered as such situation. The risk of cardiovascular (CV) disease is increased approximately two- to fourfold in adults with diabetes [19]. Improved glycemic control has been associated with a reduction in microvascular events, and there is a clear association between microvascular complications such as albuminuria and an increased risk of CV events in patients with diabetes [20]. However, the potential benefit of glucose lowering on CV events remains unclear and highly controversial [21]. Therefore, regulatory authorities have issued guidance for evaluating the long-term CV safety of new antidiabetes agents to ensure that CV safety is demonstrated with reasonable assurance [22, 23].

As a result of CV evaluation, large postmarketing CV outcome trials might become necessary. These trials are designed in close collaboration with regulators, as non-inferiority PCTs and add-on to best practice standard care. Special emphasis is on assay sensitivity, and the trial must show that the upper bound of the two-sided 95 % confidence interval for the estimated risk ratio for CV events is <1.3. Several of these studies have already been conducted and have demonstrated cardiovascular safety, i.e., comparable incidence of major adverse cardiovascular events to placebo [21].

15.5 Criteria for Justification of Placebo

As cited above, the WMA mentions placebo control in Article 33 of the current version of the DOH: “Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention” (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.
- The (cardiovascular) adverse event profile of a new intervention is uncertain (non-inferiority PCT).

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 nonresponse 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 [24]. 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 of 4 on a visual-analogue scale ranging from 0 to 10) despite maximum medical treatment for at least 6 months, and no arthroscopy of the knee during the previous

2 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 0–4 scale. Three compartments (medial, lateral, and patellofemoral) were scored and added together to generate a severity grade of 0–12. Exclusion criteria were severity grade of 9 or higher, severe deformity, and 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 and 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 theater. 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 smoothened, 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 was removed, and all torn or degenerated meniscal fragments were trimmed. The remaining meniscus was smoothened, 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 terms of body pain as measured by the SF-36-P at 2 years, with an enrollment of 180 patients and 16 or fewer lost to follow up. All statistical tests compared the treatment groups in terms of the values at each visit rather than analyzing the changes from the baseline. 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 1 year and after 2 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 1 year. The results for objectively measured walking and stair climbing were poorer in the debridement group than in the placebo group after 2 weeks and 1 year, showing a trend toward worse functioning at 2 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 generalizibility (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) [25]. 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) the 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 the benefits for the subject and the knowledge of the subject being a volunteer.

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Part IV

Topics in Clinical Pharmacology

Pharmaceutical Drug Safety

16

Martin Brunner

Abstract

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 evolution and current status of drug monitoring systems will be discussed, and recent developments in 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.

16.1 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 (ADRs) 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 16.1) [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 USA, it was even estimated that more than 100,000 deaths can be

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Table 16.1 Glossary of adverse drug reaction terms

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 the 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

Modified from Refs. [1, 21]

attributed annually to serious ADRs, and it 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. It is important to identify ADRs and their consequences, because this enables to perform cost-effectivity analyses, which in turn might have a positive impact on the public health sector, as they can be used to economically evaluate pharmacovigilance actions [7].

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 twentieth 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 [8]. 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 [8].

16.2 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 [9]. 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 USA, 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 foetus, and it is now licensed by the FDA and the European Medicines Agency (EMA) 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 [9].

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 [10].

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 USA, the FDA initiated important reforms, including giving it the power to require that a manufacturer demonstrates efficacy before a new drug can be marketed [11].

16.3 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 EMA; the equivalent in the USA 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 three 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. Usually, 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 likely to be captured and described before marketing, whereas rarer ADRs might fail to be detected. In an Institute of Medicine report, it was noted that "… a drug's risk-benefit profile necessarily evolves over the drug's life cycle" [12].

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.

16.4 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 thalidomide 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 [13]. 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 [14].

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 called VigiBase in a structured and comprehensive way to allow the detection of potential medicinal safety hazards. Currently, VigiBase contains over 10 million reports of adverse reactions [15]. 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 July 2015, 122 countries had joined the WHO Drug Monitoring Programme, and in addition, 28 "associate members" were awaiting compatibility between the national and international reporting formats [16]. In April 2015, the WHO launched VigiAccess™, a new web application that will allow anyone to access information and encourage the reporting of adverse effects from medicinal products [15].

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 [17].

In the USA the main pillars of pharmacovigilance are the FDA through MedWatch, the FDA safety information and adverse event reporting programme [18], the

pharmaceutical industry and academic non-profit organizations, such as RADAR (Research on Adverse Drug Events and Reports) [19, 20].

16.5 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" [16]. Spontaneous reporting systems have the strength to early identify signals of new, rare and serious ADRs [21]. Further advantages are that they can be used throughout the life cycle of a drug by all stakeholders at relatively low costs [15]. 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 [13]. 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 the 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 (SUSAR) must be reported to the regulatory agencies within 15 days (Table 16.1) [22, 23]. 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 stepwise 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. Analyse 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 webpage [16] and that is depicted in Table 16.2.

Spontaneous reports of suspected ADRs can only be regarded as signals of a potential hazard in the use of a drug and 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 statistical data mining techniques have gained importance, which have improved the analysis of large databases of adverse event

Table 16.2 Causality assessment of suspected adverse reactions

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 fulfil 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

Modified from Ref. [16]

reports, thereby permitting more rapid, robust and comprehensive detection of signals that indicate the possibility of safety issues [14, 21]. 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 pharmacoepidemiological studies, designed to confirm or refute the findings by hypothesis-testing techniques, such as case-control or cohort studies or randomized controlled clinical trials [14].

A special form of active, intensified surveillance, prescription event monitoring (PEM), was developed in the early 1980s [24]. 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 analysed for new signals [21]. 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 enables the incidence of ADR to be estimated, thus enabling quantification of the risk of certain ADRs [21]. 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 [14].

Although spontaneous reporting systems remain the primary and best method for identifying ADRs to newly marketed drugs [25], they have also been criticized as being fundamentally a 1950s-era approach [25] with inherent disadvantages such as poor quality of submitted reports, often with inadequate documentation and details, the potential of selective reporting and under-reporting [21]. 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 [26]. 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 [25].

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 16.3). 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 [27]. In-depth information on drug safety for the US market is summarized on the FDA homepage [28]. 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.

Table 16.3 Examples of 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	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 >30 kg/m^2) or overweight patients (BMI >27 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
2010	Sibutramine	Management of obesity (1999)	Increased risk of heart attack and stroke
2013	Nicotinic acid/ laropiprant	Mixed dyslipidaemia (2008)	No proof of significant additional benefit in reducing heart attack and stroke, compared with statin monotherapy but higher frequency of nonfatal but serious side effects

16.6 Drug Safety Issues After Lipobay and Vioxx

When cerivastatin and later rofecoxib (Vioxx®) [29] had to be withdrawn from the market due to safety problems, the thalidomide tragedy seemed almost forgotten. In the USA, 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 or 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 have been completed or even initiated [30] and that the rate of those studies declined between the 1970s and the 1990s [21]. The FDA also had no authority to legally force companies to fulfil 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 [31], 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 [11]. 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 [32]. 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 [32].

16.7 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 [33]. 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 (RMP) that describes commitments for post-marketing

pharmacovigilance in detail. This plan has to be submitted already with the application for marketing authorization [34]. 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.

In December 2010, a new European pharmacovigilance legislation was adopted, which came into effect in July 2012 [35]. The new legislation clarifies roles and responsibilities of the different stakeholders and specifies the obligation of marketing authorization holders to continuously assess the benefit-risk ratio of their products to ensure proper risk management in particular for newly authorized products for which the safety profile might not be fully characterized. For such cases, the European Union has introduced a new process to label medicines that are being monitored particularly closely by regulatory authorities, which is referred to as "additional monitoring" [36]. Medicines that are under additional monitoring have a black inverted triangle displayed in their package leaflet and the summary of product characteristics, together with a short sentence explaining the meaning of the triangle. The triangle does not imply that the product is unsafe but should raise awareness that it is monitored even more intensively and to encourage healthcare professionals and patients to actively report any observed suspected adverse reactions so that new emerging information can be analysed. To assess and analyse all aspects of the risk management of medicines for human use, the EMA has introduced a new scientific committee, the Pharmacovigilance Risk Assessment Committee (PRAC) [37]. The main responsibility of the PRAC is to prepare recommendations on any questions relating to pharmacovigilance activities and on risk management systems, including the monitoring of the effectiveness of those risk management systems. The PRAC also has responsibility for the design and evaluation of post-authorization safety studies and pharmacovigilance audits [37].

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 [14]. These strategies could include larger clinical trials, the 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 [34] as well as the conduct of post-authorization safety studies (PASS) or post-authorization efficacy studies. A PASS is defined as any study relating to an authorized medicinal product conducted with the aim of identifying, characterizing or quantifying a safety hazard, of confirming the safety profile of the medicinal product or of measuring the effectiveness of risk management measures [38]. A PASS may be initiated, managed or financed by a marketing authorization holder voluntarily or pursuant to an obligation imposed by

a competent authority [38]. 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 [14]. 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 [14]. Clinical trials and post-marketing data had identified a safety signal – natalizumab was associated with an increased risk of progressive multifocal leucoencephalopathy (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 [14].

Additionally, pharmacovigilance may increasingly rely on the use of personalized medicine. The 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 leucocyte antigen (HLA)-B*5701 allele has been shown to predict the risk of hypersensitivity in patients with HIV scheduled to receive therapy with abacavir [39]. 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 [39]. 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. The FDA has published a table of pharmacogenomic biomarkers in drug labelling on the webpage [40].

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 [21].

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 USA), the sixth 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 USA, a further 21 deaths worldwide. In addition there were 385 nonfatal cases reported among the estimated 700,000 users in the USA, 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 [30]. Bayer faced 7800 claims for compensation in the USA and about 500 in Germany [41]. 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 [42]. 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 [43].

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 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 [41].

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 [44]: 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 [44]. 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 four times the number of patients of that of any previously approved statin [44].

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) [45] 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. 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 preclinical 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 fulfil 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 checklists and a nurse administration and monitoring guide. Based on the submitted periodic safety update reports and clinical follow-up measures on pharmacokinetic data in patients with severe hepatic impairment [46], the European Summary of Product Characteristics of Mycamine® was amended several times since the initial marketing authorization [45]. In December 2012, considering the safety profile of Mycamine® and in particular the potential risk of hepatocellular carcinoma, the CHMP decided that one additional 5-year renewal is required [45].

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Drug Interactions

17

Markus Zeitlinger

17.1 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.

17.2 Relevance of Interactions

Adverse drug reactions cause more than 100,000 deaths each year in the USA 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.

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17.3 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 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 antagonistically.
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 a 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 a 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 labelled as contraindication in the SPC).

17.4 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; in 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 interindividual 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 but also due to potentially increased susceptibly towards negative drug effects. 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, antiviral 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]. Modern drug development is designed to detect clinically relevant drug-drug interactions early in order to enable go/no-go decisions before more expensive phase II and III studies are initiated.

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.
(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

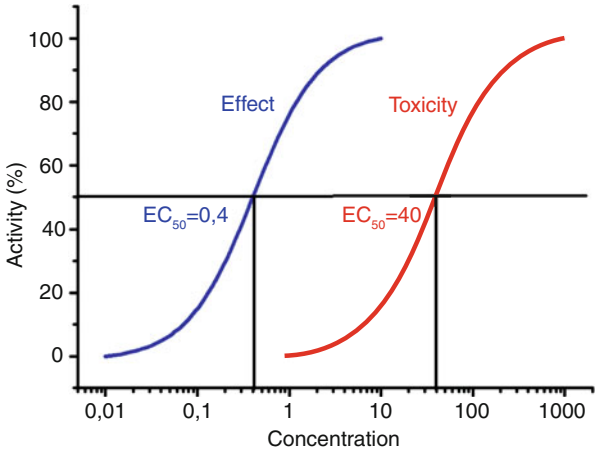

Fig. 17.1 The therapeutic index is the ratio of the concentration resulting in 50 % of lethal toxicity to the concentration necessary for 50 % effect. For optimal drugs 90 % of activity can be achieved at concentrations at which no or very little toxicity is expected

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. 17.1).

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 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].

17.5 Most Important Mechanisms of Interactions According to the ADME Schemata

17.5.1 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 the extent (percentage) of 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.

17.6 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 noncompetitive, 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 bloodstream, 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.

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. 17.2). 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

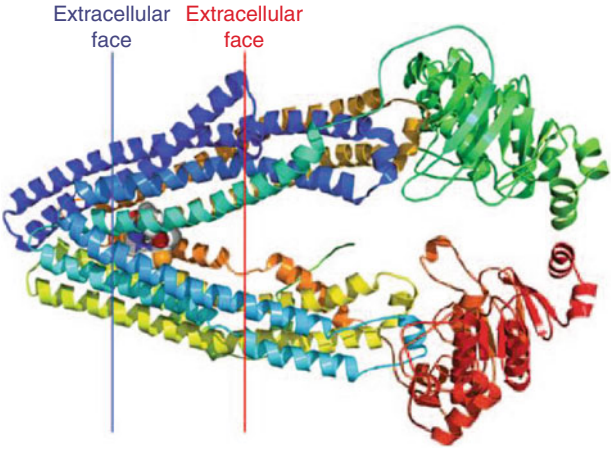

Fig. 17.2 Structure of P-glycoprotein (obtained from http://en.wikipedia.org/wiki/P-glycoprotein). The approximate positioning of the protein in the cell membrane is indicated by the blue (*extracellular face*) and red (*cytoplasmic face*) lines

Table 17.1 Examples of P-glycoprotein substrates

Antineoplastic agents	Protease inhibitors	Corticoids	Others
Vinblastine	Saquinavir	Dexamethasone	Cimetidine (H2-receptor antagonist)
Vincristine	Ritonavir	Hydrocortisone	Loperamide (antidiarrhoeal)
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)

Modified from Ref. [29]

in the kidney or into the bloodstream 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 17.1 and 17.2), further underlines the central role of P-gp in the body's 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 17.1.

