Predictive ADMET
Integrated Approaches in Drug Discovery and Development
Edited by Jianling Wang and Laszlo Urban

Empirical approaches

Physiologically based pharmacokinetic models

Log (Clearance) vs. Log (Body Weight)

Concentration vs. time
PREDICTIVE ADMET
PREDICTIVE ADMET
Integrative Approaches in Drug Discovery and Development

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When we decided to put this book together, we asked ourselves whether it was possible to address absorption, distribution, metabolism, excretion, and toxicity (ADMET) from a different angle and provide novel information and guidance on the present pharmaceutical and academic practice. This was not an easy question to answer, considering numerous books and review articles on the topic. Although many ADMET aspects are addressed during the early phases of drug discovery, it is still common that projects treat data in isolation. Thus, we started with adding “integrated” that we believe is a most significant aspect of the drug discovery process. Although poor solubility and poor permeability are recognized as significant alerts for in vitro safety pharmacology assays, it is less obvious that already mitigated scaffold-based, off-target activity could resurface in metabolites. Therefore, testing both parent and metabolite in the in vitro safety pharmacology assays will provide a better chance to avoid unexpected adverse drug reactions (ADRs).

The other major element is the predictive, translational value of the ADMET efforts. We made all efforts to incorporate clinical cases into this book to demonstrate the relevance of reverse translation for the design of early safety assessment of molecules during the drug discovery and development process. It is invaluable to connect clinical observations with ADME properties, proteins, and gene targets using information obtained from marketed compounds. This is extremely valuable to correlate ADMET features of lead molecules in the absence of clinical proof of efficacy and safety with safety performance of marketed drugs. We are considering a simple equation that translates “hazard” into “risk.”

The poor translational value of profiling efforts could come basically from two sources: (1) poor correlation of the target or measured chemical/biological characteristic of compounds with clinical features and (2) issues with assay quality. Many assays fall victim to either or both of the above and do not support optimization or mitigation. For example, hepatotoxicity assays performed on poor quality cells or within an inadequate physiological environment will have a poor predictive value and the data do not give any guidance for finding the right clinical candidate.

Another major mistake by drug discovery teams is that they look at the potency of compounds at off-targets without considering the expected therapeutic concentration. IC$_{50}$ values on their own do not mean much; they have to be put in context with the available (free) concentration of the drug at the site of action. The best way
to approach this is to consider the free effective therapeutic plasma concentration, which can be calculated in the preclinical setting from the determination of $C_{\text{max}}$ or area under concentration (AUC) and plasma protein binding. Once all of these data are available, the project team can make an estimate on the safety margin. Caution has to be taken that this is not a therapeutic index that will be only available in the clinical setting; however, it can give a good estimate of the expected clinical safety of the compound. Therefore, we tried to provide a broad array of approaches from very early in vitro testing for bioavailability and safety in drug discovery to late-phase pharmacokinetic and in vivo safety pharmacology approaches in preclinical development phase continuing into the time of clinical trials. Finally several clinical examples will reinforce the message on the value of preclinical ADMET testing.

To meet the expectation, we recruited authors who are highly regarded specialists in various aspects of ADMET or clinical aspects of drug development; they understand and practice the integrative effort and are prepared to provide a critical view on their subject. We encouraged all contributors to think “translational” and highlight the most effective and novel methods and strategies for ADMET profiling of compounds. We hope that this book will be as enjoyable as useful for a broad range of scientists working in the pharmaceutical-biotechnology industries or in academia.

Last but not least we would thank the chapter authors for their high-quality contributions and the staff at Wiley for their patient support. We also would like to thank our loved ones for being patient with us during the time of editing this book.

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INTRODUCTION TO THE CURRENT SCIENTIFIC, CLINICAL, AND SOCIAL ENVIRONMENT OF DRUG DISCOVERY AND DEVELOPMENT
1.1 THE CHANGING LANDSCAPE OF EPIDEMIOLOGY AND MEDICAL CARE

The rapidly changing landscape of epidemiology and associated medical requirements poses a significant challenge for the pharmaceutical industry, as well as for health care providers. Although the demand for new medicines is high in both developed and developing countries, the medical requirements differ considerably. In developed countries, the prevalence of diseases associated with lifestyle (e.g., obesity and type 2 diabetes) and aging (e.g., cancer and osteoporosis) is far greater than that in developing countries, whereas the latter suffer from a high frequency of infectious diseases, a large proportion of which is transmitted by vectors.

