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Shobha N. Bhattachar
John S. Morrison · Daniel R. Mudra
David M. Bender *Editors*

Translating Molecules into Medicines

Cross-Functional Integration at the Drug Discovery-Development Interface

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Volume 25

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Preface

The pharmaceutical industry, in the course of drug discovery and clinical development, is continuously challenged to simultaneously reduce costs, timelines, and the risk of attrition for clinical development compounds. These challenges persist and by many accounts continue to mount, despite decades of technological advances that have brought about improvements across all areas of pharmaceutical research and development. Over the past 10 years, much emphasis has been placed on the development and application of new and improved screens to aid in drug discovery efforts and minimize clinical development risks. As a result, pharmaceutical scientists are better able to screen out less desirable compounds and guide discovery efforts toward drug candidates better suited to achieve the clinical performance criteria across the variety of disciplines represented on the discovery team.

Development organizations have also continually improved drug product platforms and solubility enhancement technologies to address challenges associated with the absorption of poorly soluble drugs. Innovation in the fields of novel oral and non-oral drug delivery systems supports a range of molecular modalities, enabling the delivery of drug candidates to specific target sites within the body. However, application of these technologies has been mostly limited to a small number of niche products.

Venturing into the more complex and uncharted territories of druggable but non-validated pharmacological targets, discovery teams have had to retool their strategies to: (a) establish a clear understanding of the relationship between target engagement and the pharmacodynamic response, (b) develop relevant measurable biomarkers across species, and (c) understand the relationship between ADMET (adsorption, distribution, metabolism, excretion, and toxicology) parameters and the SAR (structure activity relationship) of drug candidates, in a manner that rapidly informs discovery efforts. To this end, remarkable strides have been made in the development and utilization of recombinant technologies, imaging tools,

ex vivo pharmacology models, and numerous in silico modeling and simulation tools. In addition, the need for clinical validation of novel targets has necessitated greater creativity in the design of early clinical studies in order to provide rapid feedback to discovery teams engaged with backup drug candidate efforts.

Precompetitive partnerships among companies, open innovation industry-academia collaborations, and other constructs are becoming increasingly prevalent. These interactions have helped accelerate the pace of innovation and technology development in various fields of drug discovery and development.

The digital age has also brought about significant changes to patient lifestyles and caregiver profiles. It has revolutionized our ability to generate, share, and analyze unprecedented amounts of data more efficiently than ever before. These changes, along with changes in the payer profiles across the globe, global regulatory requirements, and the large global partnership networks, have ushered in a new era in the business of pharmaceutical research. In order to be successful in this new playing field, it is essential that the pharmaceutical industry adapt to the changing environment as outlined below and described in further detail in the chapters that follow.

Once targets of interest have been identified, discovery efforts must also develop a clear understanding of the patient, care provider, and payer profiles, projected out, to the extent possible, to the estimated time of launch of the product. They must have a good understanding of how the new therapeutic agent compares with or complements the prevailing standard of care. This information must be used to guide the definition of the product profile, which should then inform the corresponding optimum molecular property space that medicinal chemistry efforts need to target.

ADME and toxicology assessments should be fully integrated with the chemistry and pharmacology trajectories in order to provide meaningful input into molecular design, as well as a sufficient understanding of translatability to clinical studies. Developability assessments require a judgment-based approach encompassing solid form, drug product design, and performance evaluations, within the context of potential challenges to clinical and commercial development, patient centricity, cost, and other business considerations.

Clinical studies must be designed to provide high-quality information on the safety, efficacy, and tolerability of the drug candidate as early as possible. Innovations in clinical and ultimately commercial drug products are also needed to ensure they are as simple and inexpensive as possible in order to rapidly inform further development and/or provide feedback for subsequent backup efforts. New modalities require drug delivery technologies that better cater to patient needs while simultaneously improving compliance and therapeutic efficacy.

This book is intended to provide pharmaceutical scientists across multiple disciplines with additional background and insights to enhance their effectiveness in their respective roles and thereby achieve greater success in the discovery and development of new drugs.

Indianapolis, IN — Shobha N. Bhattachar
Wallingford, CT — John S. Morrison
Indianapolis, IN — Daniel R. Mudra
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Shobha N. Bhattachar is a Director in Small Molecule Design and Development at Eli Lilly and Company. She has a bachelor's degree in pharmacy from Bangalore University, India, and a master's degree in pharmaceutical chemistry from the University of Kansas. Prior to joining Lilly, Bhattachar worked for 8 years at Pfizer in Ann Arbor, MI, in the Research Formulations group. She has authored/coauthored 15 publications covering wide ranging topics in the field of pharmaceutical sciences. Her current interests include the discovery-development interface, clinical candidate selection and developability assessment, clinical formulation development, and biopharmaceutical aspects of drug product design.

John S. Morrison earned his doctorate in physical organic chemistry from the University of Western Ontario studying the photochemical mechanisms responsible for the light-struck reaction in beer. After graduation John joined the Preformulation and Analytical Method Development group of Apotex Inc. evaluating drug substances and novel drug product formulations. He then transitioned to the Discovery Pharmaceutics group of Bristol-Myers Squibb characterizing early drug candidates and establishing delivery strategies for a variety of dosing routes

and molecular modalities. His current role bridges the discovery/development divide, collaboratively supporting multidisciplinary discovery teams as well as determining and mitigating developability risks for potential clinical drug candidates. John has also led several AAPS committees and co-organizes the ACS Drug Design and Delivery webinar series.

Daniel R. Mudra is Director of ADME at Eli Lilly and Company. He earned his B.S. from the University of Dayton studying pharmacokinetic/pharmacodynamic effects of drug-impregnated implants and an M.S. from Loyola University Chicago investigating the biochemical regulation of the p38 kinase pathway. He earned his doctorate in pharmaceutical chemistry from the University of Kansas developing in situ and computational models of absorption studying the effects of excipients on permeability, metabolism, and transport. Dr. Mudra began his industrial research career at XenoTech, LLC, and has published on models of P450 induction and inhibition, drug absorption, and PK. Since joining Lilly, he has contributed to projects from target identification to clinical development across a variety of therapeutic areas. He leads in the incorporation of mechanistic and physiologically based PK modeling including the use of molecular attributes to predict human clearance pathways.