Table 17.2 Important substrate, inhibitors and inducers of cytochrome P450 isoenzymes

Enzyme	Class	Substrate	Inhibitor	Inducer
CYP 3A4	Analgetics	Codeine Fentanyl Lidocaine Methadone Paracetamol		
	Antiarrhythmics/ antihypertensives	Amiodarone	Amiodarone	
		Amlodipine Digitoxin Diltiazem Nifedipine Propranolol Salmeterol Verapamil	Diltiazem verapamil	
	Antibiotics	Clarithromycin	Clarithromycin	Rifabutin
		Erythromycin	Erythromycin	Rifampin
	Antidepressives/ antipsychotics Antidiabetic	Aripiprazole Buspirone Fluoxetin Haloperidol Quetiapine Risperidone Ziprasidone	Fluvoxamine	Pioglitazone troglitazone
	Antiepileptics/ benzodiazepines	Alprazolam		Carbamazepine
		Carbamazepine Diazepam Midazolam Triazolam		Oxcarbazepine Phenobarbital Phenytoin
	Antifungals		Fluconazole Itraconazole Ketoconazole Voriconazole	
	Antivirals	Indinavir	Indinavir	Efavirenz
		Nelfinavir Ritonavir Saquinavir	Nelfinavir Ritonavir Saquinavir	Nevi rapine
	Immune modulators/ cytostatics	Cyclosporine Docetaxel Irinotecan Sirolimus Tacrolimus Tamoxifen Vincristine	Imatinib	
	Statins	Atorvastatin Lovastatin Simvastatin		
	Steroids/analogs	Dexamethasone Hydrocortisone Progesterone Testosterone		Dexamethasone

Enzyme	Class	Substrate	Inhibitor	Inducer
	Others	Caffeine Cocaine Dextromethorphan Ondansetron Warfarin	Cimetidine Grapefruit juice	St. John's wort
CYP1A2	Analgetics	Naproxen Paracetamol Ropivacaine		
	Antiarrhythmics/ antihypertensives	Propranolol Verapamil	Amiodarone	
	Antidepressives/ antipsychotics Antiepileptics/ benzodiazepines Steroids/analogs	Amitriptyline Clomipramine Clozapine Fluvoxamine Haloperidol Imipramine Olanzapine Cyclobenzaprine Estradiol	Fluvoxamine	
	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
	Antidepressives/ antipsychotics Antidiabetic Antiepileptics/ benzodiazepines Antifungals Immune modulators/ cytostatics	Amitriptyline Fluoxetine Glibenclamide Glimepiride Glipizide Glyburide Nateglinide Rosiglitazone Tolbutamide Phenytoin Tamoxifen	Fluvoxamine fluconazole	Secobarbital
	Statins	Fluvastatin	Fluvastatin Lovastatin	
	Others	Warfarin	Fenofibrate Zafirlukast	

(continued)

Table 17.2 (continued)

Enzyme	Class	Substrate	Inhibitor	Inducer
CYP2C19	Analgetics Antiarrhythmics/ antihypertensives	Propranolol	Indomethacin	
	Antibiotics	Proguanil		Rifampin
	Antidepressives/ antipsychotics	Amitriptyline	Fluoxetine	
		Citalopram Clomipramine Hexobarbital Imipramine	Fluvoxamine	
	Antiepileptics/ benzodiazepines Antifungals Antivirals Immune Modulators/ Cytostatics	Citalopram	Oxcarbazepine	Carbamazepine
		Diazepam Phenobarbital Phenytoin Nelfinavir Cyclophosphamide	Topiramate Ketoconazole	
	Steroids/analogs	Progesterone		Prednisone
	Others	Lansoprazole Omeprazole Pantoprazole Rabeprazole Warfarin	Cimetidine Lansoprazole Omeprazole Pantoprazole Rabeprazole	
CYP2D6	Analgetics	Codeine Lidocaine Oxycodone	Celecoxib	
		Tramadol		
	Antiarrhythmics/ antihypertensives	Carvedilol Metoprolol Nebivolol Propafenone Propranolol Timolol	Amiodarone	
	Antibiotics		Halofantrine	Rifampin
	Antidepressives/ antipsychotics Antifungals Antivirals	Amitriptyline	Bupropion	
		Aripiprazole Chlorpromazine Clomipramine Desipramine Duloxetine Fluoxetine Fluvoxamine Haloperidol Imipramine Nortriptyline Paroxetine Perphenazine Risperidone Thioridazine Venlafaxine	Chlorpromazine Citalopram Doxepin Duloxetine Escitalopram Fluoxetine Paroxetine Sertraline Terbinafine Ritonavir	

Enzyme	Class	Substrate	Inhibitor	Inducer
	Immune Modulators/ Cytostatics Steroids/analogs	Tamoxifen	Doxorubicin	Dexamethasone
	Others	Amphetamine Dextromethorphan Metoclopramide Nicotine Ondansetron	Cimetidine Cocaine Diphenhydramine Methadone Metoclopramide Ranitidine Ticlopidine	

Modified from University of Indiana [41] and EMEA (1997) "note for guidance on the investigation of drug interactions" [4]

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 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]. Loperamide, an opioid without central activity used for treatment of diarrhoea, was shown to cause respiratory depression when co-administered with P-gp inhibitors, which was linked to suppression of the gatekeeper 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 anthracyclines, vinca alkaloids, taxanes and epipodo-phylotoxins [32]. Resistance towards 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, in testicular 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].

On the other hand, transporters recently came in the focus of novel drug development, as acknowledged by guidelines of regulators. Presently, beside P-glycoprotein, at least the transporters OATP1B1 (SLCO1B1), OATP1B3 (SLCO1B3), OCT2 (SLC22A2), OAT1 (SLC22A6), OAT3 (SLC22A8) and BCRP (ABCG2) should be investigated in vitro. In vivo studies might become necessary if in vitro studies indicate a signal for interaction. Like for interaction studies with CYP enzymes (discussed below), these studies use "probe drugs", i.e. know substrates of the investigated transporter or enzyme and determine whether a novel drug significantly changes PK parameters of the probe drug. Likewise known inhibitors or inducers of a certain transporter or enzyme can be employed to explore, if a novel compound might be a substrate of this specific system. A detailed list of known substrates of different transporters as well-known drugs modulating them is provided by the FDA.

17.6.1 ADME: Interactions Based on Drug Metabolism

The cytochrome P450 isoenzymes (CYPs) represent a superfamily of haemoprotein enzymes localized on the membrane of the endoplasmic reticulum (Fig. 17.3). The term *P450* is derived from the spectrophotometric peak at the wavelength of the absorption maximum of the enzyme (450 nm) when it is in the reduced state and complexed with carbon monoxide.

They are 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 the 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].

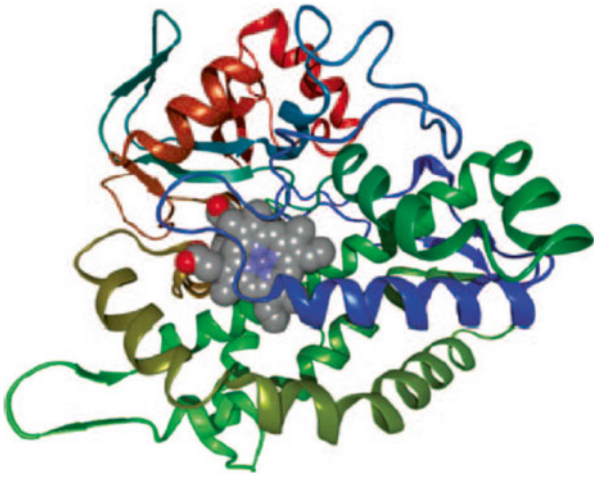

Fig. 17.3 Three dimensional structure of cytochrome P450 (Obtained from http://de.wikipedia.org/wiki/Cytochrom_P450)

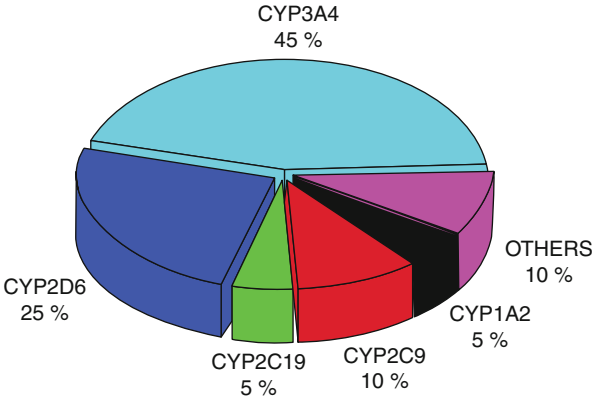

Fig. 17.4 Percentage of drugs metabolized by individual cytochrome P450 isoenzymes (Based on [36])

While in humans approximately 20 families and subfamilies and 60 genes have been identified, the majority of drugs are metabolized by families 1, 2 and 3 (Fig. 17.4). 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 CYP3 A4 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, generate 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 17.2 provides an overview of a range of substrates, inducers and inhibitors of the most import 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]. Interindividual 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 interindividual differences in metabolism of CYP2D6 substrates, while CYP2C9 shows high interethnic 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. 17.5 depicts that individual statins are differently susceptible when exposed to typical CYP3 A4 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.

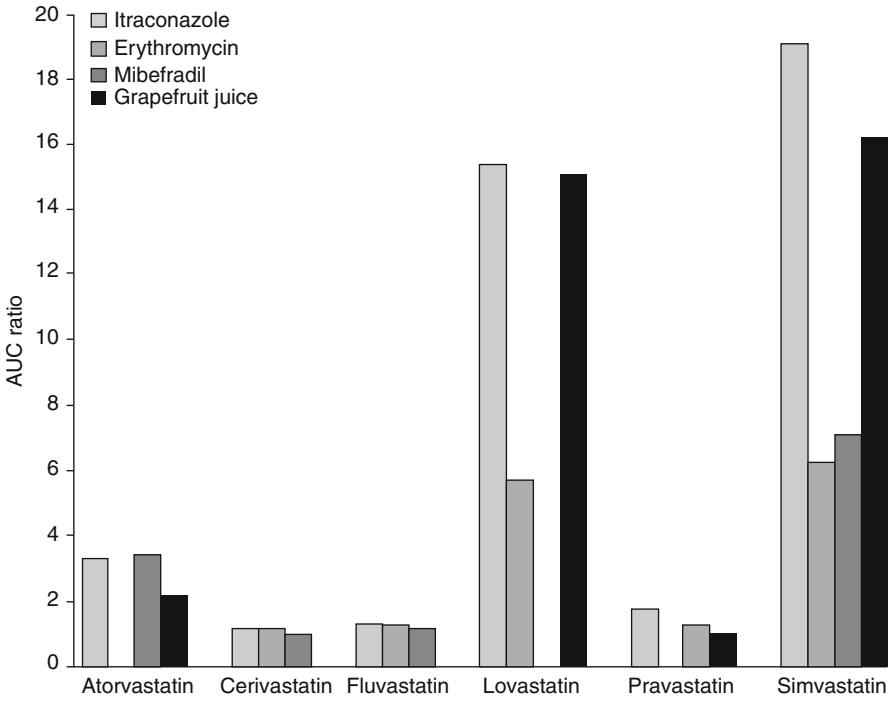

Fig. 17.5 Interactions of statins and various CYP3A4 inhibitors. "AUC ratio" is the area under the concentration-time curve (*AUC*) of the statin after combined administration divided by the AUC after administration alone; values close to 1 therefore indicate a lack of interaction [45]. The use of this figure was generously granted by Wolters Kluwer Pharma solutions

17.6.2 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 co-administered with terfenadine, astemizole, cisapride or pimozide [46]. As mentioned above, rhabdomyolysis has been reported after the co-administration of some statins and various CYP3A4 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) hypno-sedatives and CYP3A4 inhibitors [48, 49].

Likewise, induction of the CYP3A4 isoenzyme was associated with life-threatening advents. CYP3A4 inducers like rifampicin, barbiturates or some anti-epileptic 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 was even 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 unmetabolized 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.

17.6.3 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 co-administration 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 co-administration of methotrexate and nonsteroidal anti-inflammatory 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].

17.7 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].

In order to identify potentially negative drug-drug interaction, the physician must be able to indentify an adverse drug reaction as such. For this purpose, different tools, e.g. the Naranjo algorithm or the WHO-UMC causality categories, have been developed.

In particular the Naranjo score judges an event based on the following items:

1. Are there previous conclusive reports on this reaction?
   Yes (+1) No (0) Do not know or not done (0)
2. Did the adverse events appear after the suspected drug was given?
   Yes (+2) No (−1) Do not know or not done (0)
3. Did the adverse reaction improve when the drug was discontinued or a specific antagonist was given?
   Yes (+1) No (0) Do not know or not done (0)
4. Did the adverse reaction appear when the drug was re-administered?
   Yes (+2) No (−1) Do not know or not done (0)
5. Are there alternative causes that could have caused the reaction?
   Yes (−1) No (+2) Do not know or not done (0)
6. Did the reaction reappear when a placebo was given?
   Yes (−1) No (+1) Do not know or not done (0)
7. Was the drug detected in any body fluid in toxic concentrations?
   Yes (+1) No (0) Do not know or not done (0)
8. Was the reaction more severe when the dose was increased or less severe when the dose was decreased?
   Yes (+1) No (0) Do not know or not done (0)
9. Did the patient have a similar reaction to the same or similar drugs in any previous exposure?
   Yes (+1) No (0) Do not know or not done (0)
10. Was the adverse event confirmed by any objective evidence?
    Yes (+1) No (0) Do not know or not done (0)

Based on the sum of the question am event is graded as:

- ≥9 = definite adverse drug reaction
- 5–8 = probable definite adverse drug reaction
- 1–4 = possible definite adverse drug reaction
- 0 = doubtful definite adverse drug reaction

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, pre-existing 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/clinpharm/ddis/)
Drugs.com (http://www.drugs.com/drug_interactions.php)
FDA (http://www.accessdata.fda.gov/Scripts/cder/DrugsatFDA)
Medscape (http://reference.medscape.com/drug-interactionchecker)
SuperCYP (http://bioinformatics.charite.de/supercyp/)

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 addition to internet platforms, a range of software to indentify drug interactions is commercially available. It has, however, to be highlighted that none of these are certified medicinal products which might have legal implications if a physician's decision relies solely on the database and not on his own knowledge and expertise.

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 on Interactions Based on a Drug Class: Antibiotics

In the western world antibiotics frequently are given as "add 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 pre-existing 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 pre-existing 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 defence, 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. 17.6) [66–70].

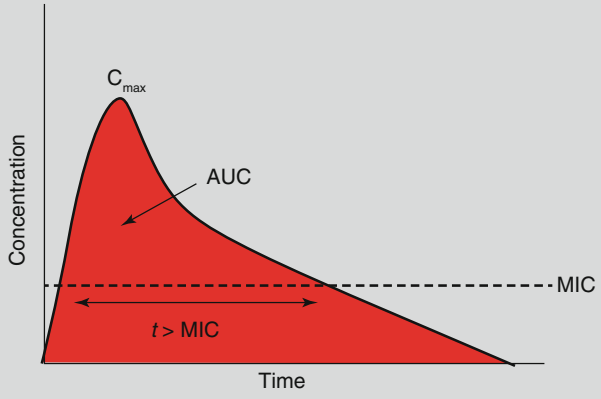

Fig. 17.6 Area under the concentration-time curve (*AUC*) and maximal concentration (*Cmax*) to the minimal inhibitory concentration (*MIC*) as well as the time period during which the concentration of the antibiotic exceeds the MIC (f > MIC) of a bacterium are considered the most important PK/PD parameters

The relevance of each of these indices for predicting antimicrobial and clinical outcome varies for different antimicrobial classes. Beta-lactam antibiotics display a "time-dependent" pattern of activity and t>MIC is considered most predictive for outcome. In contrast, for aminoglycosides, the C_{max}/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 affected. Likewise, increased elimination of filactams 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 deduction 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 belongs to the most important interactions 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 co-administered magnesium-, aluminium-,

calcium- or iron-containing products, significantly limiting absorption when co-administered 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. Co-administration 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 2 h before or after antacids and should be given with care when co-administered 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 noncompetitive, with one drug causing a conformational change in the protein molecule, which, in turn, inhibits 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 17.3 provides examples for antibiotics with binding to plasma proteins above 80 %.