The demographic change in the United States and the developed Western world is biased toward older persons. The percentage of people 55 years and older in the United States has grown from 16.8% in 1950 to 23.6% in 2008. It is even more striking when considering real numbers, rising from 26 million to 73 million during the same time period. Moreover, as average life expectancy has increased, so have health-related issues. The major disease groups affecting this population include cardiovascular,
respiratory and inflammatory diseases, cancer, neurodegenerative diseases, and psychiatric disorders. These diseases and disorders are associated with high overall drug costs. To support this, we looked at the statistics on arthritis and hypertension, which have high incidence in older persons. More specifically, according to government statistics, in 2010, more than 50 million adults of all ages in the United States suffered from doctor-diagnosed arthritis; that number is expected to exceed 60 million by 2015 [1]. However, whereas the overall prevalence of hypertension in people 18 years and older in the United States was 28.6%, it was 66.7% in people 60 years and older [2a].

Last, but not least, lifestyle, diet, and other behaviors in developed countries are associated with epidemics of diabetes, obesity, and stress-induced psychological/psychiatric disorders. There is emerging evidence that the combination of genetic and environmental factors could contribute to the development of particular mental disorders [2b]. In the United States, approximately 3.2% of boys between 6 and 17 years of age were found to have autism (National Health Statistics Report). Moreover, in the 15–17 year-old age group, the prevalence of parent-reported attentional deficit hyperactivity disorder (AD/HD) reached 13.6% in 2007, and these numbers are growing rapidly [3,4].

More and more diseases are controlled by chronic noncurative, symptomatic treatments, which contribute to persistently high medical expenses. Osteoporosis, high blood pressure, pain, atherosclerosis, diabetes, arthritis, and cancer, although not necessarily associated with an aging population, also require lifetime medications. During the past 20–30 years, indications associated with large patient populations have led to increased consumption of particular drugs and have helped create a new class of “blockbuster” products. We have learned during the past decade that this strategy has not panned out well, as new blockbusters do not come easily, and the so-called patent cliff [5] could seriously affect the financial performance of companies.

Focus on particularly “profitable” areas in health care also meant that pharmaceutical companies neglected other segments of health care where return on investment was less assured. This was in part because little was known about the pathomechanisms of diseases, so efforts failed to produce viable drugs, for example, for Alzheimer’s disease (AD), or because the disease was well managed by existing, relatively inexpensive medicines. This latter case is particularly relevant in the development of new agents to treat infectious diseases. The lack of investment into this area is resulting in an alarming dearth of therapies to treat multidrug-resistant bacterial strains.

Another contribution to the increase of expenditure on medicines is the parallel use of several medicines, either to affect various targets of the same disease, or to treat concomitant diseases. Approximately 37% of people 60 years and older in the United States takes five or more prescription medications at any time (Center for Disease Control and Prevention [CDC] statistics). Treatment regimens might also require further drugs to treat the side effects of the drugs used to treat the original disease. Cultural components are also pushing for more medications based on the commonly held belief that “pills” can provide easy and fast treatments in place of less expensive and sometimes more effective alternative methods, such as quitting
bad habits, switching to healthy diet, physical exercise, and mental relaxation. An added benefit of these behavioral approaches would be the absence of adverse drug reactions (ADRs).

Use of antidepressants and antipsychotics has also skyrocketed during the past decades. For example, at the time of the publication of this book, one in ten U.S. adults reported having depression (CDC; [6]). The population 18 years of age and older reporting prescription antidepressant drug use in a single month has shown a significant increase between the periods 1988–1994 and 2005–2008. The increase is stunning from 5% to 22%. In the absence of appropriate medical care, the off-label use of psychotropic drugs has increased in the older population, particularly in senile dementia [7]. These aspects, however, did not facilitate more investment into drug development for psychiatric disorders. Instead, many companies discontinued investing in that disease area. A major reason for this trend is the fact that little is known about the pathomechanisms of mental disorders, which makes drug discovery highly speculative with a significant chance of failure.

We believe that the previous cases reflect a culture that nurtures a consumer-based approach to medicine in developed countries. Interestingly, the contribution of drug prices relative to total medical expenses remains relatively low, i.e., 10% in the United States, and similarly in the developed world.