Part I
Discovery, Development and Commercialization of Drug Candidates: Overview and Issues

Chapter 1
Pharmaceutical Industry Performance

David C. Swinney

Abstract Good health is a priority for all. Medicines are an important aspect of maintaining good health. However, medicines are very difficult to discover, develop, and provide to patients. There are many more failures than successes resulting in high attrition rates. Analysis shows there is more than one way to discover medicines. As a consequence, the pharmaceutical industry is continuously reshaping itself to address the challenges of high attrition. This introductory chapter will highlight some of the challenges to pharmaceutical industry productivity, how they are currently addressed, and how the industry is reshaping itself to address these challenges. It is concluded that addressing these challenges creates many new opportunities for innovation.

Keywords Pharmaceutical industry productivity • Degree of innovation • Learn and confirm cycle • Mechanistic paradox • Precision medicine • Drug discovery

1.1 Introduction

Good health is a priority for all, and medicines are an important aspect of maintaining good health. The goal of the pharmaceutical industry is to continue to provide safe and effective medicines for patients. However, these new medicines are becoming more difficult and costly to discover, develop, and deliver. There are many more failures than successes, and as a consequence, the pharmaceutical industry is continuously reassessing its strategies to address the high attrition rates.

The hope of the industry and medical research has been that a greater understanding of the basis for disease enabled by new molecular technologies will lead to new medicines that address all these challenges. While the implementation of these new technologies has greatly increased the extent of disease biology knowledge and enabled more precise use of approved medicines, it has not dramatically increased

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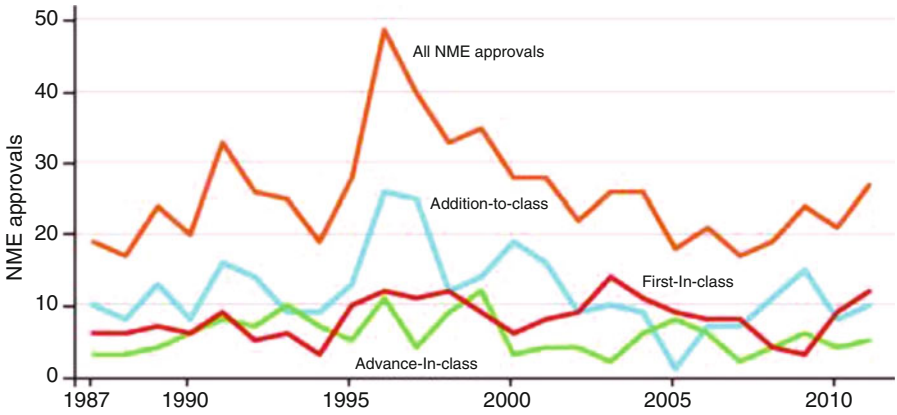

Fig. 1.1 New molecular entity (NME) approvals, by innovation category [1]

the number of new molecular entities (NMEs) approved by the US FDA [1] (Fig. 1.1). Accordingly, the adjusted cost of developing a new medicine through to regulatory approval has dramatically increased [2–4].

The consensus reason for the decrease in productivity is a high attrition or failure rate [2–4]. A large fraction of drug research and development programs fail. However, when these drug candidate failures occur at later stages in development (phase III clinical trials), the costs are significant. There have been many proposals and associated actions to address the attrition, many of which have been incorporated into the discovery and development process. Some of these processes have reduced attrition due to specific issues, but none have yet improved overall productivity [5].

Perhaps the biggest challenge to industry performance is translating molecular understanding of diseases into medicines that can be effectively and efficiently used to treat disease in patients. This is confounded by a mechanistic paradox:

> while the knowledge of mechanism (e.g. how a drug works) is very helpful to discover and precisely use medicines, paradoxically the knowledge initially available is rarely sufficiently complete to provide a blueprint for discovery and initial use of the medicines.

The current vision for medicinal health is precision medicines that customize healthcare, with medical decisions, practices, and/or products tailored to the individual patient. It is envisioned that new genetic information and advances in computation science and chemistry will enable this goal. However, knowledge of how a drug works and how it can be precisely used only becomes available after it has been discovered and tested; consequently the mechanistic paradox provides a significant long-term challenge to achieving this objective.

This introductory chapter first lays out the general background of drug discovery and development, providing some important definitions, emphasizing unmet medicinal needs, highlighting recent success rates, and describing the process that has evolved to identify safe and effective medicines. The later part of this chapter

addresses some of the knowledge gaps contributing to high attrition rates that provide challenges to implementing the vision of the Precision Medicine Initiative.

1.1.1 Definitions

Performance. Broadly defined as the action or process of carrying out or accomplishing an action, task, or function. When applied to the pharmaceutical industry, performance is typically defined as providing new medicines for patients. The performance of the pharmaceutical industry has remained relatively constant in terms of new molecular entities (NMEs) approved by the US FDA over the past several decades (Fig. 1.1), despite considerable increases in expenditures. As a consequence, the productivity (or ratio of performance/expenditures) has decreased over this time. A number of excellent articles have been written to address the decrease in productivity [2–4].

Attrition. The process of gradually reducing the effectiveness of something through sustained pressure. Attrition in the pharmaceutical industry is the failure of potential medicines to be approved for use in patients by regulatory agencies and ultimately reach the marketplace. These failures result in very significant financial losses (1) due to research expenditures on failed projects which (2) were not invested in projects that could have led to approved medicines and thereby increased performance and productivity. There are many reasons for attrition with the most common being the inability to show efficacy and the lack of tolerable safety in human clinical trials [5] (Fig. 1.2).

Process. A series of actions or steps taken in order to achieve a particular end. For example, a process has been installed in drug discovery and development across the pharmaceutical industry to ensure that medicines submitted to regulatory agencies are sufficiently safe and efficacious to be effectively used in patients. The process usually involves initial preclinical testing to identify potentially safe and efficacious drug candidates, followed by evaluating safety and efficacy in human clinical trials (Fig. 1.3). More details of this process are discussed below in Sect. 1.1.1.