Table 17.3 Protein binding of selected highly bound antimicrobials

Glycopeptides	Teicoplanin: 90–95 % Telavancin: 90 % Dalbavancin: 90 % Oritavancin: 85–90 %
Cephalosporins	Ceftriaxone: 85–95 %
Carbapenems	Ertapenem: 92–95 %
Tetracyclines	Doxycycline: 82 %
	Tigecycline: 71–89 %
Lipopeptides Oxazolidinones Echinocandins Azoles Polyenes	Daptomycin: 90 % Tedizolid: 80 % Caspofungin: 97 % Anidulafungin: 99 % Micafungin: 99 % Posaconazole: 98–99 % Amphotericin B: 95 %

Obtained from the approved labels

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 17.4).

Rifampin (rifampicin) is indicated as component of the standard drug regime 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 1–2-week period and sometimes longer, after rifampin is discontinued. Important CYP3A4 substrates are listed in Table 17.2. Rifampicin can cause acute transplant rejection in patients treated with immunosuppressive drugs, such as cyclosporin [87].

In addition, rifampicin 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 17.1). Potential drug interactions should be considered whenever starting but also when discontinuing

Table 17.4 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		
Fluconazole	2C9, 2C19, 3A4		
Voriconazole	2B6, 2C19, 3A4		
Ketoconazole	3A4, P-gp		
Itraconazole	3A4, P-gp		
Posaconazole	3A4		

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-life, has been associated with massively prolonged sedation of patients [89, 90]. Theophylline intoxications have been described when this drug with narrow therapeutic index was co-administered with erythromycin, a common treatment combination for respiratory infection 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 systemic 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].

Sometime the mode of interaction remains unknown. Although it has been reported that linezolid does not influence the metabolism or protein binding of warfarin, the anticoagulation by warfarin was demonstrated to increase significantly from after concomitant linezolid administration.

As outlined in Table 17.4, all antifungal agents of the azole class are inhibitors of different CYP systems. In contrast for echinocandins and amphotericin B, no clinically relevant PK drug interactions are described.

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 fivefold 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 co-administration 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.

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Development of Advanced Therapy Medicinal Products: A Case for Early Scientific Advice

18

Martin Brunner and Bernd Jilma

Abstract

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 multidisciplinary scientific expert committee, representing all EU member states and EEA-EFTA states, 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.

18.1 Introduction

Advanced therapy medicinal products (ATMPs) comprise gene therapy medicinal products, somatic cell therapy medicinal products and tissue-engineered products, the latter two categories of ATMPs often also referred to as cell-based medicinal products.

The affiliation of the CAT members and alternates can be found on the EMA Website (http://www.ema.europa.eu/ema/index.jsp?curl=pages/contacts/2010/02/people_listing_000008.jsp&mid=WC0b01ac05800292a6).

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(Please refer to Box 18.1 for legal definitions (Regulation (EC) No 1394/2007 [5, 18]).) 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.

Box 18.1: Definitions of Advanced Therapy Medicinal Products in the European Pharmaceutical Legislation

(See Ref. [5])

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) It contains or consists of cells or tissues that have been subject to substantial manipulation so that biological characteristics, physiological functions or structural properties 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) It 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, in particular, shall not be considered as substantial manipulations

Tissue engineered product means a product that:

(a) Contains or consists of engineered cells or tissues, and
(b) 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, 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 CAT is responsible for preparing a draft opinion on the quality, safety and efficacy of each ATMP for final approval by the Committee for Medicinal Products for Human Use (CHMP). This draft opinion is issued on any scientific assessment of ATMPs necessary to draw up the scientific opinions by the CHMP relating to granting, variation, suspension or revocation of an authorization to place an ATMP on the market in accordance with Regulation (EC) No 1394/2007 and pharmacovigilance. At the request of the Executive director of the Agency or the Commission, an opinion is also drawn up on any scientific matter on 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;
- 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 EMA: http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000294.jsp&mid=WC0b01ac05800241e0

Information on the Committee for Advanced Therapies (CAT): http://www.ema.europa.eu/ema/index.jsp?curl=pages/about_us/general/general_content_000266.jsp

Information on Medicines and Emerging Science: http://www.ema.europa.eu/ema/index.jsp?curl=pages/special_topics/general/general_content_000339.jsp

EMA Innovation Task Force (ITF): http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000334.jsp

EMA SME Office (micro-, small- and medium-sized enterprises): http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000059.jsp

EMA Scientific Guidelines for Biologicals, including guidelines specific to ATMPs: http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000082.jsp

EMA Multidisciplinary Guidelines, including guidelines specific to ATMPs: http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000086.jsp

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 [13]. 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 was at the European Medicines Agency (EMA) in London: the Committee for Advanced Therapies (CAT), which is a multidisciplinary 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, to regulate the development of ATMPs in Europe 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). There have only been few ATMPs authorized so far, but many products are in the pipeline. Therefore, the CAT is concerned with many pre-authorization activities, such as scientific recommendations on ATMP classification, the certification procedure of quality and non-clinical data of ATMPs generated by micro-, small- or medium-sized enterprises (SMEs) and scientific advice requests (*vide infra*).

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 ATMPs 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 end points.

This chapter highlights some major regulatory challenges.

18.2 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 be administered to humans to prevent or treat a disease or to make a diagnosis, by a metabolic, immunological or pharmacological mode of action of its cells or tissues (for legal definition, see Box 18.1). 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 Box 18.1). 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).

18.3 Efficacy and Safety Challenges

18.3.1 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 microenvironment. However, this is often species and/or disease specific, which complicates 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 tumorigenic risk of undifferentiated or incompletely differentiated stem cells that needs to be eliminated before clinical use [14].

18.3.2 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 [8]. Non-clinical studies in a relevant animal species are required to assess toxicity due to dedifferentiation, cell transformation, tumorigenicity 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 tumorigenic potential. The use of immunodeficient 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 maybe 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. End points that were originally validated for other product types may sometimes have to be adapted for a cell-based product [12], e.g. cancer immunotherapies may transiently increase tumour size by T-cell influx, oedema 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 EMA. A general guideline on stem cell-based products has been developed [10] to provide guidance on the conduct of pharmacodynamic/pharmacokinetic studies, dose finding and clinical efficacy and safety studies and to describe the special consideration that should be given to pharmacovigilance aspects and the risk management plan for these products.

18.4 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 18.1 and Ref. [5]). 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 GTMPs.

18.5 Development Challenges and Strategies to Address Them

18.5.1 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.

18.5.2 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].

18.5.3 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 nontarget 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 [10, 16], and ICH considerations on oncolytic viruses have been released (EMEA/CHMP/GTWP/607698/2008).

As far as safety is concerned, insertional mutagenesis, which 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 disactivation 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 [15].

Considering these various challenges, various scientific guidelines that address adherent problems have been prepared, which can be found on the homepage of the EMA (http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000410.jsp&mid=WC0b01ac058002958d).

18.6 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 [6, 7]. 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.

18.7 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 complexities and technical specificities of gene- and cell-based medicinal products. The regulators provide a variety of opportunities for early interaction with developers of ATMPs to enable them to have early regulatory and scientific input to ensure compliance with the regulatory and legal framework for the authorization of ATMPs.

Examples for direct interactions are briefing meetings of individual manufacturers with the EMA Innovation Task Force, CAT's routine involvement in all scientific advices on ATMPs or scientific workshops with SMEs and developers from academia, hospitals and non-for-profit organizations [4]. Scientific advice is open to all applicants at any stage of development. As an incentive to boost the development of ATMPs, a reduced fee is payable [11]. 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 not legally binding *classification procedure* is to determine whether a given product meets the scientific criteria which define ATMPs. It is open to all developers and shall help to address, as early as possible, questions of borderline with other areas such as cosmetics, medical devices or tissue/cell transplantation [11]. So far, the CAT has classified more than 100 products and a summary of the classifications is given on the CAT homepage. The second new procedure the *certification procedure* is a scientific evaluation of available quality and non-clinical data. It is restricted to SMEs. Evaluation by CAT and certification by EMA give SMEs a possibility to attract financial support for the further development of their product. By scientific input from the CAT, companies 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.

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 that is mandatory for ATMPs [9]. 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 EMA website [11].

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.

Disclaimer Although Bernd Jilma has been and Martin Brunner is 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).

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Biologics

19

Bernd Jilma and Markus Müller

19.1 Introduction

Historic Perspective One of the first successful attempts to employ a "biologic" 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. At the beginning of the twentieth century, however, a revolution in chemistry and pharmacology overshadowed the "biologic" by a "xenobiotic" concept and led to an explosion of our therapeutic options by providing many thousands of traditional chemicals that we employ in medical practice today. Historically, biologics have been developed in three increasingly bigger waves. 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 anymore 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 third wave which resulted in hundreds of potential therapeutics in development. In fact, biologics may soon enter a new era of widespread use because of the marketing authorization of the cholesterol-lowering antibodies alirocumab and evolocumab [1, 2], which effectively lower low-density lipoprotein due to the

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Table 19.1 The diverse spectrum of "biologics"

Drug class	Example
Blood products	Fresh frozen plasma, platelets
Recombinant proteins	Insulin
Antibodies	Infliximab
Soluble receptors	Etanercept
Oligonucleotides*	Aptamers*
Vakzines	β-Amyloid-vakzine
Gene therapy	PEG-adenosine deaminase
Stem cells	Embryonic stem cells

Adapted from Ref. [5]
Examples of "biologics." Biologics 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
* Aptamers are mostly synthethtic

inhibition of proprotein convertase subtilisin–kexin type 9 (PCSK9). In contrast to the more traditional small molecular chemicals, biologics are derived from living organisms. These include bacteria (e.g., for production of granulocyte colony-stimulating factor), yeast (such as HPV vaccines), mammalian cell lines mainly from Chinese hamster ovary cells (many fully human antibodies), and recently human embryonic kidney cells (FVIII products), and occasionally biologics are produced transgenically, e.g., recombinant antithrombin in goat milk [3].

Definition A biopharmaceutical, or biologic medical product or biologic, is any medicinal product manufactured in, extracted from, or semisynthesized from biological sources. Biologics comprise a heterogenous group of pharmaceutical products, including blood, or blood components, allergenics, vaccines, cell or gene therapy products (see chapter on advanced medicinal products (ATMPs)), tissues, recombinant therapeutic protein, and living cells used in cell therapy. The official FDA definition of "biological products" or "biologics" can be summarized as "any virus, therapeutic serum, toxin, antitoxin, or analogous product applicable to the prevention, treatment, or cure of diseases or injuries of man" [4]. European Union regulations define "biological medicinal products" as "a protein or nucleic acid-based pharmaceutical substance used for therapeutic or in vivo diagnostic purposes, which is produced by means other than direct extraction from a native (nonengineered) biological source" (Table 19.1).

Due to their specific characteristics, biologics introduce major challenges to our traditional concepts of drug development and routine practice of therapeutic medicine. Biologics 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, biologics 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.

Scope This chapter focuses on more general aspects of protein therapeutics. Vaccines, although highly effective, lifesaving, and a major success story of human medicine, will not be covered in this chapter. Their particular mechanism of action is the induction of a prophylactic immune response against invading pathogens after only 2–3 injections usually, and they generally have a good safety profile (except for local and systemic reactogenicity). Thus, their clinical development is fundamentally different and other regulatory frameworks apply. A complete list of FDA-approved vaccines may be found at http://www.fda.gov/cber/vaccine/licvacc.htm. More information on gene and cellular therapies can be found in Chap. 21 on advanced therapy medicinal products. For in-depth discussion of biologics in particular medical specialities, the reader is referred to recent reviews on biologics such as allergy [6], asthma [7], dermatology [8, 9], oncology [10], and rheumatology [11]. Given the rapid development in the field of monoclonal antibodies, tabulation of antibodies on the market may have to be updated frequently in the next years. Therefore it is useful to refer to free resources for the latest updates. A table of compounds of monoclonal antibodies may be found at http://www.biologics.clinimmsoc.org/table-of-compounds. A description of the nomenclature of monoclonal antibodies may be found at http://www.biologics.clinimmsoc.org/nomenclature

19.2 Specificity Versus Pleiotropy

Despite the obvious and tremendous success of traditional (small) 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 [5, 12]. 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 (Chap. 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 [13]. Many problems have been identified as a result of drug action on the HERG K(+) channel and QTc prolongation [14] or drug interactions via cytochrome P450. In late clinical development, more than 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 [15]. Liver toxicity problems were recently observed with troglitazone, trovafloxacin, leflunomide, telithromycin, tolcapone, or ximelagatran [16]. 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 biologics relates to the fact that they do not undergo metabolism *by hepatic enzymes* and therefore are unlikely to lead to the above described spectrum of drug interactions *and adverse drug reactions unless they strongly alter cytochrome p450 activity*.

19.3 Preclinical Development of Biologics

In Europe, biologics have to undergo a centralized approval process at the European Medicines Agency (EMA). Although guidance documents on the development of biologics are evolving [17], there is still considerable uncertainty about different aspects.

Preclinical Standard preclinical testing, e.g., genotoxicity studies, is not always appropriate for biologies [18, 19]. Inter alia, this relates to the fact that *some* biologics *may* exert bell-shaped rather than sigmoid dose–response relationships. Therefore, the definition of an optimal biologic dose (OBD) is more appropriate than definitions of thresholds like "no-observed-adverse-effect level" (NOAEL) or "maximum tolerated dose" (MTD). Also, toxicological concerns for biologics are closer related to exaggerated pharmacology than "true" toxicology in a strict sense. One of the lessons of the trial with an activating CD28 T-cell super-antibody discussed in Chap. 7 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 is not expressed in animals. Thus, lack of severe toxicity in animal models should never be viewed as a guarantee of safety in man [20, 21]. 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 biologics, rather than rats or dogs.

Clinical The traditional phase 1–4 concept appears somewhat obsolete due to the specific complexities of biological drug development. In oncology, for example the well-established concept of dose escalation to the maximum tolerated dose (MTD) is not always appropriate for many biologics [19]. 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 [22]. 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 1 studies, a decision must be made on whether to test the new product in volunteers or patients. In case of targeted biologics, several factors favor 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 [23] 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.