Cost becomes even more of a pressing issue in the developing world, where there is likely to exist a large burden of epidemics of infectious, parasitic, and vector-carried diseases; lifestyle-associated diseases (e.g., smoking); or inappropriate nutrition and exposure to environmental hazards. Just to highlight the important contribution of infectious diseases to general health care issues, we looked at two diseases that overwhelmingly are associated with the developing world: dengue fever and drug-resistant tuberculosis. According to the World Health Organization (WHO), nine countries had reported severe dengue epidemics prior to 1970. Today the disease is endemic and affects more than 100 countries, with most cases reported in Southeast Asia and the western Pacific [8]. More recently, the number of reported cases has continued to rise. Because there is no treatment, and the most severe cases are life threatening or cause permanent disability, dengue fever remains a major neglected disease.

As another example, tuberculosis (TB) does not just remain a burden on its own, but more often it manifests in a multidrug-resistant format (MDR-TB). TB is the second highest cause of mortality worldwide as a result of a single infectious agent, resulting in an estimated 1.4 million death in 2010 alone (for further details and combination of HIV and TB, see Reference 8). A major problem is that MDR-TB does not respond to first-line standard anti-TB treatments. Second-line treatments are costly, significantly depend on compliance, and carry serious side effects. Alarmingly “extensively drug-resistant TB” (XDR-TB) is spreading and does not respond to any existing treatment. Of the greater than half million cases of MDR-TB in 2010, an estimated 9% progressed to XDR-TB, with an estimated annual death toll of 150,000. These numbers highlight the demand for action from health care professionals, governmental authorities, and the pharmaceutical industry.
1.2 COST OF DRUG DEVELOPMENT

The pharmaceutical industry faces challenges to develop affordable medications for diseases with high prevalence, regardless of whether they affect the population of a developed or developing country. Although hypertension, hyperlipidemia, and arthritis could be well controlled with relatively low-cost, generic products, new drugs for cancer with breakthrough results often demand high prices, which are likely to be unsustainable to health plans and other payers. Furthermore, diabetes, asthma, and mental disorders, to name a few conditions, are poorly managed even at higher cost.

During the last three decades, the cost of health care increased significantly worldwide, becoming a major strain on government resources [9]. National health expenditures in the United States skyrocketed during the past 10 years, from US$1,377.2B in 2000 to US$2,495.8B in 2009, according to CDC statistics [10]. The proportional rise in prescription drug spending during the same period (from US$120.9B to US$256.1B, respectively) contributed to increasing health care costs as well. The rise in prescription drug prices is driven by several factors, with a main contribution from three sources: the decline in overall industry productivity; the rise in safety issues during clinical testing and after approval; and the increase in regulatory requirements necessary to obtain market approval. We will look at each of these factors individually.

1.2.1 Decline in Industry Productivity

There has been considerable concern over the past two decades about the productivity of the pharmaceutical industry [10–13]. A recent analysis reveals that the average cost to bring a new molecular entity (NME) to the clinic today is US$1.3B [14]. Some estimates place the figure substantially higher, at an average of between US$5.5B and US$6B. Even the most productive companies, such as Amgen (Thousand Oaks, CA) and Novartis (Basel, Switzerland), spend more than US$3.5B to bring a novel drug to the clinic. In the worst case, this figure is greater than US$10B [15]. This latter calculation is based on simple mathematics: basically dividing research & development (R&D) expenditures by the number of new NMEs brought to the clinic by a company.