Precision medicine. Precision medicine refers to the tailoring of medical treatment to the individual characteristics of each patient [6]. It does not literally mean the creation of drugs or medical devices that are unique to a patient but rather the ability to classify individuals into subpopulations that differ in their susceptibility to a particular disease, in the biology and/or prognosis of those diseases they may develop, or in their response to a specific treatment. Preventive or therapeutic interventions can then be concentrated on those patients most likely to benefit, sparing expense and side effects for those who will not. Although the term "personalized medicine" is also used to convey this meaning, that term is sometimes misinterpreted as implying that unique treatments can be designed for each individual. The discovery and precise use of medicines is a long-term goal of medical research.

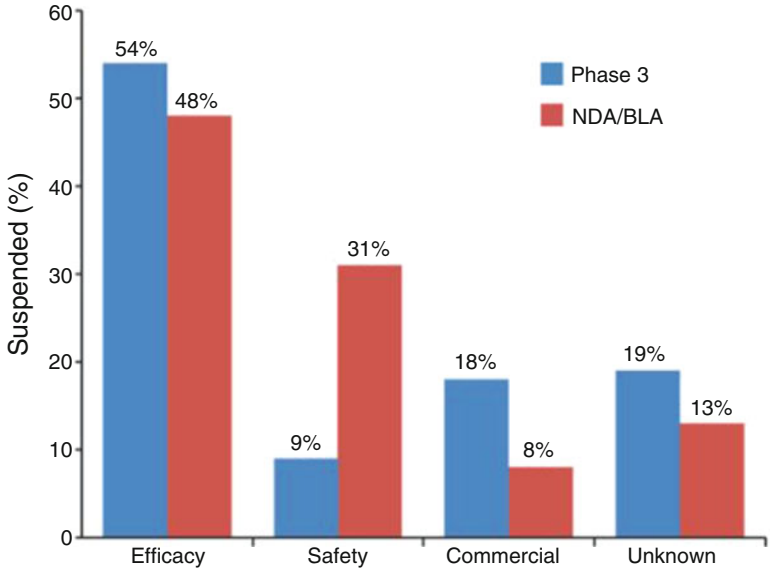

Fig. 1.2 Root-cause analysis for 359 phase 3 and 95 NDA/BLA suspended programs. A program was designated as "suspended" when conclusive evidence had been gathered regarding a company's plans to discontinue development or communications with regulators were not reinitiated for several years [5]

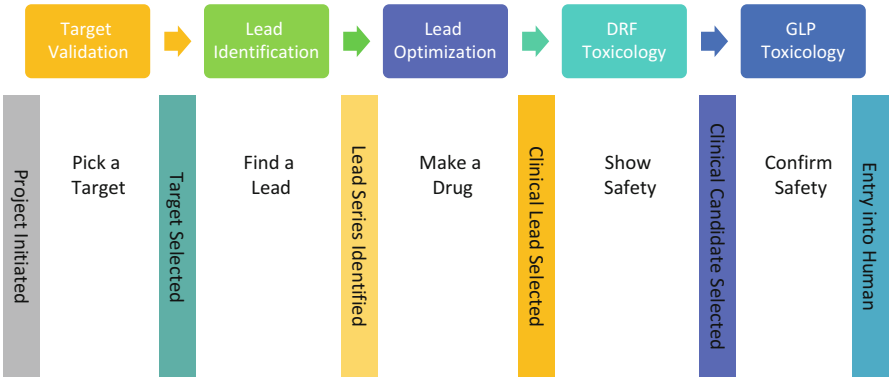

Fig. 1.3 Drug discovery and development: a product development process

Innovation. The introduction of something new. Innovation is commonly confused with invention and creativity. Creativity is the ability to generate original ideas, concepts, and objects. It spurs invention, which is most evident in the areas of technology and business. Artists enjoy creativity, whereas engineers and scientists focus on inventions. But innovation demands an additional ingredient: market success.

In a recent opinion in *Nature*, Kuziak stated that the path to innovation is currently more art than science, which might explain why it is shockingly inefficient: the chance of an invention attaining enough commercial or social success to be recognized as an innovation reaches no more than low single percentages. In the US Small Business Innovation Research program, a very low proportion of grants results in a viable economic activity, product, or service. In markets that are saturated, such as those of mobile phones or medical discoveries, the success rate is even lower [7].

The goal to discover, develop, and market innovative new therapies is arguably the grand challenge of medical research. As noted above, there is a clear process to ensure therapies that reach the market are safe at effective doses; however, this process does not ensure that new therapies will be innovative. This aspect occurs earlier in the basic research and discovery phases and will be discussed in more detail in Sect. 1.1.2.

1.1.2 Unmet Need

The primary goal of the pharmaceutical industry is to provide medicines for unmet medical needs. The priority unmet medical needs for Europe and the world in 2013 were identified in a report from the WHO (World Health Organization) [8]. The report identifies:

- The population of Europe and the world is aging, with more people—especially women—living beyond the age of 80. Since 2004, for the first time in Europe, there are now more people over the age of 65 than under 15 years. With this aging, there is a marked increase in diseases of the elderly such as osteoarthritis, lower back pain, hearing loss, and Alzheimer's disease.
- Ischemic heart disease, stroke, depression, chronic obstructive pulmonary disease (COPD), and alcoholic liver disease were all considered as areas for priority research as was the need for specific biomarkers which could be used to identify potential pharmaceutical products, diagnose and monitor the progression of disease, or assess the effect of treatment.
- Tobacco use, alcohol abuse, and obesity are risk factors that underlie many of the most common serious noncommunicable diseases (NCDs) affecting both Europe and the world. While prevention efforts must take precedence, the report stated that research is needed on pharmaceutical methods to address these risk factors and the pathologies exacerbated by these risk factors (e.g., COPD, various cancers, alcoholic liver disease, osteoarthritis, and diabetes).
- Antibacterial resistance and pandemic influenza remain major threats to global public health. Malaria and tuberculosis (TB) represent major threats, especially in low- and middle-income countries. Antimicrobial resistance will remain a threat until primary prevention with vaccines occurs. Diarrhea, pneumonia,

neonatal conditions, and maternal mortality are major contributors to the global burden of disease. For neglected tropical diseases and rare diseases, establishing new mechanisms to promote the translation of basic research into clinically important products remains a priority. The report identified that while progress has occurred since 2004 in the treatment of Buruli ulcer, other diseases such as leishmaniasis, trypanosomiasis, and dengue still require substantial research.