19.4 Specific Safety Consideration

Adverse Effects Evolutionary forces have shaped the extremely diverse human cytochrome P450 system as a defense strategy to herbal nutrients and exogenous toxins. Therefore human beings 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. In contrast to chemicals, biologics display "atypical" side effects, some of which are discussed below. General toxicity of biologics is very low and no dose-limiting toxicity can be observed even in a majority of first-in-man trials with anticancer biologics [19]. However, there are several pitfalls with the preclinical safety evaluation of biologics [24] not only because of limited cross-species reactivity of antibodies. Several adverse drug reactions are relatively specific for biologics and have been classified according to their pathophysiology in five subgroups [25, 26], which are discussed below:

(a) Immunostimulation (e.g., infusion reaction, cytokine release)
(b) Immunogenicity (e.g., hypersensitivity type I–IV, antibody induction)
(c) Immunodeviation (immune suppression, opportunistic infection or autoimmunity)
(d) Cross-reactions (e.g., acneiform exanthema due to epidermal growth factor antibodies)
(e) Non-immunologic adverse reactions or not yet classifiable reactions

Cytokine (Release) Reactions Infusion reactions occur very often after administration of cytokines such as interferons or interleukin-2 but typically become milder with subsequent infusions. Some antibodies such as the CD28 antibody have even caused a severe cytokine storm and have led to novel guidelines for first-in-man studies (see Chap. 7). However, similar reactions had already been observed with muromonab (OKT3) more than two decades before this unnecessary event. Since then severe infusion reactions have been observed after rituximab infusion in approximately 10 % of treated patients, which are rarely fatal usually during the first infusion. Rituximab causes complement activation-dependent adverse events [27], and similarly cytotoxic antibodies may induce tumor lysis syndromes. In contrast to anaphylactic reactions, cytokine (release) reactions are dose dependent and therefore may be mitigated using very low start doses and slow dose escalation in early-phase clinical trials.

Cytokine-Induced Inhibition of CYP450 Various cytokines may inhibit biotransformation by cytochrome P450 as previously reviewed [28]; however this is of little concern for most of the other biologics.

Immunogenicity and Hypersensitivity Reactions These are difficult to predict based on animal models. EMEA/CHMP/BMWP/14327/2006: An overview of the methodology used to detect immunogenicity has recently been provided [29].

Immunogenicity depends on the degree of humanization, the route of administration (less when given intravenously), and concomitant immunosuppression [26]. Allergic hypersensitivity reactions typically occur after repeated exposure. However, they may also occur upon first exposure, e.g., preexisting anti-IgE antibodies have been found against the chimeric antibody cetuximab or more specifically its 1,3-α galactose glycosylation which induced anaphylaxis [30]. Severe anaphylactoid reactions have more often been reported with chimeric antibodies such as abciximab, basiliximab, cetuximab, infliximab, omalizumab, and rituximab [31, 32]. Even omalizumab which has been used off label to prevent anaphylaxis causes anaphylaxis in 1–2 per 1000 patients [32], although polysorbate, one of the excipients, may be responsible in individual cases. Of note, severe allergic hypersensitivity reactions cannot be prevented by antihistamines or glucocorticoids [33]. Pseudoallergic reactions can occur due to nonantigen-specific activation of the complement cascade (complement activation related pseudoallergy). Complement activation, which leads to rituximab-induced infusion reactions in >70 % of treated patients [33], may be encountered with any cytotoxic antibodies [34].

Immunosuppression Safety-related regulatory actions for approved biologics are necessary in 14 and 29 % of cases after 3 and 10 years post approval and are often due to immunomodulatory effects (infections) [35]. Antitumor necrosis factor antibodies were among the first recognized to induce infections such as tuberculosis, and the use of high-dose biologics increases the risk of infections in patients with rheumatoid disease [36]. Other antibodies such as the CD52 alemtuzumab have frequently been associated with severe infections, and the alpha4 integrin inhibitor natalizumab has led to rare but lethal progressive multifocal leukoencephalopathy [24]. Luckily, neoplasia appears to be less of a problem for antibodies targeting specific parts of the immune system as compared to small molecule immunosupressants that induce a global immunosuppression. *Autoimmunity*: Patients treated with either interleukin-2 or interferon alpha have developed various autoimmune diseases.

Cross-Reactivity An example is the occurrence of acneiform exanthems and folliculitides induced by inhibitors of the epidermal growth factor receptor (EGFR) such as cetuximab. As EGFR is also expressed on basal keratinocytes and in follicles, this is in fact a predictable side effect. This “skin toxicity” correlates with the extent of the response of the tumor and can also be observed with tyrosine-kinase inhibitors such as erlotinib and gefitinib [37].

Other Reactions Interestingly, some of the infusion reactions to biologics are actually caused by excipients such as polysorbate 80, and this not only applies to various types of biologics including vaccines [38]. Of note, polysorbate frequently causes adverse events when higher doses of this excipient (up to 4 g) are used, for example, to dissolve chemotherapeutic agents such as paclitaxel, docetaxel, or etoposide.

For a summary of design aspects of recent first-in-human trials with monoclonal antibody, please see Chap. 7 or the publication by Tosi et al. [19].

19.5 Pharmacokinetic–Pharmacodynamic Properties

General Considerations on Absorption, Distribution, Metabolism, and Excretion (ADME) Lack of efficacy in phase II trials is considered a primary reason for drug failure. Three key questions should be answered before a drug candidate is selected for clinical trials. (1) Does the drug reach the target organ at sufficient concentrations? (2) Does the drug bind to the target in vivo with the coverage needed for biologic activity? (3) Does the drug functionally modify the target? To address these questions, in-depth characterization of ADME properties and pharmacokinetic and pharmacodynamic (PK–PD) relationship is necessary (e.g., by the use of biomarkers) in preclinical and clinical studies. Due to their large molecular size, the ADME process of large proteins usually differs from that of small molecules.

Distribution Biologics display limited distribution to tissues. It is crucial to comprehend the penetration mechanism and the ensuing relationship between tissue concentration and efficacy particularly for antibodies that do not target circulating molecules or cells but solid tissue. We refer to open-access publications by Xu and Vugmeyster [39] for more details on methods used for distribution studies in preclinical studies and a discussion of tissue distribution of monoclonal antibodies by Boswell et al. [40]. Biodistribution is not only of interest for antibodies targeting solid tumors but also in the case of enzyme replacement therapy. For example, laronidase effectively stabilizes pulmonary function and physical endurance in mucopolysaccharidosis type I. However, intravenously administered laronidase is unable to correct central neuron system disease [41] due to limited penetration to the central nervous system. This is likely applicable also for different other enzymes treating various forms of mucopolysaccharidosis.

Metabolism or catabolism of biologics generates peptides or amino acids, which can be recycled for protein synthesis. The half-life is dependent largely on the protein properties particularly molecular sizes. Smaller peptides have shorter half-lives (including insulin, hematopoietic growth factors), and larger molecules tend to have longer half-lives, particularly antibodies, due to binding of the neonatal Fc receptor (FcRn) and endocytotic recycling, which can lead to a half-life as long as 3 weeks.

Table-specific modulation of ADME profiles of biologics

	Examples of key modulators
Absorption	Subcutaneous route of administration (contribution of lymphatic absorption)
Distribution	Target binding, FcRn binding
Metabolism	Not cytochrome dependent but proteolysis
Elimination	Target-mediated clearance, nonspecific endocytosis, immune complexes, protection by FcRn
Nonlinear kinetics	Saturable target-mediated clearance, immunogenicity
Species differences	Immunogenicity, target-binding affinity, off-target effects FcRn/IgG interactions [42]
Subjects	Prior exposure to biologics (e.g., preformed human anti-chimeric antibodies)

Modified from Xu and Vugmeyster [39]

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. Biologics are characterized by specific pharmacokinetic (PK) features [18]. The delivery of biologics 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 biologics also exhibit nonlinear 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. 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, biologics 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. Thus, "biosimilars" are being developed, several of which have gained approval including growth hormones, hematopoietic growth factors, and recently the biosimilar antibody infliximab.

19.6 New Advances

There are currently also several technical advances yielding new molecules. Those include recombinant proteins with reduced size due to removal of functionally nonessential domains (e.g., B-domain deleted FVIII). *Antibody fragment technologies* are also emerging which include antigen-binding fragments (e.g., ranibizumab), nanobodies such as caplacizumab, and single-chain variable fragments such as efungumab (Fig. 19.1).

Further, the number of conjugates is increasing and cytotoxic drugs are conjugated to monoclonal antibodies such as brentuximab vedotin or trastuzumab emtansine. Therefore, after many years "magic bullets" eventually seem to become true. Antibody–cytokine fusion proteins (also termed immunocytokines [43, 44]) shall deliver immunomodulatory cytokines specifically to tumors aiming to induce antitumoral responses while simultaneously limiting systemic toxicity.

Bispecific antibodies: Bispecific antibodies (bsAbs) combine specificities of two antibodies and simultaneously bind different antigens or epitopes [45]. Thereby they can interfere with multiple surface receptors or ligands. Bispecific antibodies can also place targets into close proximity, to support protein complex formation on one cell or to trigger contacts between cells which then help to catalyze specific reactions. One such example is an antibody which binds to both coagulation factors

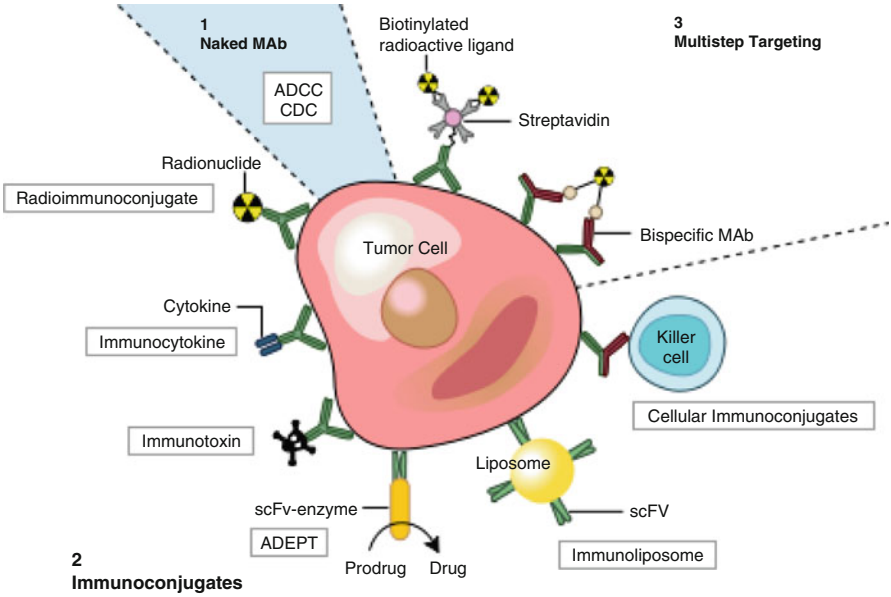

Fig. 19.1 Potential target-modulating strategies with monoclonal antibodies are presented. These range from immune-dependent target modulation via antibody-dependent cytotoxicity (*ADDC*) to targeting of conjugates, e.g., radioisotopes, to target structures. Dependent on sequence variations, monoclonal antibodies may be categorized as chimeric(-ximab), humanized(-zumab), murine(-momab), and human(-umab) antibodies

FIXa and FXa mimicking FVIII activity. This yields a long-acting procoagulant molecule with a duration of action outlasting that of factor VIII many times.

Half-life extensions can be achieved by coupling of large biologic or synthetic nonbiologically produced molecules such as polyethylene glycol (PEGylation, e.g., pegfilgrastim or PEG interferons) and hydroxyethyl starch (HESylation; [46]), Fc fusion, immunoglobulin (Ig) binding, enhanced FcRn binding, albumin binding or fusion, and eventually glycosylation such as polysialation.

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Generics, Biosimilars, Enantiomers, and Me-Toos

20

Dieter Deforce, George Wade, and Brigitte Bloechl-Daum

20.1 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) and 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 indefinitely. Generic medicines are basically copies of these innovative medicines which have been marketed for several years with proven 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,

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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 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 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 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 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 multicenter 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.

20.1.1 Regulatory Background

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, decentralized, and centralized (i.e., EU-wide) levels. The centralized procedure was set up in 1995 to deal primarily with new molecules, new technology, biotechnological medicines, new clinical indications, etc. 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" or 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. Generic 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 colors, 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 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 SmPC. This defines the approved clinical indications, population to be treated, dose, precautions, warnings, contraindications, 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 SmPC, they may expose themselves to issues of liability. Not surprisingly, the SmPC for a generic should be identical to that of the reference product as far as possible, but important differences may exist [2] (see also the next section with regard to patented indications).

20.1.2 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 pharmaceutical 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 cannot 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 cannot 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 favorable 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 SmPC 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 SmPC 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.

20.1.3 Salts and 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 and amlodipine maleate
- Clopidogrel hydrogen sulfate and 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 and can result in a BCS (Biopharmaceutics Classification System)-based biowaiver. Over the last years the EMA has issued (draft) product-specific bioequivalence guidance indicating for these specific products what bioequivalence studies are expected and if a biowaiver could be applicable [3]. In general BCS class I and III compounds are likely to be acceptable candidates for a biowaiver; on the contrary, for BCS class II and IV drug substances in vivo studies will most likely be mandatory.

20.1.4 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 predefined 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 (biowaiver)
- Comparative bioavailability (bioequivalence) studies
- Comparative pharmacodynamic studies in humans
- Comparative clinical trials

Bioequivalence is not normally needed when both the generic and reference products contain a water-soluble drug which is already in solution in the product, but it is particularly relevant for the following types of products 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
- Unfavorable 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) [4]. 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 fixed 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 nonlinear drugs. Studies should be carried out in accordance with provisions of EU requirements for Good Clinical Practice, Good Manufacturing Practice, and 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 metabolizers
  - 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. Two sets of data are gathered, one for the generic (test) product and the other for the reference product. According to current EU guidance [5], 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, $AUC_{(0-72h)}$. 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 [6]. These are compared in terms of the ratio between the means 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 [7]. No particular attention or weighting factor is given to the values of the means themselves; within the confidence interval, all values have equal probability and equal weight, and the confidence interval must be wholly contained within the defined acceptance range.

90 % CI around the mean $(C_{max})_{test}/(C_{max})_{ref}$ should be within 80.00–125.00 %.
90 % CI around the mean $(AUC)_{test}/(AUC)_{ref}$ 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 (Fig. 20.1).

20.1.5 Bioequivalence: Some Special Issues

20.1.5.1 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 are required to be contained in the range of 90.00–111.11 %, and this also applies to mean C_{max} if considered necessary. There is no official list of NTIDs; the judgment is made case by case according to clinical considerations, but for, e.g., sirolimus product-specific guidance is available [3].