Indeed, the number of new medicines registered with the health authorities seriously declined during the first decade of the 21st century [12] followed by criticism of work practices, innovative spirit, and inadequate R&D strategies of the pharmaceutical companies [16–18]. The high expectations for the Human Genome Project did not seem to materialize and led to impatient cries of mismanagement. The slow utilization of new information was blamed for sluggish industry output. Now that the figures on new medicines have increased, it is obvious that there has been a certain time lag resulting from the adaptation and translation of new discoveries into drug discovery practices. In general, an increase in pharmaceutical productivity is expected to push down development costs, with the hope of producing less expensive medicines. Although the above is an important factor in drug prices, the development of novel cancer treatments and some special new treatments for rare diseases have
been achieved by large investments. For example, the development of small-molecule kinase inhibitors took a long time because of the development of new tools for this class of targets and understanding the role and mode of action of kinases in disease pathomechanisms, particularly in cancer [19]. New technologies and work processes, such as high-throughput screening (HTS), helped to examine millions of compounds for interaction with protein targets and exert therapeutic effects. Although HTS made it possible to discover hits with considerable chemical diversity, it also flooded the assays with compounds with poor physicochemical properties. Molecules with low solubility, high lipophilicity, and poor stability cause major problems in absorption, distribution, metabolism, and excretion (ADME) assays, which result in inaccuracies, discrepancies between biochemical and cellular assays, etc. [20]. These properties lead to extended timelines and a higher cost during the discovery process and could be the reason for project termination or production of a poor quality clinical candidate [21]. To avoid these problems, profiling of biopharmaceutical properties has been included in early drug discovery road maps with effective results demonstrated by decreased attrition rates as a result of poor drug metabolism and pharmacokinetic (DMPK) properties [22]. Thus, ADME profiling proved to be highly effective particularly as it is done in parallel with testing for pharmacological potency and efficacy. Implementation of early profiling for ADME and toxicology (T) proved that simple assays could have great translational value at a low cost [23]. Another benefit of these efforts was the development and implementation of in silico tools, which greatly enhanced the predictive power of ADME profiling and gave direction for chemistry laboratories to generate compounds with better physicochemical properties.

1.2.2 Rise in Safety Issues

Another main factor contributing to increased drug-related expenses also has roots in early drug discovery; the cost, however, will appear in the clinic. This relates to safety issues, which are realized during clinical trials and in some cases postapproval only. Safety assessment of drug candidates is a late event in drug discovery, whereas early hazard identification was completely neglected in the past. The side effects of individual drugs or drug combinations generate a significant expense in health care either by rehospitalization; secondary treatments, such as management of concomitant hypertension; or number of lost working days. There are two categories of side effects: those that are associated with the primary target, and those associated with unintentionally encountered off-targets.

Many serious side effects are associated with primary targets and are difficult to avoid or manage. Recent withdrawals of anti-diabetic drugs (rosiglitazone), obesity treatment (rimonabant), and black box labels for many new drugs, in particular in cancer management, highlight this problem. For example, almost all kinase inhibitors carry box warnings for cardiovascular safety matters. In addition to acute effects, delayed post-treatment cardiological symptoms have been observed after treatment with tyrosine kinases inhibitors (TKIs; [24, 25]). We will address these new aspects and more issues associated with TKI treatment in the following chapters.
Cardiac and hepatic toxicities have remained the leading reasons for attrition and labeling for decades [26, 27]. These side effects largely develop because of the off-target activity of drugs and are a result of their metabolism by common enzymes. Idiosyncratic drug-induced liver injury (DILI) has been a dreaded, unpredictable threat until recently. Today newly developed in vitro and in silico technologies can assess the major metabolic route of compounds and predict possible drug–drug interactions in the liver, detect toxic effects of reactive metabolites, and link hepatobiliary hepatotoxicity to several bile transporters. Nevertheless other, currently not well-understood pathomechanisms or species differences in safety pharmacology keep hepatotoxicity prediction difficult. In the meantime, cardiotoxicity predictions improved significantly with the recognition of off-target effects at cardiac ion channels, particularly at the human ether-a-go-go related gene (hERG) potassium channel [28]. The hERG channel is a notoriously promiscuous protein with easy access for many compounds, and the block of its pore causes long QT (LQT) which can lead to life threatening ventricular arrhythmias, known as Torsades de Pointes. This off-target effect is well understood, and the regulatory assessment has high predictive value [29–31]. However, although rarely, we still encounter cases of hERG-related arrhythmias with compounds that are used for treatment of life-threatening diseases where the risk is accepted and managed by thorough examination of patients for prevalence of arrhythmia and monitoring during treatment.

In general, preclinical assessment of cardiac ion channel inhibition is well established and supports mitigation prior to clinical candidate selection and basically weeds out compounds with effects at these channels. Although mitigation of arrhythmias has become routine in preclinical development, the acute and delayed cardiotoxic effects of the TKIs has not been addressed satisfactorily [25]. This is in part a result of the poor preclinical assessment of cardiac inotropy, which could signal pathological changes in cardiomyocyte contraction and predict congestive heart failure [26, 32].