The report also stated that pharmaceutical innovation should encompass special groups of patients such as the elderly, women, and children, who have particular needs in relation to dosage forms and products. The development of appropriate formulations for children and the elderly needs to be supported. Progress has been made in some oral forms but more is needed. Furthermore, research is needed on the use of electronic health records (EHRs) to deliver much-needed information on safety and effectiveness of medicine use in these populations.

1.1.3 NMEs and the Degree of Innovation

Pharmaceutical performance for all novel therapeutics approved by the FDA between 2005 and 2012 was evaluated using a framework established by the FDA to classify the degree of innovation: first-in-class, advance-in-class, and addition-to-class [9]. Although innovation can be measured in different ways, drugs with novel mechanisms of action (first-in-class) are largely considered to be the most innovative. Drugs that provide important clinical benefits despite not being mechanistically novel (advance-in-class) may be equally important innovations in terms of their clinical promise [1]. The report compared the use of priority review and accelerated approval regulatory pathways, regulatory review times, and characteristics of pivotal trials, including number, design, primary end point, duration, and size, for novel therapeutics stratified by degree of innovation (Fig. 1.4). Between 2005 and 2012, the FDA approved 188 novel therapeutics: 70 (37%) were first-in-class, 42 (22%) were advance-in-class, and 76 (40%) were addition-to-class. Over half of the biologics (56%, 19 of 34) were first-in-class; nearly half of the small molecules (46%, 70 of 154) were additions-to-class and accounted for 73% (51 of 70) of first-in-class therapeutics. Almost two-thirds of therapeutics approved for autoimmune and musculoskeletal diseases (64%, 7 of 11) were first-in-class, as were one-third of therapeutics for cancer (36%, 14 of 39) and less than one-quarter of therapeutics for psychiatric disease (22%, 2 of 9). The authors concluded that the FDA was consistently applying existing regulatory levers to support and accelerate the review and approval of drugs considered mechanistically innovative (i.e., first-in-class therapeutics) as well as those anticipated to provide substantial clinical advances (i.e., advance-in-class therapeutics acting through existing mechanisms of action) [1].

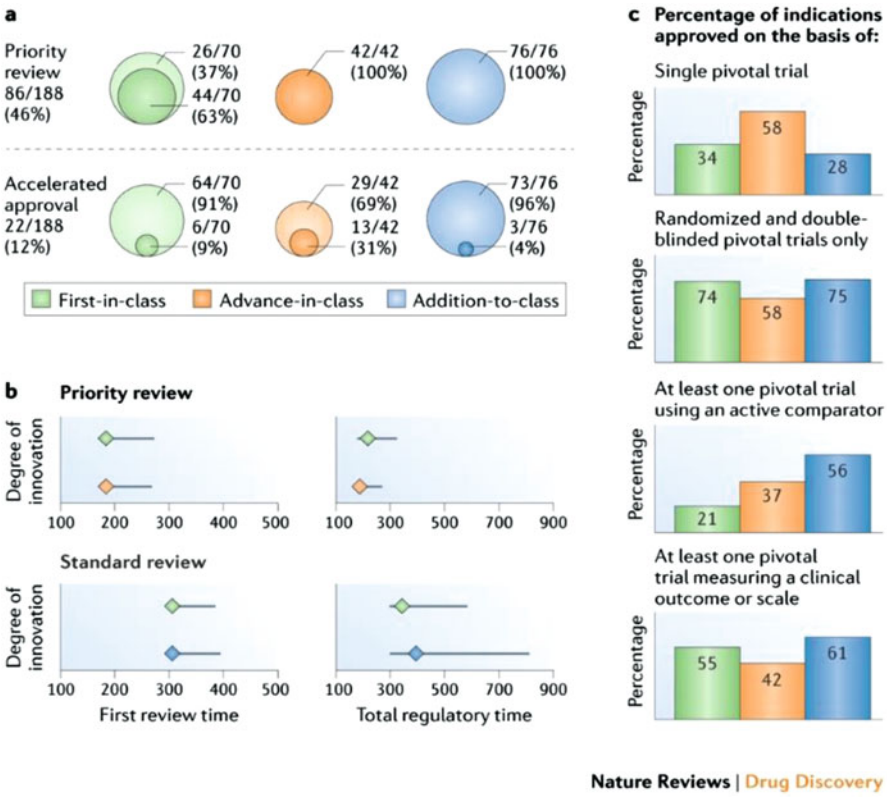

Fig. 1.4 Characteristics of novel therapeutics approved by the FDA between 2005 and 2012, stratified by degree of innovation. Data for first-in-class therapeutics are shown in *green*, advance-in-class therapeutics in *orange*, and addition-to-class therapeutics in *blue*. (**a**) Use of special regulatory pathways. *Lighter circles* represent the total number of therapeutics, whereas the *darker circles* nested within the *lighter circles* illustrate the proportion of therapeutics approved through the special regulatory pathways. (**b**) First review and total regulatory time. (**c**) Characteristics of pivotal efficacy trials, aggregated to account for all trials supporting the FDA indication approval [9]

1.2 Drug Discovery and Development Overview

1.2.1 Learn and Confirm Cycle

The creation of medicines to treat unmet medical needs involves an iterative cycle of testing and learning. Figure 1.5 describes some of the important phases of this process in which research, discovery, and development activities are initiated to provide a treatment for disease. Physiological, genetic, and chemical knowledge are generated and used to understand the disease, and this knowledge helps identify translation biomarkers to evaluate the effectiveness of the potential medicine. These activities represent the research phase (12 to 3 o'clock in Fig. 1.5).