20.1.5.2 Highly Variable Drug Products (HVDPs)

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

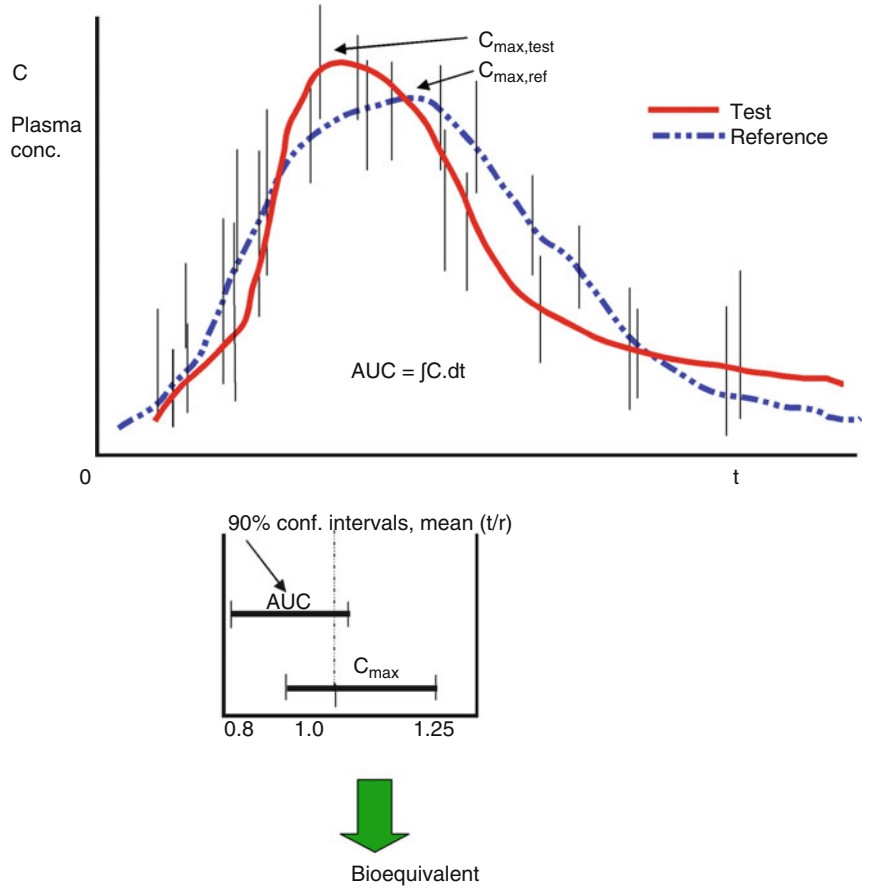

Fig. 20.1 Oral immediate-release products. Mean drug plasma levels measured in a single-dose crossover study. Generic (test, f) is bioequivalent to the reference product (reference, *r*), even though the mean C_{max} "appears" to be higher and the mean AUC lower. The confidence interval boundaries do not cross the 0.8–1.25 envelope

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.

20.1.5.3 Chiral Drugs and 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.

20.1.5.4 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 nonparametric 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}.

20.1.5.5 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 end points 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.

20.1.5.6 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 are 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 end point like FEVV.

20.1.5.7 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 exists 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 are 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 end points 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 tumors – the EPR effect [8] (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 of 10–100 nm are retained by the tight endothelial junctions of normal

vasculature, but tumor vasculature 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 tumor – 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.

20.2 Biosimilars

20.2.1 Complexity

Biological medicines, including large peptides and proteins, are complex molecules, not only in terms of primary structure but also secondary, tertiary, and quaternary. Arising from this complex nature, the manufacturing process must be very tightly defined, because small changes in processing conditions may have an impact on the nature of the resulting molecule and have an impact on 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 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 (Da)	Biological	MWt (Da)
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 and blood
- A "nonchemical 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)

20.2.2 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, they are also open to competition. However showing essential similarity and bioequivalence as for generics will in general not suffice. 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, and now current EU legislation [9] 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 in general will need appropriate preclinical and clinical testing before being delivered to the market; the recent EMA guideline suggests a stepwise approach from quality to nonclinical to clinical and opens the door to reduced clinical programs where appropriate and justified [10].

The safety and efficacy profile of a biosimilar established for one therapeutic indication can be extrapolated to other therapeutic indications of the originator if justified [11]. The European Medicines Agency has several guidance documents on biosimilars, and both the European Generic Medicines Association (EGA) and EMA have produced useful Question and Answer documents on their websites [12, 13].

The concepts established for chemical generics, e.g., essential similarity and 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 posttranslational modifications such as the glycosylation profile can be significantly altered by manufacturing 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.

20.2.3 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 physicochemical and biological methods, clinical studies demonstrated similar clinical efficacy for the biosimilar and the reference product. The incidence of antisomatropin 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 product-specific guidelines for biosimilars covering several different types of recombinant proteins, including insulin, granulocyte colony-stimulating factor (G-CSF), somatropin, erythropoietin, interferon, follicle-stimulating hormone, as well as low-molecular-weight heparins and monoclonal antibodies. These product-specific guidelines for biosimilar development give indications on what kind of studies will be expected nonclinically and clinically. Information is given on PK/PD studies and expected end points, for clinical efficacy, preferences are given on patient population and preferred end points, and for clinical safety, information is provided on, e.g., expected duration of immunogenicity testing. Up until now, at least nineteen biosimilars have been authorized in the EU, including biosimilars for insulin, somatropin, follitropin, epoetin, interferon, filgrastim, and infliximab (a monoclonal antibody), and many more biosimilars are in the pipeline.

20.3 Enantiomers

20.3.1 Sophisticated Nonsense?

The stereochemistry of organic molecules has been known and studied since the early nineteenth century, and terminology has evolved to be quite confusing, with a plurality of systems in use for the differentiation of chiral forms:

d, l 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 center 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 Ariens at the University of Nijmegen published an article with the provocative title "Stereochemistry, a basis for sophisticated nonsense in pharmacokinetics and clinical pharmacology" [14]. 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 maybe expected to have a more uniform action, although many of the claimed benefits have yet to be clearly realized in the clinic. In addition some enantiomers will interconvert.

20.3.2 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.

20.3.3 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 an over 100-fold more potent inhibitor of cyclooxygenase 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 an over 100-fold more potent 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.

20.3.4 Recent Regulatory Experience of the Chiral Switch: A Word of Caution

There has been dispute in the regulatory authorities through the 1980s and 1990s 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 favorable.

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"? 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 [15]:

Proton Pump Inhibitors: Omeprazole exists 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.

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 favorable 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 program 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 judgment 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, no protection period could be granted under EU pharmaceutical legislation, and the application was withdrawn by the applicant. However it was registered in the USA, and it is a quite successful drug, for which the FDA has meanwhile also accepted a generic.

20.4 Me-Toos

20.4.1 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 [16]. 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 sulfanilamide, a common industrial chemical that was no longer patented and that no one had ever thought to test against bacteria [17, 18].

Within months, nearly every drug company in the world began synthesizing their own versions of sulfanilamide – 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 sulfonamide drugs plunged [19]. The pattern was repeated after World War II. The US 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" [20].

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 fueled 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 of 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 [21].

20.4.2 What Are "Me-Too" Drugs?

The term "me-too" drug first came up in the 1960s, following increasing concerns over the abovementioned "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 [22].

In the 1970s the median time between the innovator and first "me-too" drug was 10.2 years, and in the 1990s it was only 1.2 years. The median period between the first and second "me-too" drug in the 1970s was 4.2 years and in the 1990s it was 1.7 years. The interval between the second and third me-too drug dropped from 3.7 years in the 1970s to 0.9 years in the 1990s [22]. Taking into consideration that the development of a new drug from bench to bedside is estimated to be between 10 and 15 years [23], 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" [22]. So the availability of "me-too" drugs does not necessarily mean that imitation has replaced innovation in healthcare. 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 [24].

20.4.3 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) [22]. 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.

20.4.4 What Is a Drug Class?

The US Food and Drug Administration (FDA) uses class labeling 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 [25].

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 [26]

20.4.5 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" [26]. 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" [27].

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 [27].

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-analysis 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 [28]. In another study the authors found no difference between five PPIs (esomeprazole, lansoprazole, omeprazole, pantoprazole, and rabeprazole) for relief of symptoms and healing of gastroesophageal reflux disease [29].

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 [30].

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Orphan Drugs for Rare Diseases

21

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21.1 What Are Rare Diseases?

In the European Union rare diseases have been defined as life-threatening or chronically debilitating conditions that affect no more than 5 in 10,000 people in the EU [1].

The European Union has recognised that '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 socioeconomic potential. While the prevalence number seems relatively small, currently it translates into approximately 250,000 persons in the EU with 28 Member States.

To date, 5000–8000 distinct rare diseases have been described in the medical literature, affecting between 6 and 8 % of the population in total [2], which means that between 30 and 40 million people in the European Union are affected by a rare disease.

Florence Butlen-Ducuing and Jordi Llinares-Garcia: 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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21.2 What Are Orphan Drugs?

The lack of investment and 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 stimulate the development and marketing 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 [1]:

- The severity of the disease in terms of its life-threatening or chronically debilitating nature
- The rarity of the condition based on a prevalence not higher than 5 in 10,000 patients
- The intention to treat, prevent or diagnose the disease
- Either a demonstrated insufficient return on investment or the rarity of the condition or the absence of satisfactory method of diagnosis, prevention or treatment of the condition concerned is authorised or, if such method exists, the assumption that the product will be of Significant Benefit to those affected by the condition

21.3 The Orphan Drug Legislation

Under normal market conditions, the pharmaceutical industry has had 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 recognised 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 [2]. Therefore, specific legislation to stimulate the discovery and development of drugs for rare diseases – the so-called orphan drugs – has been introduced in the United States in 1983 [4], in Japan in 1993 [5], in Australia in 1997 and in the EU in 2000 [6].

21.4 The Committee for Orphan Medicinal Products (COMP)

In April 2000 a new Committee for 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 European Medicines Agency and appointed by the Commission. The committee meets 11 times per year. The COMP is responsible for reviewing applications from individual sponsors (usually researchers) and companies seeking ‘orphan medicinal product designation’ for the products they intend to develop for the diagnosis, prevention or treatment of rare diseases [7].

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.

21.5 Orphan Incentives [1]

Sponsors with an orphan designation for a medicinal product are entitled to incentives such as:

- Market exclusivity: For 10 years after the granting of a marketing authorisation, 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 unless some specific derogations defined in the regulation are accepted (lack of supply of the first product, superiority over the first product or consent from the original sponsor). It is not possible to extend an existing authorisation of a similar product for the orphan indication.
- Mandatory and direct access to centralised marketing authorisation
- Protocol assistance
- The agency can provide scientific advice to optimise development and guidance on preparing a dossier that will meet European regulatory requirements. This helps applicants to maximise the chances of their marketing authorisation application being successful. Protocol assistance (i.e. scientific advice for orphan medicines) is considered a priority, as it is a way to both improve and facilitate the applications for marketing authorisation of orphan medicinal products and therefore to increase the chances for the patients affected by rare diseases to have access to effective treatments in a timely manner.
- Protocol assistance is one of the most utilised and important incentives for orphan medicine development.
- Fee reductions [8]
- 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 is considered for all types of centralised activities, including applications for marketing authorisation, inspections, variations and protocol assistance. For small- and medium-sized enterprises (SMEs), additional fee reductions are applicable.
- 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. Recently the European Commission has considered orphan designation as a key element for applications to the Research Framework Programmes of the European Union.

21.6 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 [9]. 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 costs 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 [2].
- 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 [2].
- 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 over authorised 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 [10] may have been based, for example, on an alternative mechanism of action which might result in an improved clinical outcome, more favourable pharmacokinetic properties or potentially better clinical efficacy. 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 improvement to patient management or quality of life or, for instance, a significant and clinically valuable decrease in the number of intakes/day with the same clinical outcome, which improves patient care or compliance.

So far, more than 60 % of positive opinions adopted on orphan designations were based on the assumption of Significant Benefit [2]. The concept of Significant Benefit seems to be closely related to that of added therapeutic value [11].

Significant Benefit has to be demonstrated at the time of marketing authorisation in order to maintain the orphan status once the product reaches the market and is eligible for further incentives. 'If it is established before the market authorisation is granted that the criteria laid down in Article 3 (criteria for designation) are no 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 to assess Significant Benefit, 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 authorisation. 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 authorisation.

21.7 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.
- Recognised 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 and 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 subset 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 [2].

21.8 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 authorisation, but a 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.

21.8.1 Some Examples for a Positive Opinion for Orphan Designation

Human reovirus type 3 Dearing strain for the treatment of pancreatic cancer [14]:

- Pancreatic cancer affects less than 103,000 persons in the European Union, which is below the ceiling of 5 people in 10,000.
- This 'oncolytic' virus called reovirus might be able to target, infect and destroy cancer cells, but does not infect normal cells. When inside a cancer cell, the virus is expected to take over the cell's replication apparatus and use it to make more copies of itself. This is expected to kill the cell, leaving the virus to spread to neighbouring cancer cells. These characteristics may be of potential Significant Benefit over the existing authorised medicinal products → positive opinion.

Chimeric 2'-O-(2-methoxyethyl)/DNA modified oligonucleotide targeted to huntingtin RNA for treatment of Huntington's disease:

- The condition is chronically debilitating due to progressive motor dysfunction and severe behavioural and cognitive disturbances and life-threatening and affects approximately 1 in 10,000 persons in the European Union.
- The product is an antisense oligonucleotide that is expected to reduce HTT expression via hybridization to the cognate mRNA and avoid the abnormal protein from being produced, which is expected to reduce damage to brain cells and hence improve symptoms and slow the progression of the disease. The alternative mechanism of action to existing therapies and the sound pharmacological data were deemed as of potential Significant Benefit over existing authorised 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.

21.8.2 Reason for Negative Opinions: Subsetting, No Significant Benefit

21.8.2.1 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 subset should be submitted; otherwise this would not be sufficient to receive orphan designation, subsetting into different stages, and severities of diseases are not allowed.

One issue has become relevant given advances in personalised medicine where a subset of the patient population can be defined by the existence of a biomarker [16]. This might lead to the fact that applicants for orphan designation will 'salami slice' treatment indications in order to qualify for orphan status [17].

Example

Transglutaminase-1-deficient autosomal recessive congenital ichthyosis:

- Transglutaminase-1-deficient autosomal recessive congenital ichthyosis is an inherited skin disorder caused by abnormalities in a gene called TGM1, which produces the enzyme transglutaminase-1. This is a subset of autosomal recessive congenital ichthyosis (ARCI), a group of rare and severe skin diseases (Russell et al. 1995; Oji et al. 2010). Up to date nine different genes have been associated with ARCI.
- The product designated is an amino acid sequence of recombinant human transglutaminase 1 (rhTG1) which works as an enzyme replacement therapy.

The specificity of the mode of action for the missing enzyme justified the exclusive use in the subset of ARCI lacking the enzyme that characterises transglutaminase-1-deficient autosomal recessive congenital ichthyosis.

→ positive opinion (valid condition)

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.

21.8.2.2 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 authorised 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 sound pharmacological principles and data, the level of which depends on the stage of development, i.e. well-justified assumptions and supportive data at the time of designation and confirmation with results from trials at the time of the confirmation prior to marketing authorisation.

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 authorisation, more than 65 % of 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, versus 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. i.v. application: 'the burden of i.v. 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.