It is certainly more challenging to bring drug candidates to the clinic than a decade ago. The estimated average time of the preclinical phase is approximately 6 years, which includes preclinical safety studies. There is more expectation for both better efficacy than existing medications for a particular indication, and importantly, safety became a more sensitive issue. Although some drugs make it through the safety tests, labels can cripple their clinical applications, with disappointing return on investment. In general, there has been a notable move toward preclinical safety mitigation by the introduction of broad-scale safety profiling, including both target- and pathway-based safety assessments. There is good agreement within the pharmaceutical industry concerning target-based profiling practices [33] and associated predictive in silico tools [34]. Although this move could provide clinical candidates with higher safety margins, it costs more and prolongs timelines.

1.2.3 Increasing Regulatory Requirements

Regulatory requirements surrounding clinical trials have become significantly more stringent in recent years, demanding larger patient populations and including more
diverse investigations [35, 36]. As an example, off-targets linked with suicidality can trigger demand for clinical evaluation of suicidal intent, which will add extra expense and prolong the trial. Large-scale trials to prove superior activity in comparison with existing treatments could demand thousands of patients in multiple centers, with the uncertainty that the result would be favorable for the drug (e.g., the case of Avandia; GlaxoSmithKline, London, U.K.).

1.3 THE NEW PARADIGM OF ADME/PK ASSESSMENT

1.3.1 Recent Advancement of ADME/PK Assessment of Drug Candidates in Discovery and Development

The introduction in the 1990s of the “rule of five,” or drugability concept, by Lipinski et al. [37, 38] greatly facilitated the early assessment of the ADME properties of NMEs with a wealth of newly developed in silico, in vitro, and in vivo tools [39]. Not only does the availability of integrated application of in vitro and in vivo ADME/PK data enhance the optimization of efficacy for the designated therapeutic targets [40], but it also offers a wealth of knowledge for the potential development of tomorrow’s more predictive in silico assay-free tools [41]. More predictive models require intelligent evaluation of dynamics, associations and disconnects within the historic data collection.

Today, tiered approaches are the standard, eliminating an earlier stepwise routine. First-line ADME assays provide “sentinel” for bad behavior and identify those factors that would prevent the advance of molecules toward clinical candidate status. These assays are relevant for structure activity relationship (SAR) support at a high-throughput rate. However, they mostly lack the sophistication for deeper analysis of individual components of ADME characteristics. High-throughput solubility, permeability, and microsomal stability tests would come under this category [42]. Mechanistic studies regularly are used as follow-up assays (e.g., tier 2,3…).

The application of state-of-the-art laboratory automation and bioanalytical technologies have resulted in high-quality ADME/PK data and have enhanced the predictability of downstream assays [41–43]. Centralization of assay facilities in pharmaceutical companies and contract research organizations (CROs) largely improved the overall efficiency and cost effectiveness of absorption, distribution, metabolism, excretion, and toxicity (ADMET) assessment. Multitiered PK tools range from fast snap-shot PK or cassette dosing all the way through to detailed mechanistic studies, such as sampling via the bile duct, sampling/dosing to the hepatic portal vein, or use of humanized or gene-knocked out animal models to address specific questions in early drug discovery.

Today, researchers in the pharmaceutical and biotechnology industry are trained for fundamental understanding of ADMET/PK principles that are typically incorporated into the drug discovery process for fast and efficient selection of the highest quality clinical candidate.
1.3.2 New Challenges and Emerging Fields of ADME/PK Development

From an overall ADMET perspective, early ADMET models offer a set of comprehensive “surrogate” filters to enable potentially problematic new chemical entities to be weeded out in the early discovery phase. Although effective in reducing ADME-related attrition in clinical phases, the current ADMET “justice” system is far from perfect and may transgress against “innocent” or “imperfect but manageable” candidates. As a matter of fact, a reasonable fraction of currently marketed drugs might have never qualified for clinical use if they were simply applied to rigid ADMET filters.