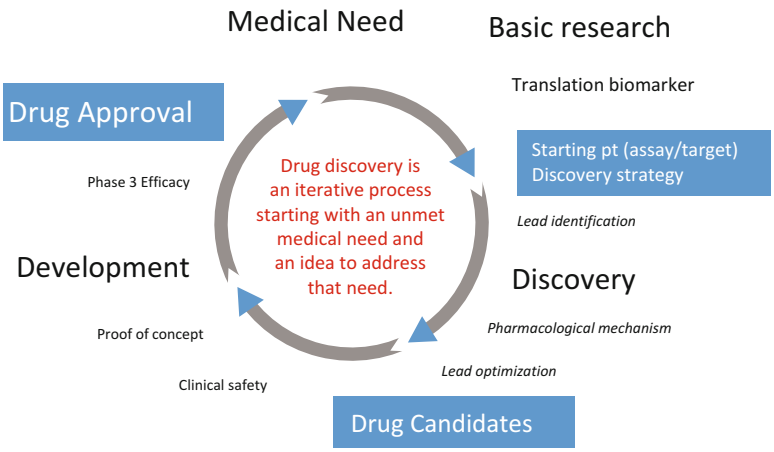

Fig. 1.5 Learn and confirm cycle of drug discovery and development. Drug discovery and development cycle. The approval of a medicine to treat an unmet medical need including a rare disease involves an iterative cycle of testing and learning. This figure describes some of the important phases in the process. The process of discovery and development of a new medicine is initiated in response to an unmet medical need to treat a disease. Physiological, genetic, and chemical knowledge provide an understanding of the disease. This knowledge will lead to the identification of translation biomarkers that are used to evaluate the effectiveness of a potential medicine. The available knowledge informs drug discovery strategies which are used as starting points for the practical process of discovering a new medicine. Target-based drug discovery (TDD) is associated with modulating a specific gene product known as the target, and phenotypic drug discovery (PDD) is a strategy driven by assays which measure phenotypes associated with the disease. Ideally these phenotypes will be associated with the translational biomarkers. These two strategies generally are focused on small molecules and are medicinal chemistry intensive, in contrast to biologics which use recombinant proteins and antibodies as therapeutics. It should be noted that the knowledge to choose a strategy is generally incomplete; however, the more iterations that occur in the drug discovery/development cycle, the more complete the knowledge and the better chance that a molecule will make it to registration. The discovery strategies will result in a lead molecule, ideally with activity against the translational biomarker. The molecule will work by a molecular mechanism of action (MMOA) that provides an optimal therapeutic index. These molecules will then be optimized for biopharmaceutics properties and safety to provide a drug candidate. At this point, the process of drug discovery is complete, and the molecule should succeed or fail based on its own merit. Opportunities to improve efficiency in drug discovery will increase the probability that clinical candidates will make it to registration. The *left hand* of the *circle* (from 6 to 12 o'clock) is the development phase of drug discovery which involves testing for safety and efficacy in humans leading to registration. Multiple iterations are generally required before a medicine with sufficient efficacy at a safe dose is discovered, tested in humans, and registered

The objective of the research phase is to generate knowledge that will inform the discovery phase. In the most effective processes, the discovery and development phases further refine this knowledge and provide feedback for continued research. There are many types of knowledge important to drug discovery and development: the cause of the disease, including the genetic contributions, pharmacological mechanisms of action that can safely modulate the disease, the most appropriate therapeutic

molecular modality (small molecules, biologics, nucleic acids), and the pharmaceutical properties of these molecules. Initially there are gaps in this knowledge base, and processes have been put in place to bridge or de-risk these gaps. An important feature of a productive R&D paradigm is to efficiently use the available knowledge and to effectively integrate new knowledge as it becomes available.

The knowledge obtained during the research phase is used to inform the discovery phase (3 to 6 o'clock in Fig. 1.5) and establish strategies that will be used as the practical starting points for creating new medicines. Target-based drug discovery (TDD) is associated with modulating a specific gene product or target, whereas phenotypic drug discovery (PDD) is a strategy driven by assays which measure phenotypes or observable characteristics associated with the disease. Ideally these phenotypes are also associated with translational biomarkers. Both of these strategies are primarily small molecule focused and medicinal chemistry intensive, in contrast to biologics which use recombinant proteins and antibodies as therapeutic moieties. The knowledge required to choose a specific strategy is initially incomplete; however, further iterations in the research/discovery/development cycle add to the knowledge base and improve the chances that a molecule will possess sufficient efficacy and safety to survive to registration. Successful discovery strategies result in lead molecules with a molecular mechanism of action (MMOA) that provides an optimal therapeutic index and which ideally provides activity against translational biomarkers. These lead molecules are then further optimized to improve biopharmaceutics properties and safety which yield a drug candidate for clinical assessment. At this point, the drug discovery phase is complete, and the molecule must succeed or fail based on its own merit with no further structural modification.

The left-hand portion of the cycle (from 6 to 12 o'clock in Fig. 1.5) represents the development phase of the process in which the drug candidate is tested for safety and efficacy in humans and if successful ultimately leads to registration. Multiple iterations of the entire research/discovery/development cycle are often required before a medicine is created with sufficient efficacy safety.

1.2.2 Process to Identify Safe and Effective Medicines

As noted above, a key challenge of drug discovery is to identify molecules that are safe at efficacious doses. Since it is impossible to a priori predict all interactions of a medicine within a patient, a process has evolved that first identifies potential drug candidates and then de-risks these molecules in numerous tests.

This process of drug discovery involves the identification of molecular structures, synthesis, characterization, and screening in assays for therapeutic efficacy and safety. Compounds demonstrating beneficial activity in these tests begin the process of drug development leading to clinical trials. This process has evolved over time to identify molecules that are safe as well as efficacious as further outlined below (Fig. 1.3).

Target identification and validation. This is the typical starting point for drug discovery programs. There is currently much debate regarding the role of this step in the process, which is discussed further in Sect. 1.4. Armed with an idea, researchers work to identify biological targets for a potential medicine. A drug target is a molecular structure in the body that, when it interacts with a potential drug compound, produces a clinical effect (e.g., treatment or prevention of a disease). The investigators conduct studies in cells, tissues, and animal models to determine whether the target can be influenced by known medicinal agents.

Lead identification. After learning more about underlying disease pathways and identifying potential targets, researchers seek to narrow the large field of potential compounds to one lead compound. This promising molecule provides activity against the target and has the potential to become a medicine. Candidate molecules are created from living or synthetic material and tested with high-throughput screening techniques.