21.8.2.3 All Negative Opinions [21]

Medicinal product	Condition	Reason for the negative opinion
Ibritumomab tiuxetan/90yttrium	B-cell non-Hodgkin's lymphoma	Prevalence not below 5 in 10,000
Chlorproguanil hydrochloride and dapsone	Acute uncomplicated *Plasmodium falciparum* malaria	No Significant Benefit
Mycobacterial cell wall complex	Superficial bladder cancer	Prevalence not below 5 in 10,000
Midazolam hydrochloride (for oromucosal use)	Seizures which continue for at least five minutes	Prevalence not below 5 in 10,000
Histamine dihydrochloride	Malignant melanoma	Prevalence not below 5 in 10,000
Sudismase	Active phase of Peyronie's disease	Prevalence not below 5 in 10,000
Ibuprofen L-lysinate (salt)	Treatment of patent ductus arteriosus in premature neonates of less than 34 weeks of gestational age	Prevalence not below 5 in 10,000
Ibuprofen L-lysinate (salt)	Prevention of patent ductus arteriosus in premature neonates of less than 34 weeks of gestational age	Prevalence not below 5 in 10,000
Tramadol hydrochloride	Painful HIV-associated neuropathy	Not considered as a valid subset
Capsaicin	Painful HIV-associated neuropathy	Not considered as a valid subset
Chelidonii radix spec. liquid extract – Ukraine	Treatment of pancreatic cancer	No Significant Benefit

Medicinal product	Condition	Reason for the negative opinion
Molgramostim	Treatment of cystic fibrosis	No Significant Benefit
Gastrin 17C diphtheria toxoid conjugate	Treatment of pancreatic cancer	No Significant Benefit
Lentiviral vector expressing the truncated form of human tyrosine hydroxylase gene, human aromatic L-amino acid decarboxylase gene, human GTP cyclohydrolase 1 gene	Treatment of 'off' periods in adult patients with advanced Parkinson's disease	Not a distinct, recognisable medical entity but a stage of a broader, medical condition, namely, Parkinson's disease, which is not rare Not a valid subset for orphan designation
Nabilone	Treatment of amyotrophic lateral sclerosis	No data on the effect
Tariquidar	Treatment of P-gp-positive breast cancer	Not a valid subset for orphan designation. Prevalence of the broader medical condition (breast cancer) is more than 5 in 10,000 people
Zoledronic acid	Treatment of complex regional pain syndrome	No medical plausibility
5-Chloro-N2-[2-isopropoxy-5-methyl-4-(4-piperidinyl) phenyl]-N4-[2-(isopropylsulfonyl) phenyl]-2,4-pyrimidinediamine	Treatment of non-small cell lung cancer (NSCLC) that is anaplastic lymphoma kinase (ALK)-positive	Not a distinct, recognised medical entity, not a valid subset, it cannot be established that the medicinal product would only work in patients in this subset as opposed to other patients with NSCLCs. Prevalence of NSCLC is estimated to be about 6 in 10,000
Sodium ascorbate and menadione sodium bisulfite	Treatment of autosomal dominant polycystic kidney disease	Prevalence not below 5 in 10,000

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 reapply 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 transformed into a Commission decision, unless an appeal procedure is triggered. A summary of the negative opinion will be published on the agency website [21]. About one-quarter of all applications are withdrawn, before a negative opinion gets adopted and published (see Table 21.1).

Table 21.1 Overview of the status of orphan designation applications since 2000

	2000–2005	2006–2010	2011	2012	2013	2014	Total
Applications submitted	548	686	166	197	201	329	2127
Positive COMP opinions	348	500	111	139	136	196	1430
Negative COMP opinions	8	6	2	1	1	2	20
EC designations	343	485	107	148	136	187	1406
Withdrawals during assessment	156	144	45	52	60	61	518

21.9 Scientific Advice: Protocol Assistance [22]

Scientific advice (SA) provided by the European Medicines Agency (EMA) was initiated in 1996 as a tool to improve communication between sponsors and regulators throughout drug development. Its aim is to support sponsors to provide adequate data for benefit-risk assessment at the time of marketing authorisation application (MAA) and thereby to facilitate the introduction of new, safe and effective medicines. SA is voluntary and nonbinding and may be given on all aspects of drug development programmes by the Scientific Advice Working Party (SAWP) of the EMA's Committee for Medicinal Products for Human Use (CHMP) [23].

The agency gives scientific advice by answering questions posed by companies. The advice is given in the light of the current scientific knowledge, based on the documentation provided by the company. It is not the role of the CHMP to substitute the industry's responsibility for the development of their products. Scientific advice is prospective in nature. It focuses on development strategies, rather than pre-evaluation of data to support a marketing authorisation application.

Protocol assistance is the special form of scientific advice available for companies developing designated orphan medicines for rare diseases.

In addition to scientific advice, companies developing an orphan medicinal product can receive answers to questions relating to the criteria for authorisation of an orphan medicine. These include:

- The demonstration of Significant Benefit within the scope of the designated orphan indication
- Similarity or clinical superiority over other medicines

This is relevant if other orphan medicinal products exist that might be similar to the product concerned and that have market exclusivity in the same indication.

21.10 Confirmation of Orphan Status at the Time of Marketing Authorisation [24]

Designated orphan medicinal products have mandatory access to the centralised procedure marketing authorisation. The assessment of the benefit/risk balance of the applications for marketing authorisation is done by the Committee for Medicinal

Products for Human Use (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 authorisation 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 authorisation by the European Commission, orphan medicinal products will benefit from 10 years of market exclusivity for the authorised 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 [25], orphan medicinal products may be granted a 2-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.

21.11 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 No 847/2000:

- 'Similar medicinal product' means a medicinal product containing a similar active substance of substances as contained in a currently authorised 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 No 141/2000 three derogations to break market exclusivity for similar products are laid down. These are:

- If the holder of the marketing authorisation for the original orphan medicinal product has given his consent to the second applicant
- If the holder of the marketing authorisation 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 authorised, 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) [26]

- 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.

21.12 Success of the Orphan Programme

21.12.1 Designated Orphan Medicinal Products

In the first 10 years since the EU Orphan Legislation, 720 medicinal products were officially designated as orphan by the European Commission [27]. Now 15 years after implementation of the Orphan Legislation, the number has more than doubled and has reached now 1430.

The distribution of positive COMP opinions by therapeutic area is provided below in Fig. 21.1.

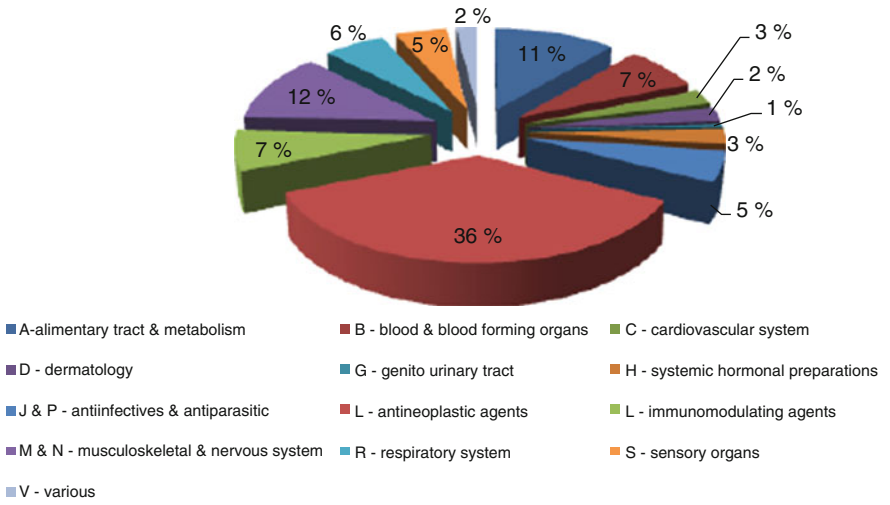

Fig. 21.1 Distribution of positive COMP opinions on designation by therapeutic area

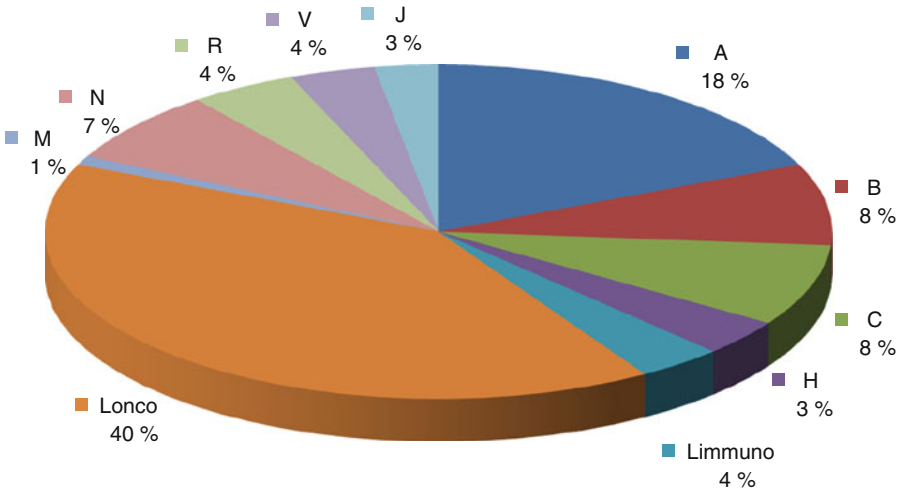

Fig. 21.2 Distribution of orphan marketing authorisations per therapeutic area

Table 21.1 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 329 applications received in 2014.

Furthermore, 261 designated products have been removed from the register on request of sponsors, for administrative reasons or when development was discontinued.

21.12.2 Marketing Authorisations for Orphan Medicinal Products

Up to December 2014 100 designated orphan medicinal products have received marketing approval in the EU so far. More than two-thirds of the authorised orphan medicinal products were antineoplastic and immunomodulating agents (Fig. 21.2).

Of the 100 orphan medicinal products authorised, 28 % of the marketing authorisations were granted 'under exceptional circumstances' and 10 % as 'conditional approval' (Table 21.2). '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', further studies will be needed to maintain the marketing authorisation; this will be reviewed annually by the European Medicines Agency.

Table 21.2 List of the 100 orphan medicinal products approved through the centralised procedure since 2001

Year	Orphan marketing authorisation	Significant benefit	Type of marketing authorisation
2001	*Fabrazyme* for Fabry disease	No	Exceptional circumstances
	Replagal for Fabry disease	No	Exceptional circumstances
	Glivec for chronic myeloid leukaemia	Yes	Exceptional circumstances
2002	*Tracleer* for pulmonary arterial hypertension	Yes	Exceptional circumstances
	Trisenox for acute promyelocytic leukaemia	Yes	Exceptional circumstances
	Somavert for acromegaly	Yes	Normal
	Zavesca for Gaucher's disease	Yes	Exceptional circumstances
2003	*Carbaglu* for hyperammonaemia	No	Exceptional circumstances
	Aldurazyme for mucopolysaccharidosis	No	Exceptional circumstances
	Busilvex for haematopoietic progenitor cell transplantation	Yes	Normal
	Ventavis for pulmonary arterial hypertension	Yes	Exceptional circumstances
	Onsenal for familial adenomatous polyposis	No	Exceptional circumstances
2004	*Litak* for hairy cell leukaemia	Yes	Normal
	Lysodren for adrenal cortical carcinoma	Yes	Normal
	Pedea for patent ductus arteriosus	Yes	Normal
	PhotoBarr for Barrett's oesophagus	No	Normal
	Wilzin for Wilson's disease	Yes	Normal
	Xagrid for thrombocythaemia	Yes	Exceptional circumstances

Table 21.2 (continued)

Year	Orphan marketing authorisation	Significant benefit	Type of marketing authorisation
2005	*Orfadin* for hereditary tyrosinaemia type 1	No	Exceptional circumstances
	Prialt for chronic pain requiring intrathecal (IT) analgesia	Yes	Exceptional circumstances
	Xyrem for cataplexy in patients with narcolepsy	Yes	Normal
	Revatio for pulmonary arterial hypertension	Yes	Exceptional circumstances
2006	*Naglazyme* for replacement therapy in patients with mucopolysaccharidosis VI	No	Exceptional circumstances
	Myozyme for glycogen storage disease type II (Pompe's disease)	No	Normal
	Evoltra for acute lymphoblastic leukaemia	Yes	Exceptional circumstances
	Nexavar for advanced renal cell carcinoma	Yes	Normal
	Sutent for gastrointestinal stromal tumour and metastatic renal cell carcinoma	Yes	Conditional approval
	Savene for anthracycline extravasation	No	Normal
	Thelin for idiopathic pulmonary arterial hypertension or pulmonary arterial hypertension	Yes	Normal
	Exjade for chronic iron overload due to blood transfusions	Yes	Normal
	Sprycel for acute lymphoblastic leukaemia and chronic myeloid leukaemia	Yes	Normal
	Diacomit for severe myoclonic epilepsy in infancy	Yes	Conditional approval
	Elaprase for mucopolysaccharidosis type II (Hunter syndrome)	No	Exceptional circumstances
	Inovelon for Lennox-Gastaut syndrome	Yes	Normal
	Cystadane for homocystinuria	Yes	Normal
2007	*Revlimid* for multiple myeloma	Yes	Normal
	Soliris for paroxysmal nocturnal haemoglobinuria	No	Normal
	Siklos for sickle cell syndrome	No	Normal
	Atriance for acute lymphoblastic leukaemia	Yes	Exceptional circumstances
	Increlex for primary insulin-like growth factor-1 deficiency due to molecular or genetic defects	No	Exceptional circumstances
	Gliolan for intraoperative photodynamic diagnosis of residual glioma	Yes	Normal
	Yondelis for soft tissue sarcoma	Yes	Exceptional circumstances
	Tasigna for chronic myeloid leukaemia	Yes	Normal
	Torisel for renal cell carcinoma	Yes	Normal

Table 21.2 (continued)

Year	Orphan marketing authorisation	Significant benefit	Type of marketing authorisation
2008	*Thalidomide Celgene* for multiple myeloma	Yes	Normal
	Volibris for pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension	Yes	Normal
	Firazyr for angioedema	Yes	Normal
	Ceplene for acute myeloid leukaemia	Yes	Exceptional circumstances
	Kuvan for hyperphenylalaninaemia	Yes	Normal
	Mepact for osteosarcoma	Yes	Normal
	Vidaza for acute myeloid leukaemia and myelodysplastic syndromes	Yes	Normal
2009	*Nymusa* for primary apnoea in premature newborns	Yes	Normal
	Afinitor for renal cell carcinoma	Yes	Normal
	Mozobil to mobilise progenitor cells prior to stem cell transplantation	Yes	Normal
	Cayston for gram-negative bacterial lung infection in cystic fibrosis	Yes	Conditional approval
	Arcalyst for cryopyrin-associated periodic syndromes (CAPS), including familial cold autoinflammatory syndrome (FCAS) and Muckle-Wells syndrome (MWS)	No	Exceptional circumstances
	Ilaris for cryopyrin-associated periodic syndromes (CAPS), including familial cold autoinflammatory syndrome (FCAS) and Muckle-Wells syndrome (MWS)	No	Exceptional circumstances
	Firdapse for treatment of Lambert-Eaton myasthenic syndrome		Exceptional circumstances
	Nplate for idiopathic thrombocytopenic purpura (ITP)	Yes	Normal
2010	*Revolade* for chronic immune (idiopathic) thrombocytopenic purpura (ITP)	Yes	Normal
	Tepadina for conditioning treatment prior to autologous or allogeneic haematopoietic progenitor cell transplantation	Yes	Normal
	Azerra chronic lymphocytic leukaemia (LL)	Yes	Conditional
	VPRIV for long-term enzyme replacement therapy (ERT) in patients with type 1 Gaucher's disease	Yes	Normal