With the increasing costs and challenges in discovering new drug targets and the more crowded intellectual property space, the pharmaceutical industry can no longer afford to abandon a promising drug candidate simply as a result of a single unfavorable ADMET property. False positives derived from current ADMET models as a result of lack of robust in silico–in vitro–in vivo (ISIVIV) links have a further negative impact on the discovery of new drugs, although this imperfect approach may be valuable to reject the worst offenders. As an example, the assay applied to determine covalent binding, despite being a gold standard in vitro approach for identifying reactive metabolites, cannot reliably predict the occurrence of liver toxicity. Its correct role should be defined as flagging potential toxic structural alerts. Most of the existing in silico ADMET models seem to work well when used to predict simple physicochemical properties, such as ionization constant, LogP, and polar surface area, which are primarily governed by the intrinsic molecular descriptors of scaffolds and molecules. They, however, are less applicable to more complex interactions within the physiological environment (e.g., gastrointestinal solubility, clearance, bioavailability, etc.; [39]).

During optimization, overinterpreting ADMET data or overemphasizing the role of a single ADMET parameter is damaging. With tons of data generated in multiple ADMET models, the “more is better” approach is less of a dream and more of a nightmare for drug discovery teams. They may get buried in a flood of data and lost within seemingly “contradictory” messages. The strategy of “box-checking” or simple “all-around” profiling passed its time as it failed to deliver better candidates, faster to the clinic. For instance, one shortcoming of single-minded heat map tables is that a similar weight gets applied to each pixel of colors (e.g., red). A further limitation is that the possible interplay between remote pixels that impacts a pathway and may be potentially important in driving an undesired physiological effect is not reflected in the heat map. Therefore, integrating data within heat maps with consideration of biological networks, or pathways that interact, will make it easier and more reliable for the user to identify and mitigate risk. Clearly, success seems to rely not just on volume of the data but also on the meaningful integration of emerging patterns with translational relevance as a project matures (e.g., human exposure, PK/pharmacodynamics (PD)/efficacy in humans, therapeutic index, etc.). Data should be used to generate knowledge and drive testable hypotheses to enable right, reliable, and decisive conclusions. Thus, it is beneficial to connect all ISIVIV models together.
Gaps in the ISIVIV correlations (ISIVIVCs), within a single scaffold or across series, can be valuable to identify missing pieces of the puzzle and to generate a new and reiterated hypothesis.

An inferred algorithm based on experiences drawn from the chemotypes’ history may offer greater advantage. Meanwhile, it should be kept in mind that the existing models might have been developed from historic or global chemical spaces and they might not necessarily apply to drug candidates from new series or local chemical space. Sometimes, global empirical rules might be misleading and their applications should be optimized in nontraditional ranges of physical properties. Although multiple statistics tools such as quantitative structure activity relationship (QSAR), principal component analysis (PCA), and partial least squares (PLS) are frequently used in the analysis of ADMET data, their limitation is that they need to be trained against a set of truly relevant data. In the early days of a project, there may be little alternative to reliance on more basic guidance such as the “rule of five,” “absorption model,” and some ADMET diagnostic models that are mostly applicable on a “global” basis. As measured ADMET data accumulate and get enriched, a better understanding of the chemotype emerges with the consequence of an increased confidence level for “local” models. This certainly extends to sophisticated modeling of exposure and effect relationships during preclinical and early clinical assessment with a better determination of safety margins.

In the current book, various data integration tools are introduced ranging from ADME diagnosis model (Chapter 3), PATH—a hypothesis generation tool (Chapter 4), PK-Matrix (Chapter 5), QSAR/PCA/chemoinformatics (Chapters 6 and 7); multiparameter optimization (Chapter 8), PBPK models (Chapters 9 and 10), and PK/PD models (Chapter 11). The comprehensive utility of the data integration models in real drug discovery and development projects to address specific ADMET issues are also presented (Chapters 12–21) along with successful stories and lessons learned (Chapters 22–26).