Lead optimization. Lead investigational compounds that display sufficient potency to survive the initial screening are then "optimized" or structurally altered to improve efficacy and safety. By changing the structure of a compound, scientists can modulate its properties. Hundreds of different variations or "analogues" of the initial leads are produced and then tested and ranked in multiple assays. The resulting "best" compound that meets the required profile criteria becomes a drug candidate and undergoes extensive further testing and analysis before potentially being reviewed for approval by regulatory agencies.

Preclinical safety testing. Scientists carry out both in vitro and animal tests to assess the compound's safety. Through these techniques, researchers strive to understand what potential side effects may occur in humans. Techniques for making a drug on small-scale preclinical stage may not translate easily to larger production. Therefore, during this stage, scientists must also determine how sufficiently large quantities of the drug candidate can be produced for toxicity studies as well as to support clinical trials. Further production will also be required once the medicine is approved for use in the general patient population.

Investigational new drug application and clinical trial planning. Before any clinical trial can begin, drug sponsoring organizations must file an investigational new drug (IND) application with the FDA. The application includes the results of the preclinical work, the candidate drug's molecular structure and properties, details on how the investigational medicine is thought to work in the body, a listing of any potential side effects indicated from the preclinical studies, and manufacturing information. The IND also provides a detailed clinical trial plan that outlines how, where, and by whom the studies will be conducted.

Phase I clinical trial. In phase I trials, the candidate drug is tested in people for the first time. These studies are usually conducted with a small number of healthy volunteers, generally 100 individuals or less. The main goal of a phase I trial is to assess the safety of the medicine when used in humans. Researchers explore the human pharmacokinetics of a drug: how it is absorbed, distributed, metabolized,

and eliminated (ADME) from the body. They also study the drug's intended and unintended pharmacodynamics (potential side effects). These closely monitored trials are designed to help researchers determine a safe dosing range and if the candidate medicine warrants advancement to the next stage of development.

Phase II clinical trial. In phase II trials, researchers evaluate the candidate drug's effectiveness in 100 to 500 patient volunteers with the disease or condition under study. Many phase II trials evaluate patients receiving the drug candidate versus a comparator treatment, either an inactive substance (placebo) or a different drug that represents the standard of care for the disease. At this stage, researchers analyze optimal dose strength and dosing schedules. Possible short-term side effects (adverse events) and risks associated with the drug are also investigated. Drugs that continue to show promise are advanced to much larger phase III trials.

Phase III clinical trial. Phase III trials generate statistically significant data about the safety, efficacy, and the overall benefit-risk relationship of the investigational medicine. Phase III trials may enroll 1000 to 5000 patients or more across numerous clinical trial sites around the world. This phase of research is essential in establishing whether a drug is safe and effective. It also provides the basis for labeling instructions to help ensure proper use of the drug (e.g., information on potential interactions with other medicines, specific dosing instructions, etc.).

FDA review and approval of marketing application. Once the clinical trials have demonstrated that the drug candidate is both safe and effective, the sponsoring company submits a new drug application (NDA) or biologics license application (BLA) to the FDA requesting approval to market the drug. These applications contain the results and data analysis from the entire clinical development program, as well as the earlier preclinical testing and proposals for manufacturing and labeling of the new medicine. These documents can run 100,000 pages or more.

1.3 How Medicines Work

The process of drug discovery evolved to ensure that drug candidate molecules address an unmet medical need without compromising patient safety. Knowledge of how a drugs works (e.g., its mechanism of action) is helpful to translate understanding of the disease to treatment for the patient. For instance, a specific mutation in a gene that results in cancer can inform the selection of a target as well as the patient population for clinical testing. As an example, c-Abl is a kinase that when mutated causes chronic myelogenous leukemia and can be treated with the inhibitor imatinib [10]. Some forms of melanoma are caused by a mutation in BRAF kinase, which are treated with the BRAF inhibitor vemurafenib [11].

It has been long recognized that pharmacological action begins with an interaction between two molecules (a drug and a target). Ehrlich noted in 1913 that a substance will not work unless it is bound, *corpora non agunt nisi fixata* [12]. However, target binding alone is not always sufficient for a substance to initiate the

desired physiological process. For example, two similarly structured molecules can bind to:

1. An enzyme with similar affinity; however, only the molecule that binds in a suitable manner will initiate the catalytic reaction.
2. A receptor with similar affinity; however, an agonist will initiate a response, whereas an antagonist will block the response.

The molecular mechanism of action (MMOA) through which binding is coupled to the pharmacological response affects dose-response relationships and the therapeutic index. But simply knowing the parts of an efficient machine, be it a watch, automobile, or computer, is not sufficient to understand how it works. The parts must collaborate in precise ways to provide the desired accurate outcome: time, reliable transportation, or processed information.

Analogously an MMOA is the interaction between a drug and its target (or targets) that creates a specific response. These specific molecular interactions link structure to function in such a manner as to provide a therapeutically effective and safe response. As such, an MMOA differs from a mechanism of action (MOA), which only describes the process from the context of the physiological response (such as antihistamines, anti-inflammatory, etc.). There are many facets to this interaction that ultimately result in the desired therapeutic outcome. For example, the particular site of interaction (allosteric or orthosteric), molecular descriptors of the binding interaction (such as affinity and binding kinetics), the functional impact (receptor agonism, modulation, or antagonism), and the specificity of the functional outcome (activation of specific signaling pathways) all contribute to the MMOA and affect the ultimate pharmacological response. Possible MMOAs at a target are listed below, together with selected examples of drugs that act through these MMOAs.

(a) *Kinetic mechanisms*.

For kinetic mechanisms, a pharmacological response to the drug is primarily driven by binding kinetics and residence time at the target [13–17].