(continued)

Table 21.2 (continued)

Year	Orphan marketing authorisation	Significant benefit	Type of marketing authorisation
2011	*Esbriet* for mild to moderate idiopathic pulmonary fibrosis (IPF)	No	Normal
	TOBI Podhaler for the suppressive therapy of chronic pulmonary infection due to *Pseudomonas aeruginosa* in adults and children aged 6 years and older with cystic fibrosis	Yes	Normal
	Votubia for the treatment of patients with subependymal giant cell astrocytoma (SEGA) associated with tuberous sclerosis complex (TSC)	No	Conditional
	Plenadren for adrenal insufficiency	Yes	Normal
	Vyndaqel transthyretin amyloidosis in patients with symptomatic polyneuropathy	Yes	Exceptional circumstances
2012	*Xaluprine* for acute lymphoblastic leukaemia (ALL)	Yes	Normal
	Bronchitol for the treatment of cystic fibrosis	Yes	Normal
	Signifor for the treatment of Cushing's disease	Yes	Normal
	Kalydeco for treatment of cystic fibrosis	Yes	Normal
	Jakavi for: treatment of chronic idiopathic myelofibrosis and treatment of myelofibrosis secondary to polycythaemia vera or essential thrombocythaemia	Yes	Normal
	Revestive for treatment of short bowel syndrome	No	Normal
	Dacogen for treatment of acute myeloid leukaemia	Yes	Normal
	Glybera for treatment of lipoprotein lipase deficiency	No	Exceptional circumstances
	Adcteris for: treatment of Hodgkin's lymphoma and anaplastic large cell lymphoma	Yes	Conditional
	NexoBrid for treatment of partial deep dermal and full-thickness burns	Yes	Normal
2013	*Bosulif* for treatment of chronic myeloid leukaemia	Yes	Conditional
	Iclusig for: Treatment of chronic myeloid leukaemia Treatment of acute lymphoblastic leukaemia	Yes	Normal
	Pomalidomide for treatment of multiple myeloma	Yes	Normal
	Procysbi for treatment of cystinosis	Yes	Normal
	Orphacol for treatment of inborn errors in primary bile acid synthesis	No	Exceptional circumstances
	Defitelio for treatment of severe hepatic veno-occlusive disease	No	Exceptional circumstances
	Opsumit for treatment of pulmonary arterial hypertension	Yes	Normal

Table 21.2 (continued)

Year	Orphan marketing authorisation	Significant benefit	Type of marketing authorisation
2014	Cometriq for treatment of adult patients with progressive, unresectable locally advanced or metastatic medullary thyroid carcinoma	Yes	Conditional
	Sirturo indicated for use as part of an appropriate combination regimen for pulmonary multidrug-resistant tuberculosis (MDR TB) in adult patients when an effective treatment regimen cannot otherwise be composed for reasons of resistance or tolerability	Yes	Conditional
	Adempas for treatment of chronic thromboembolic pulmonary hypertension and pulmonary arterial hypertension	Yes	Normal
	Deltyba for treatment of multidrug-resistant tuberculosis	Yes	Conditional
	Granupas for treatment of tuberculosis	Yes	Normal
	Kolbam for treatment of inborn errors of primary bile acid synthesis	No	Normal
	Vimizim for treatment of mucopolysaccharidosis, type IVA (Morquio A syndrome, MPS IVA) in patients of all ages	No	Normal
	Sylvant is indicated for the treatment of adult patients with multicentric Castleman's disease who are human immunodeficiency virus negative and human herpesvirus-8 negative	No	Normal
	Gazyvaro for treatment of patients with previously untreated chronic lymphocytic leukaemia	Yes	Normal
	Translarna for treatment of Duchenne muscular dystrophy	No	Normal
	Ketoconazole Lab HRA Pharma for treatment of Cushing's syndrome	Yes	Normal
	Imbruvica Indicated for the treatment of adult patients with relapsed or refractory mantle cell lymphoma. Indicated for the treatment of adult patients with chronic lymphocytic leukaemia	Yes	Normal
	Cyramza is indicated for the treatment of patients with advanced gastric cancer or gastro-oesophageal junction adenocarcinoma after prior chemotherapy	Yes	Normal
	Lynparza is indicated for the maintenance treatment of adult patients with platinum-sensitive relapsed BRCA-mutated ovarian cancer who are in response (complete response or partial response) to platinum-based chemotherapy	Yes	Normal
	Scenesse for treatment of phototoxicity in adult patients with erythropoietic protoporphyria	No	Exceptional circumstances

(continued)

Table 21.2 (continued)

Year	Orphan marketing authorisation	Significant benefit	Type of marketing authorisation
2015	Ofev for the treatment of idiopathic pulmonary fibrosis	Yes	Normal
	Cerdelga for the treatment of Gaucher's disease	Yes	Normal
	Holoclar for the treatment of lesions associated with corneal (limbal) stem cell deficiency due to ocular burns	Yes	Conditional
	Lenvima for the treatment of thyroid neoplasms	Yes	Normal
	Hetlioz for the treatment of non-24-h sleep-wake disorder	No	Normal
	Unituxin for the treatment of neuroblastoma	Yes	Normal
	Strensiq for the treatment of hypophosphatasia	No	Exceptional
	Kanuma for the treatment of inborn errors in lipid metabolism	No	Normal
	Farydak for the treatment of multiple myeloma	Yes	Normal
	Raxone for the treatment of Leber's hereditary optic neuropathy	No	Exceptional
	Cresemba for the treatment of aspergillosis	Yes	Normal
	Kyprolis for the treatment of multiple myeloma	Yes	Normal
	Kolbam for the treatment of inborn errors in primary bile acid synthesis	Yes	Normal
	Blincyto for the treatment of acute lymphoblastic leukaemia	Yes	Conditional
	Ravicti for long-term use to manage urea-cycle disorders	Yes	Normal
Total	*100*	*SB 73*	*Normal: 61* *Exceptional circumstances: 29* *Cond. app: 10*

21.13 Discussion

Despite the obvious benefits of the Orphan Legislation, some criticism has been raised. The most common criticism of the orphan product legislation has been the very high cost of treatment with some of the drugs.

One of the concerns is that some incentives could also promote the creation of new drugs at prices that are so high that the actual accessibility of these drugs for patients might be an illusion [28].

Drugs such as imiglucerase, an enzyme replacement therapy developed to treat Gaucher's disease, and eculizumab [29], a humanised monoclonal (IgG2/4κ) antibody produced in NS0 cell line by recombinant DNA technology for the treatment of paroxysmal nocturnal haemoglobinuria (PNH) and atypical haemolytic uremic syndrome (aHUS) with costs in the range of 500,000 US dollars [30] or £330,000

[31] per patient per year, and other expensive orphan drugs have led to calls for modification of the legislation. The suggestion was made to place a cap on revenues from orphan drugs, to shorten exclusivity provisions or to review exclusivity provisions, when a drug becomes profitable [32].

Cote and Keating [28] point to the fact that companies developing orphan drugs are often very profitable and that orphan drugs are viewed as a good business opportunity. Others opined that while it is important that orphan drugs are as profitable as non-orphans if society wants them, they should not be disproportionately more profitable [17]. It has been estimated that the average cost of bringing a pharmaceutical product to market is approximately $1.3 billion USD, mostly distributed between different stages of clinical development [33]. However it has been suggested that, in general, R&D costs for orphan drugs are 25 % of the costs of standard drugs [34] as the costs associated with clinical trials are also lower due to the smaller number of patients involved [28].

In a recent publication by Lincker et al. [35], medicinal products for human use containing a new active substance (NAS) were investigated ($n=94$). For each approved marketing authorisation application (MAA), between 2010 and 2012, the originator organisation was profiled. For orphan products, 61 % of originators were SMEs, with large- or intermediate-sized pharmaceutical companies accounting for 22 % and academic/public body/public-private partnerships accounting for 11 %. This suggests that a sizable fraction of the basic development costs were publically funded. Also the contribution of patient organisations in the funding, research and development of orphan molecules should be taken into account. For example, the American Cystic Fibrosis Foundation has invested more than $300 million in the development of nearly all treatments approved in the United States for this rare disease [34]. Therefore it has been concluded by Cote et al. [28] that pricing is often not based on the actual development costs, but is often based on what patients and/or third-party payers are willing to pay.

There actually seems to be an inverse correlation between the price per capita of an orphan drug and the prevalence of the indication; the rarer the indication, the more expensive the treatment is. Development and production costs have not been found to correlate with the price of an orphan drug. Prices of orphan drugs are influenced by factors such as the availability of an alternative drug treatment, the length of treatment, the administration route, the presence of multiple indications and the impact on overall survival and quality of life [30].

Nevertheless, 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].

Fairness of access is generally regarded as a fundamental principle for healthcare systems; access to orphan drugs therefore is often seen as a right by patients and a challenging obligation for managers of public health programmes. With recent technological and scientific advances and the general success of the orphan programme, however, the number of treatments and treatable patients is rising. This, coupled with the high cost of treatment, has an increasingly significant impact on national budgets devoted to the reimbursement of drugs – so much some authors fear that it may jeopardise the viability of these programmes [28].

One concern that has been mentioned [17] about current policies for orphan drugs is that the policies for stimulating research and for providing reimbursement are at odds with one another and might lead to inefficiencies if scarce resources are devoted to the research and development of drugs that are not going to be used because of lack of funding from the healthcare provider, and pharmaceutical companies might eventually cease responding to the incentives to develop orphan drugs, because they will increasingly be uncertain whether the drugs, if developed, will be reimbursed.

Therefore the changes that the regulation has introduced to the marketing and development business models of pharmaceutical companies deserve attention, so the different decision-makers in the path to patient access apply the necessary controls to keep the value for the incentives for development and balance them against the profit that orphan medicines generate.

However, despite some misgivings, 15 years after the inception of the orphan regulation in Europe, there is clear evidence for success, with 100 new medicinal products having reached the market and being available for patients with rare diseases. Furthermore, with 1430 products for orphan conditions designated in Europe, and several ongoing MA applications, more orphan medicinal products are expected to be authorised in the coming 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 [2]. But as there is an estimate of 5000–8000 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.

21.14 Useful Links

European Medicines Agency website: http://www.ema.europa.eu/

Guidance documents, COMP information, public summaries of opinions for designated products and more are available on the agency's website Human regulatory > Orphan designation.

Community register of orphan medicinal products: http://ec.europa.eu/health/documents/community-register/html/index_en.htm

DG Health and Consumers, 'Rare diseases' page: http://ec.europa.eu/health/rare_diseases/policy/index_en.htm

European Organisation for Rare Diseases (EURORDIS): http://www.eurordis.org

Orphanet: http://www.orpha.net/

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Special Situations, Market Fragmentation II: Sex Differences

22

Ghazaleh Gouya

22.1 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 focused on reproductive health issues, such as contraception, pregnancy, menopause, and breast cancer and was relegated mainly to obstetricians and gynecologists. 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

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argue that geriatric medicine is essentially women's medicine. Female consumers are an increasing force likely to have substantial influence in the prescription marketplace.

22.2 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 follows:

- *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.

22.3 Sex Matters

22.3.1 Physiological 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].

Physiological 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, and 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].

22.3.2 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 plasma protein binding of drugs. Relevant differences between women and men in the unbound fraction of highly plasma protein-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 CPY2C9 and CYP2D6 activity [15–18].

22.3.3 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.

22.3.4 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].

22.3.5 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. Among 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, have 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 antipsychotics 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 antiarrhythmic 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].

22.3.6 Clinical Trials: Women's Representation

22.3.6.1 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 focused 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 (EMEA) 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).

22.3.6.2 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.

22.4 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 childbearing 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.

22.5 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 preexistent 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 safely during pregnancy as quickly as possible after a new drug is marketed.

22.6 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 three billion base pairs, and for every thousand base pairs, there is a variable base pair that gives rise to an SNP, resulting in three 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. Pharmacogenetic 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. 22.1). Cardiovascular death rates tend to slightly decline in men within the past two decades but are constantly high in women [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 ischemic 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

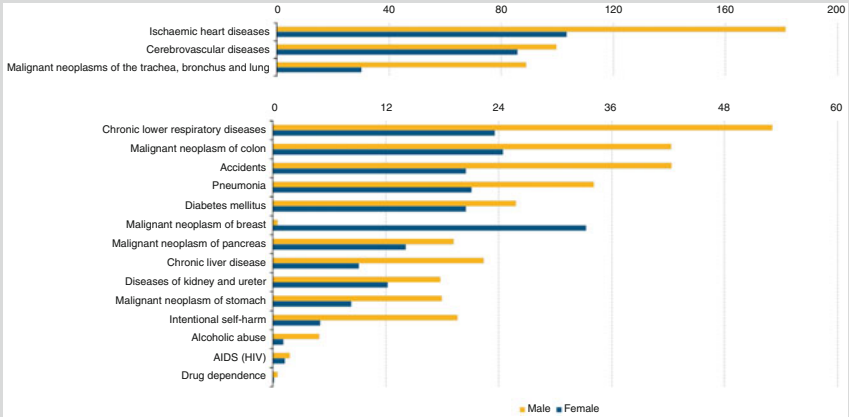

Fig. 22.1 Causes of death — standardised death rate, EU-28, 2012 (per 100 000 inhabitants) (http://ec.europa.eu/eurostat)

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 hypoestrogenemia [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 remodeling).
- 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 remodeling.
- 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 results 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. *Beta-blockers:* Myocardial beta-1 receptors are upregulated in case of estrogen deficiency, without effects on binding affinity [49]. Hormone supplementation with estrogens and progestins can prevent such upregulation. 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 postmenopausal 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 favorable 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 aspects of these evident differences are suspected gender-specific differences in cellular sodium and calcium handling [54]. A subgroup analysis of Heart and Estrogen-Progestin Replacement Study (HERS), which investigated the effect of postmenopausal 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 unfavorable 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 metabolization), and fluvastatin (predominantly CYP2C9 metabolization), 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 CYP3 A4 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 underrepresented 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.

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Special Situations III: Medicines for Children

23

Christoph Male

Abstract

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 different adverse events as in adults. 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 USA 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 authorisation extension. Paediatric development is rewarded with a 6-month 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 and transparency and to stimulate research into paediatric medicines. Key points to consider for a PIP are the definition of relevant paediatric indications(s), development of age-appropriate formulation(s), juvenile animal studies, paediatric PK and PD studies, clinical efficacy and safety studies, and the possibility of extrapolation from adults. A case study on a PIP is provided.

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23.1 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-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 from adults are applied to children. Such implicit extrapolation is usually not appropriate because of physiological, pathophysiological, and pharmacological 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, metabolisation, 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, and leukaemia.

Use of unlicensed drugs in children carries risks such as ineffective dosing, overdosing, lack of efficacy, and unknown safely 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 metabolisation, these doses lead to accumulation and life-threatening toxic effects in the neonates [9]. Child-appropriate formulations are usually lacking, and extemporaneous formulations are used instead, e.g. crushed tablets and pharmacy-prepared liquid formulations, which carry risks of contamination and unprecise dosing. Finally, marketing authorisation 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 % versus 3.9 % in the outpatient setting [10] and 3.4 % versus 1.4 % in the inpatient setting [11].