1.4 INCREASED SAFETY EXPECTATIONS

1.4.1 Early Awareness of Safety Hazards

Preclinical safety assessment of compounds has been significantly enriched during the past decades [44]. In vivo safety testing now routinely incorporates genetic toxicity, LQT-related (long QT interval) studies, and refined biomarkers for both DILI and nephrotoxicity are available (see Sections 2.7–2.10 of Chapter 2). Also, early assessment of hazards associated with well-defined molecular targets and pathways are now gaining ground and entering the domain of registration expectations [45,46]. Although in vivo safety pharmacology provides the backbone of preclinical safety evaluation of drug candidates, it has had considerable “misses” in the past. The best known cases are associated with 5-HT2B receptor agonism and inhibition of 3’,5’-cyclic-nucleotide phosphodiesterase (PDE3) inhibition (see Section 3.4 of Chapter 3), which are well publicized [47,48]. The reasons for these undetected ADRs are
various; some are the result of species differences, whereas others occurred because side effects associated with targets were not known and not detected by routine regulatory assays. Mechanisms that were not known prior to clinical observations include selective stimulation of the 5-HT$_{2B}$ receptor that is associated with the gradual, time-dependent development of general fibrosis that manifests in cardiac valvular disease (CVD) and serious heart conditions [48]. Because of the slow development of this disease and the need for echocardiography for its early detection, CVD is difficult to diagnose in vivo. To the contrary, in vitro detection of 5-HT$_{2B}$ agonism is easy and highly predictive for CVD. Therefore, compounds with agonism at 5-HT$_{2B}$ can be de-prioritized before candidate selection or the activity engineered out of molecules [49]. Another example is inhibition of the biliary salt export protein (BSEP), which is associated with hepatobiliary DILI [50]. Although there is high homology between rat and human BSEP, the function of this transporter in the rodent differs from that in humans. BSEP in rats does not have a significant contribution to bile secretion, unlike in humans [51,52]. These examples highlight the challenges surrounding the predictive value of the in vitro safety pharmacology assessment of drug candidates. Furthermore, the cost and speed of the in vitro assays provide a very powerful tool for hazard detection and mitigation and give guidance for the in vivo safety pharmacology studies in the regulatory panel [33,49]. Certainly, once the cause is known and the link between the human molecular target and the adverse reaction has been established, simple in vitro assays can identify compounds acting on these targets. For these reasons, it was introduced into the drug discovery process during lead selection and lead optimization. As an added benefit, data accumulated by in vitro safety profiling gave way to the development of insilico tools with the predictive power of ADRs [34].

It is important to emphasize that in vitro safety pharmacology data provide alerts for safety hazards. The potency at the off-targets should be considered in the context of exposure, which is defined by the maximum free available drug concentration at the site of action in the organism. Most often the free $C_{\text{max}}$ is used for this purpose, but area under concentration (AUC) can be considered if more relevant [53]. This simple calculation will define whether the hazard would represent a safety risk during clinical application. Once the risk was established, the in vitro profiling assays can lead mitigation by available SAR during lead optimization and, in case the hazard still persists to some extent at candidate selection, guide the preclinical safety assessment to explore no observed effect level (NOEL) for the associated ADR. Using the 5-HT$_{2B}$ example again, if a clinical candidate with high potency at the therapeutic target shows weak agonist activity at this off-target, then a long-term rodent experiment for echocardiography should be considered within the preclinical assessment [54].

One particular area that is difficult to predict from nonclinical in vivo observations is suicidal ideation. It needs dedicated expert analysis and complex in vivo evaluation. However, once critical evidence emerges from clinical observations—in particular, from the U.S. Food and Drug Administration (FDA) adverse event recording system (AERS)—on high incidence of suicidal ideation of various drugs with a common central nervous system (CNS) target, one can establish links between the target and the observed ADR. Testing compounds at this target will give an opportunity for early warning for suicidal ideation and guide to surrogate in vivo testing and, if necessary, mitigation.
1.4.2 Logistics for In Vitro Safety Profiling

To set up the in vitro safety profiling panels is not trivial: Target selection should be guided by clinical information that is by observation of side effects during clinical trials and postapproval. Ranging from the discovery of the association of QT prolongation, to ventricular arrhythmias with hERG inhibition, to the suicidal ideation linked with vesicular monoamine transporter-2 (VMAT2) inhibition, a large volume of data has been derived from clinical observations and used in a reverse translational format to identify targets for in vitro safety assessment panels [33, 49]. Obviously, this is possible only if there are drugs that enter clinical development and/or proceed to full registration. When no drug-related data are available, one can use information obtained from human genetic diseases, pharmacological data in animal experiments, and knock-out/knock-in (KO/KI) animal models. Once targets associated with ADRs are identified, the next step is to establish panels for safety assessment.