- *Equilibrium binding*. The response is determined by the equilibrium dissociation constant (K_i) of the drug to the target. Binding has sufficiently rapid association and dissociation rates (k_{on} and k_{off}) that allow equilibrium to be reached, and this process is therefore sensitive to competition with physiological substrates and/or ligands. Examples include bosentan, an endothelin receptor antagonist, and aliskiren, a renin inhibitor [18, 19].
- *Slow kinetics*. Non-equilibrium and irreversible mechanisms involve slow association and/or dissociation rates (k_{on} and k_{off}) that do not allow equilibrium to be reached, and these processes are less sensitive to competition with physiological substrates and/or ligands. Examples include orlistat which binds irreversibly to the active site serine of pancreatic lipase, azacitidine which irreversibly binds to DNA methyltransferases, and candesartan which has a slow dissociation rate from the angiotensin II receptor [13–17, 20–22].

(b) *Conformational mechanisms.*

For conformational mechanisms, drug binding results in a conformational change in the target that elicits a response. Examples include sirolimus which binds to the peptidyl-prolyl isomerase FKBP12 and stabilizes a conformation that subsequently inhibits the kinase activity of mammalian target of rapamycin and fulvestrant which induces a conformation of the estrogen receptor that is subsequently degraded [13, 23–27].

- *Noncompetitive inhibition and/or antagonism.* This MMOA involves drug binding to a target at a site that is distinct from the physiological substrate, and/or ligand-binding site, and results in an inhibition of the response. Caspofungin is believed to be a noncompetitive inhibitor of 1,3-β-D-glucan synthase, owing to the observation that its IC_{50} (half-maximal inhibitory concentration) is not influenced by substrate concentrations [28].
- *Uncompetitive inhibition and/or antagonism.* An uncompetitive MMOA is contingent on prior activation of the target by a physiological effector (the substrate or the ligand). As a consequence, the same amount of drug blocks the response to a greater degree at higher versus lower concentrations of the physiological effector. Memantine is an uncompetitive antagonist that binds only to the activated form of the NMDA receptor. The potency of the inhibition of the NMDA receptor by memantine increases at higher concentrations of glutamate (the physiological ligand [29, 30]).
- *Full agonism.* Maximal efficacy is produced following drug binding to a receptor and subsequent receptor activation. For example, ramelteon mimics the activity of melatonin for the melatonin receptor through binding at the orthosteric site with efficient coupling to activate specific signaling pathways [31].
- *Partial agonism.* This form of MMOA produces only partial efficacy following drug binding to the orthosteric site on the receptor. Examples include aripiprazole as a partial agonist of the dopamine D_2 receptor and varenicline as a partial agonist of the nicotinic acetylcholine receptors [32–34].
- *Allosteric modulation.* This mechanism involves regulation of the biological activity of the target by binding of a drug at a site distinct from the site for the endogenous substrate and/or ligand (allosteric site). Cinacalcet is an allosteric modulator of the calcium receptor by binding to the allosteric site [35].

(c) *Redox mechanisms.*

Reduction-oxidation (redox) reactions produce a pharmacological response to the drug as a consequence of electron transfer between the drug and a physiological target. For example, generation of hydroxyl radicals by verteporfin is thought to contribute to its ability to damage cells, and the antiprotozoal activity of nitazoxanide is believed to be due in part to interference with the pyruvate ferredoxin oxidoreductase enzyme-dependent electron transfer reaction, which is essential to anaerobic energy metabolism [36, 37].

A major challenge in the identification of safe medicines is to identify molecular mechanisms of action (MMOAs) that provide both sufficient efficacy and safety [13, 24, 38–41]. These MMOAs can be thought of as "pharmacological hot spots." Due to the dynamic complexity of physiology both at the molecular and systems level, it is difficult to a priori predict the exact interactions and molecules that will elicit a safe, therapeutically useful response.

1.4 Drug Discovery Strategies: How Medicines Are Discovered

Medicines come in many forms (biologics, small molecules, etc.) and are discovered in different ways. The generally preferred drug discovery process involves utilizing whatever knowledge of a molecular mechanism is available to help identify potential new medicines. Unfortunately, in many cases, this knowledge is incomplete, most notably for first-in-class medicines. As a result, one of two drug discovery strategies to increase this knowledge base is pursued:

1. *Target-based drug discovery (TDD).* For target-based drug discovery, a "therapeutic hypothesis" refers to the concept that perturbing a particular target in a given manner will benefit patients with minimal (or at least acceptable) toxicity. Ideally, the data for validating such a therapeutic hypothesis is derived from the patient population of interest as a result of direct perturbation of a target with a known function. However, strictly speaking, the only truly validated targets are those that are already successfully modulated by a safe and effective therapeutic [42].
2. *Phenotypic drug discovery (PDD).* Phenotypic assays measure a phenotype response in a physiological system (e.g., animals, cells and biochemical pathways) [43, 44]. A phenotype is the physical appearance or biochemical characteristic of an organism as a result of the interaction of its genotype and the environment. Phenotypic assays provide an empirical method to probe effects in physiological systems with minimal assumptions as to the molecular details of how the system works. The phenotype most relevant to the practice of drug discovery is a phenotype that directly translates to the clinical disease (translational biomarker).

Phenotypic assays have always played an important role in drug discovery [40, 45]. Much of early pharmacology and drug discovery was based on phenotypic assays, which were used to identify lead compounds that provided the desired efficacy. In his Nobel lecture entitled "Selective Inhibitors of Dihydrofolate Reductase," George H. Hitchings Jr. stated "Those early, untargeted studies led to the development of useful drugs for a wide variety of diseases and has justified our belief that this approach to drug discovery is more fruitful than narrow targeting" [45]. In the final decades of the twentieth century, the emphasis of drug discovery

changed to a more reductionist, target-based approach, with phenotypic assays used primarily to confirm efficacy and evaluate safety. This was driven by the molecular and genetic revolutions with the capabilities to identify many new drug targets and the potential toprovide numerous new medicines. Though not explicitly stated, the drug targets were also intended to be biomarkers for the disease. In this paradigm, the central features are (1) identification of a molecule that binds to the selected target and (2) optimization of the biopharmaceutics properties such that the drug concentrations in the body are sufficient to ensure that the drug is available to bind to the target throughout the dosing interval. This target-based paradigm was envisioned to provide a more rational approach to drug discovery, analogous to a design and engineering approach in other industries.