23.2 Hurdles to Drug Development for Children

23.2.1 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 data on paediatric medicines 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 two decades 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 which are likely to yield more valid results. Moreover, the directive ensures special protection of the child, e.g. by permitting only research questions that cannot be addressed by studying adults and that are related to the child's disease and requiring documented informed consent by proxies and minimisation of risks and burden to the child.

23.2.2 Lack of Public Acceptance

In public perception and the attitude of families 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.

23.2.3 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 diseases. 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.

23.2.4 Lack of Research Infrastructures and Paediatric Research Networks

The special methodological challenges of clinical studies in children require special expertise, infrastructures, and organisational 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 personal 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.

23.2.5 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.

23.3 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').

23.3.1 Paediatric Initiatives in the USA

In 1997, the *Food and Drug Agency Modernization Act* (*FDAMA, Paediatric Exclusivity Rule*) introduced a 6-month prolongation of market exclusivity for authorised drugs when paediatric studies were performed (incentive). This was followed in 1998 by the *Final Paediatric Rule* which authorised the 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 the 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] and made permanent in 2012 by the *FDA Safety and Innovation Act (FDASIA)* [15]. The US paediatric legislation was found to be very successful leading to added information to the drug label concerning the safe and effective use of more than 400 drugs in children [16].

23.3.2 EU Paediatric Regulation

The *EU Regulation on Medicinal Products for Paediatric Use* (*EC No 1901 & 1902/2006*) came into force in January 2007 [17]. It built 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 authorised medicines for children, and (iii) improve information available on medicines for children. These objectives should be achieved, (iv) without unnecessary trials in children and (v) without delaying authorisation 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

23.3.2.1 Requirements and Rewards

The requirements are similar for all new medicinal products (article 7) or for authorised on-patent medicinal products when applying for a new indications, new pharmaceutical forms, and new routes of administration (article 8): applicants must agree to a PIP with the PDCO/EMA, the results of which must be submitted at the time of marketing authorisation application. Alternatively, applicants may apply for a deferral or waiver of paediatric studies (see Sect. 23.3.2.2). The reward for article 7 and 8 applications is a 6-month patent extension, on the conditions of compliance with the 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 market exclusivity for compliance with the PIP.

For medicinal products already off-patent, the Paediatric Regulation established a new type of marketing authorisation, 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', homoeopathic drugs, and traditional herbal medicines.

23.3.2.2 Paediatric Investigation Plan

A *Paediatric Investigation Plan* is basis for the development and authorisation 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 age-appropriate formulations. A PIP needs to be proposed by the applicant by end of phase 1 of development for a new product because paediatric development should be early. The PIP is scientifically reviewed, amended, and eventually agreed or refused by the PDCO. An agreed PIP is legally binding for the applicant and the agency. However,

if new information evolves during development of the drug which impacts on the PIP, modifications of the PIP may be agreed with the PDCO.

A *waiver* may be granted by the PDCO for a class of drugs, for a specific product, for an indication, or for certain age subsets, based on (i) expected lack of efficacy or safely concern, (ii) if the condition only occurs in adults, and (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 different indications and age subsets.

23.3.2.3 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), Norway and Iceland, three representatives of health professionals and three representatives of patient organisations, 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 the inventory of paediatric needs and a priority list of off-patent paediatric drugs, and support the agency in the coordination of the European Network of paediatric research network.

Until end of 2014 the PDCO has received about 1800 PIP/waiver applications, covering more than 2200 indications. Of these applications, 78 % were for new medicinal products, 20 % for authorised on-patent medicinal products, and only 2 % for off-patent medicinal products. The PDCO has adopted 788 positive opinions on PIPs, 35 negative opinions on PIPs, and 366 opinions on full waivers. Additionally, 861 modifications of agreed PIPs were adopted. The most frequent therapeutic areas covered by PIPs were endocrinology and metabolism, cardiovascular system, oncology, and infectious diseases [18].

23.3.2.4 Collateral Measures

The Paediatric Regulation includes a number of *collateral measures* intended to improve information and 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, (iii) an inventory of paediatric therapeutic needs, (iv) public access to information on paediatric studies in the EudraCT database, (v) grants for paediatric studies on off-patent drugs (a special call in the EU 7th framework programme), and (vi) a European paediatric research network coordinated by the EMA (EnprEMA).

23.3.2.5 Achievements of the Paediatric Regulation

The Paediatric Regulation has clearly changed the environment for paediatric medicine development in Europe [19]. All collateral measures have been implemented. The Regulation has led to a paradigm shift in the pharmaceutical industry who now considers paediatric investigations as integral part of medicine development. Although about 800 PIPs have been agreed, a minority has been completed yet because paediatric developments take a long time. As result of the regulation, a number of medicines have already received new paediatric authorisations, new age-appropriate formulations, or updates of product information with new paediatric data, but their number is still relatively small. Limitations of the Paediatric Regulation are that there are several exceptions, such as generics and herbal medicines. There have been very few PIP applications on off-patent medicines, apparently because the incentives are not sufficient. There are ethical, methodological, and practical challenges to the successful and timely implementation of PIPs. Finally, the need for paediatric studies is not yet universally accepted among patients, parents, and health professionals [20]. In summary, the Paediatric Regulation has implemented important measures to improve medicine development for children. To achieve broad availability of authorised medicines for children will need more time.

23.4 Points to Consider for a Paediatric Investigation Plan

23.4.1 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 (i) the expected therapeutic benefit of the drug for children, (ii) the timing of paediatric development, and (iii) 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 *Inventories of Paediatric Therapeutic Needs* for all relevant therapeutic areas compiled by the EMA [21]. Based on the survey of medicine use in children in all EU member states mandated by the Paediatric Regulation [22], the inventories are being 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 [23]. For extrapolation of data from adults to children, see Sect. 4.5 and the PIP case study.

The International Conference of Harmonization (ICH E11) has defined the following *paediatric age groups* in relation to developmental stages: prematures (<37th weeks of gestation), term newborns (age 0–27 days), infants and toddlers (age 2–23 months), children (age 2–11 years), and 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).

23.4.2 Child-Appropriate Formulations

There is a need for age-appropriate formulations that assure accurate dosing and reliable administration across all targeted paediatric age groups [24]. 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. Colour and taste must also be considered to optimise 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 minimise the risk of medication errors [25].

23.4.3 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 [26, 27]. 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, 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 endpoints 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.

23.4.4 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 [28]. 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 modelling and simulation approaches [8, 29]. *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 [30]. 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, the predictions 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 burden of paediatric studies (number of patients, number of samples) 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 are 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* [31]. In contrast to standard (full) PK studies where multiple samples are taken from each individual 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 build prediction models, e.g. for younger age groups. Population PK models can be used in combination with PBPK models.

Practical challenges 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 samples should be taken during routine blood sampling where possible. To minimise pain, local anaesthetic cream and oral saccharose (in infants) and, for repeated samples, indwelling lines should be used. Blood samples volumes can be minimised 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.

23.4.5 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, 23]. 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 children in sufficient numbers into clinical trials is difficult because many paediatric diseases are rare and children are heterogenous with respect to age, development, and comorbity. 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. Because of the dynamic development process, some adverse events may not manifest 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, behavioural, cognitive, sexual, and immune maturation and development [23].

23.4.6 Extrapolation

Extrapolation of data from adults to children or between paediatric age groups may allow reducing the data required from studies in children [32]. The primary rationale for extrapolation is to avoid unnecessary studies in children for ethical reasons. Alternatively, in situations where sufficiently large studies are not feasible in children, extrapolation may be a means to optimise the use and interpretation of all available data. To what extent extrapolation is possible depends on the similarity of disease (aetiology, manifestation, progression) between adults and children, similarity of drug disposition and effect, and similarity of clinical response to treatment.

A structured approach to extrapolation should involve the following general steps:

- *Extrapolation concept*: quantitative synthesis or modelling of available data in adults, other sources, and children, to develop explicit predictions on expected similarities between adults and children.
- *Extrapolation plan*: proposed set of studies in children considered necessary to complement the information extrapolated from adults. In accordance with the predicted similarities between the populations, paediatric development may be reduced in types of studies and/or numbers of study patients (no, partial, full extrapolation).
- *Validation*: emerging data from study children should be used to validate the extrapolation concept or, if not consistent with the initial predictions, to update the extrapolation concept and amend the extrapolation plan. This may be an iterative process of predicting and confirming, or adapting study plans, when moving through the phases of clinical development and down age groups.
- *Extrapolation*: interpretation of the limited data generated in children in the context of data extrapolated from all other sources.

To mitigate the risk of false conclusions due to assumptions and uncertainties underlying an extrapolation procedure, further validation may be required from post-authorisation studies and pharmaco-epidemiological data.

For an example of extrapolation, see the PIP case study on rivaroxaban.

Case Study: Paediatric Investigation Plan for Rivaroxaban

A Paediatric Investigation Plan (PIP) for rivaroxaban was agreed with the PDCO in 2009 and underwent a number of subsequent modifications [33]. Rivaroxaban is an oral, direct inhibitor of clotting factor Xa. Studies in adults with rivaroxaban have shown dose-proportional effects and predictable anticoagulation not affected by food intake and with few drug interactions [34]. Rivaroxaban used in fixed dosing regimens with no dose adjustment or routine coagulation monitoring was demonstrated to be efficacious and safe in adults [35]. Anticoagulants currently used in children, such as heparins and vitamin K antagonists, have several shortcomings, including lack of age-appropriate formulations, unpredictable PK, antithrombin dependence, food and drug interactions, and the need for therapeutic monitoring and frequent dose adjustments. The pharmacological properties of rivaroxaban demonstrated in adults have particular appeal for its use in children. Thus, rivaroxaban has the potential to significantly improve anticoagulant treatment for children.

Rivaroxaban is authorised in adults for the indications: (i) treatment and secondary prevention of venous thromboembolism (VTE) and (ii) primary

prevention of VTE in patients undergoing orthopaedic surgery and medically ill patients and primary prevention of stroke and systemic embolism in patients with atrial fibrillation. The agreed PIP indication is *treatment and secondary prevention of VTE in children from birth to <18 years*. A waiver for paediatric development was granted for all indications of primary prevention of thrombosis.

Venous thromboembolism, although much rarer than in adults, does occur in children as secondary complication of severe underlying diseases and their treatment, such as cancer, congenital heart disease, prematurity, and others. The most important risk factor for VTE in children is the presence of central venous access devices (CVAD). Most VTE in children occur in the central and upper venous system reflecting the most common location of CVAD. So VTE in children have different aetiology and manifestations compared to adults where a large proportion of VTE are spontaneous and most occur in the lower extremities. Nevertheless, there is a common pathophysiological pathway of thrombotic vessel occlusion and a risk of embolism. The mechanism of anticoagulant drugs is inhibition or reduction of clotting factors, an effect which may be quantitatively different in young infants. Similar clinical endpoints for anticoagulant treatment are used in adults and children: recurrent VTE for efficacy and clinically relevant bleeding for safety. Thus, there is a certain degree of similarity of VTE and anticoagulant treatment between adults and children.

The PIP includes the following measures [33]:

1. *Development of an age-appropriate liquid oral formulation of rivaroxaban*
2. *Two non-clinical toxicology studies in juvenile rats*
3. *Study of relative bioavailability and food effect of the oral formulation suspension in healthy adults*
4. *Phase I: single-dose PK/PD, safety, and tolerability study in children 6 months to <18 years*
5. *Phase II: multiple-dose PK/PD, safety, randomised, and active-controlled (rivaroxaban versus standard of care) study of 4 weeks VTE treatment in children 6 to <18 years, and separate for children 6 months to <6 years*
6. *Phase III: efficacy, safety, randomised, and active-controlled (rivaroxaban vs. standard of care, 2:1 ratio) trial of 3 months VTE treatment in children from 6 months to <18 years and separate for* infants 0 to <6 months*, n = 150 children.*

Discussion: Development of an age-appropriate formulation is considered state of the art for any PIP targeting children younger than 6 years to assure reliable administration and exact dosing. In preparation of the clinical studies in children to estimate age-appropriate doses, the applicant developed a

paediatric PBPK model of rivaroxaban [36]. The model predicted that weight-normalised adult doses would lead to underexposure in younger children due to age-specific differences in metabolisation of rivaroxaban, indicating that relatively higher doses are required in children. Moreover, an *in vitro concentration-response study* was performed by spiking rivaroxaban in various concentrations into plasma from healthy children of different age groups [37, 38]. There were no differences in the exposure-response relationship between adults and children of all age groups except for neonatal plasma.

The clinical study program takes a cautious approach to exposing children to rivaroxaban with an initial single-dose PK study to confirm dose predictions and assess safety and tolerability. All studies have age-staggered recruitment, from adolescents down to younger age groups. The results of each age group are incorporated into the PK model to improve precision of predictions for younger age groups. Only after a data monitoring committee has reviewed the results of one age group will enrolment in the next age group be permitted. The second step is a multiple-dose PK, dose confirmation, and safety study in the last month of VTE treatment, a period where the risk of recurrent VTE has already decreased. Studies in infants <6 months of age are performed separately. The final step is an efficacy and safety study for the full period of VTE treatment. Although this is a randomised study comparing rivaroxaban versus standard-of-care anticoagulation, the study has a limited sample size, not powered for an independent proof of efficacy in the paediatric population. Thus, the efficacy evaluation will be based on partial extrapolation of efficacy from adult data, given that there is reasonable similarity in VTE treatment between these populations. The clinical studies of the PIP are still ongoing, and only part of PK data has been reported in abstract form so far [39, 40].

In summary, this PIP is an example of using state-of-the art methodology to systematically evaluate pharmacological properties of rivaroxaban in children as compared to adults with the aim to establish age-appropriate doses in children. On top of these data, there is only limited assessment of efficacy and safety in a small-scale comparative study, an example of partial extrapolation of efficacy and safety from adult data.

The PIP for apixaban, another oral direct factor Xa inhibitor, targets primary prevention of VTE in children [41]. The specific PIP indication is *prevention of VTE in children with acute lymphoblastic leukaemia with CVAD from birth to <18 years*. Although VTE are very frequent in patients with CVAD, currently available anticoagulants have not unequivocally been demonstrated to be beneficial for primary prevention of VTE in this setting, neither in children nor adults, as evidenced by several meta-analyses [42]. The rationale for targeting this indication with apixaban is that the many advantages of an oral direct factor Xa inhibitor, particularly for children, may potentially translate in an improved benefit to risk ratio. However, because there is no proof of efficacy in adults in this setting, the proof of concept must solely rely

on the paediatric studies. Consequently, the PIP for apixaban consists of a set of studies to develop an age-appropriate formulation and establish age-appropriate dosing and a pivotal study fully powered to demonstrate efficacy and safety based on clinical endpoints. In summary, with no adult data available in the specific PIP indication, there is no option for extrapolation.

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