The second most important criterion is to investigate the incidence and severity of the side effects associated with the targets and the hit rate of compounds at the selected targets, a phenomenon that is referred to as pharmacological promiscuity. By these standards a relatively slim panel can be identified and used for regular, iterative safety assessment [33] as an integral component of the design-make-test-analyze drug discovery cycle. Targets not included into such a panel still could be screened at the stage when clinical candidates are nominated. As this “secondary” or follow-up panel contains targets with links to less serious side effects and with a very low hit rate, it is unlikely that clinical candidates would be seriously affected.

Once the panels are assembled, the next question is concerned with the application of the panels to the different requirements during the drug discovery process and what is the most relevant assay format. These panels are used most extensively during lead selection and optimization when there is a chance for risk mitigation and produce molecules with the least off-target effects by clinical candidate stage. This requires rapid iterative cycles, which could be performed at the time of the primary target assay. In vitro safety profiling requires multiparallel assembly of assays, where a large number of compounds (from hundreds to several thousand/year) can be screened in an iterative manner. The assay cycle is set to the synthetic chemistry cycle, which allows testing for safety at the same frequency as for primary activity; thus, it does not introduce delays for the projects.

The cost should be minimal and the assays should not consume a large volume or quantity of material as it is rarely available at this stage. These readouts can indicate hazards, which should be considered in conjunction with other data, such as in vitro and in vivo data predicting human exposure at efficacious doses (see subsequent discussion).

Assay formats vary, but whenever possible robust biochemical assays are used for primary profiling, particularly if both receptor agonist and antagonist effects are associated with ADRs. As the panels are used for SAR, it is important to generate high-quality data that can be relied on for compound prioritization; therefore, only IC₅₀ or Ki values are acceptable. Selection of the appropriate ligands and most full-length proteins will have an impact on the data and are crucial for both biochemical and functional assays. Functional assays are mostly considered for the primary panel
when the modality is important: In some cases, only antagonist or agonist effects are associated with serious ADRs; in these instances, biochemical assays do not provide the crucial information.

*In vitro* safety profiling is primarily executed during lead selection and optimization when compounds have not been optimized for physicochemical properties and some synthetic impurities may exist. Therefore quality control (QC) and solubility testing is an essential component of the *in vitro* safety assessment.

### 1.4.3 Relevance and Confidence in Profiling Data

Relevance of preclinical safety profiling has two major aspects: (1) Predicted association with ADRs and (2) predicted manifestation. Early *in vitro* data are available at the time when no *in vivo* information exists and no clear safety window can be determined. However, iterative testing of compounds can determine pharmacological promiscuity and provide SAR for off-targets. These properties rarely warrant a Go/No-Go decision; however, they will initiate safety-related mitigation. This can be done at high confidence if an *in vivo* assay is available that links the *in vitro* data to clinical ADRs and provides a preclinical readout at a relatively low cost. Take hERG channel inhibition as an example: The hERG channel is a particularly promiscuous potassium channel, which attracts a large proportion of molecules and is associated with QT prolongation and ventricular arrhythmia. Potency at hERG only identifies the hazard; it does not predict the real risk in clinical settings. Although most disease models used rats or mice, rodents are not appropriate to evaluate the hERG-mediated pro-arrhythmic risk because the rodent hERG equivalent is not expressed in the adult cardiac tissue. Therefore, a nonrodent *in vivo* cardiotoxicity model (usually the dog or nonhuman primate) has been introduced [55] to translate the *in vitro* observation to clinical relevance [29]. The information from the nonrodent model also integrates any other off-target effects associated with cardiotoxicity, which can be the feature of the compound. Thus, this model provides integration of any cardiovascular effect and a predictive safety index. Still, clinical pathological conditions might modify the predicted index, which can be assessed, at least for the time being, only in the clinical setting.

Although nonrodent telemetry is incorporated into the regulatory safety assessment, many off-target effects could slip unnoticed into the clinical phase, such as PDE3 inhibition or 5-HT$_{2B}$ receptor agonism. Positive finding at these targets should be followed up separately once the signal is identified. In case of positive findings in these assays, the predicted ADR will be high and could trigger a Go/No-Go decision.

### 1.5 TRANSLATIONAL VALUE OF *IN VITRO* PROFILING DATA

The true value of *in vitro* profiling manifests during integration. Target-based safety profiling, together with ADME, PK, and SAR or QSAR data, will provide the full picture and generate knowledge about clinical expectations. For example PK issues, such as blood-brain barrier (BBB) penetration, will determine which effect at a CNS site would manifest. This was clearly demonstrated during the development of