The general lack of productivity with the TDD approach has led to a reemerged interest in the last few years for using phenotypic assays to drive discovery. Swinney and Anthony analyzed the discovery strategies for NMEs approved by the US Food and Drug Administration (FDA) between 1999 and 2008 [41]. Of the 259 agents identified, 75 were first-in-class drugs with new MMOAs, and of these, 50 (67%) were synthetically derived small molecules versus 25 (33%) that were biological agents produced in cells. The results also showed that the contribution of phenotypic screening to the discovery of first-in-class small-molecule drugs exceeded that of target-based approaches—with 28 and 17 of these drugs coming from these two approaches, respectively. This discrepancy is especially notable in an era in which the major focus was on target-based approaches. A more recent analysis by Swinney and Xia showed a similar trend of success with phenotypic strategies. Between 1999 and 2012, there were 102 NMEs approved for rare diseases. Within the first-in-class NMEs, 15 used phenotypic drug discovery, 12 used target-based drug discovery, and 18 were biologics [46]. The Swinney and Anthony analysis suggested that compound identification using a phenotypic approach can also be effective (function-first/phenotypic drug discovery (PDD)). It was concluded that the function-first approach was valuable for uncovering new molecular mechanisms of action (MMOAs) that a priori were difficult to identify, and this contributed to the success of phenotypic assays for first-in-class medicines [41, 47].

Clearly the pharmaceutical industry and medical research are heavily invested in the target-based approach for both technical and intellectual reasons. When this process works (in other words when the target/MMOA are validated), it provides a rational approach for discovering and developing medicines, analogous to engineering. The ability to apply structure-based design to a specific target allows optimization of efficacy and drug-like properties in a rational way. A target-based approach also aligns with the potential for genetics to explain the cause of disease and provide biomarkers for discovery and clinical evaluation. This in turn allows better selection of patients for clinical trials and increases the probability of success. The clinical pharmacology directly relates dosing to target occupancy in order to maximize the therapeutic index. And finally, a target provides an understandable metrics to communicate mechanism of action to the stakeholders across the value

chain, from researchers in discovery biology and medicinal chemistry to drug developers and program funders.

Despite these benefits, there are two major challenges for the TDD approach. First is the necessity of identifying and validating the target. Second, as described earlier in this chapter, the MMOA of an effective medicine must provide a safe and effective response. This process is more complex than simple occupancy of a drug target and is difficult to a priori predict. The renewed interest in phenotypic screening provides new opportunities to address these challenges through integration of the systems (PDD) approach with the current target-centric (TDD) approach.

Two recent manuscripts by Moffat et al. and Eder et al. provided two different perspectives to this issue [48, 49]. The Moffat paper addresses the challenges of relating a target and genetics to a well-defined phenotype [48], while the Eder work focuses on the processes that successfully led to identification of first-in-class medicines [49].

Moffat et al. investigated the contribution of phenotypic screening toward oncology therapeutics, an area in which target-based approaches have been particularly prominent. In many cases, disease-causing genes also provided patient-specific biomarkers for clinical evaluation [48]. The authors defined pure phenotypic screening to be a discovery process identifying chemical entities able to produce desirable biological and therefore phenotypic effects on cells or organisms without prior knowledge of the biological activity or mode of action against a specific molecular target(s). However, the authors noted that in practice many projects are not target agnostic and conversely many target-based discoveries rely heavily on phenotypic assays. They concluded that recent phenotypic screening in cancer drug discovery has been hampered by a reliance on "classical" nonspecific drug phenotypes such as cytotoxicity and mitotic arrest. They instead proposed that mechanism-informed phenotypic drug discovery (MIPDD) provides a basis to better identify the causal relationships between target inhibition and phenotypic effects. Such mechanistically informed phenotypic models can provide some confirmation that the targeted agents have the necessary MMOA. Additionally knowledge of the drug target enables diagnostic hypotheses and the development of pharmacodynamic biomarkers [50].

Eder et al. performed a very thorough analysis of the origins of first-in-class new drugs, emphasizing the processes that led to identification of the new drugs [19]. To this end, phenotypic screening was defined as the testing of compounds in a systems-based approach such as cells, tissues, or animals using a target-agnostic assay that monitors for a phenotypic change. This definition assumes that no mechanistic information is available. The analysis by Eder shows that the majority of first-in-class drugs were discovered with target-based approaches as opposed to the finding in the Swinney and Anthony 2011 paper in which the majority were categorized as being discovered by phenotypic screening. This discrepancy is partially resolved by Eder as being due to the categorization, in which they included biologics as target based as well as drugs discovered using a chemocentric approach. The category "chemocentric" was used to categorize systems-based approaches in which an active component had been identified previously, such as

isolation of aspirin from willow bark. Eder et al. concluded that phenotypic screening and target-based screening were complementary strategies not requiring researchers to choose between the two [49].

Ultimately the conclusions of the Moffat and Eder groups depended on the definitions used for phenotypic screening, and it is the differences in the definitions and corresponding interpretations that provide interesting insights. In the Swinney and Anthony 2011 paper [41], the term "phenotypic screening" was used to describe any approach in which the MMOA that provides a tolerable therapeutic index is not assumed. In this context, phenotypic screening is empirical and includes all screening that is not target based. This definition focused on synthetic small molecules and excluded biologics as well as natural substance-based medicines. This is primarily because the question addressed in the analysis was "what type of mechanistic knowledge led to the identification of MMOAs that provide safe and effective medicines." The work by Moffat et al. recognized this issue and specifically addressed the concern with a new category that bridges between TDD and PDD, mechanism-informed PDD (MIPDD) [48]. The paper by Eder et al. did not address this aspect [49].

Many of the features important for successful drug discovery are relevant to both the PDD and TDD strategies. This includes the necessity of progressing forward with an incomplete understanding of the disease pathobiology, chemistry, and mechanisms of drug action. It remains a continued challenge to relate the molecular aspects of drug action to a safe and therapeutically useful response in patients. Moffat and coworkers ultimately conclude that very few recent cancer drug discovery success stories can be described as purely TDD or PDD, and PDD therefore remains a crucial activity in selecting, validating, and developing cancer drugs with optimal MMOAs [50]. Accordingly an integrated view of drug discovery that links molecular drivers to molecular targets to well-defined phenotypes is recommended. While Eder et al. highlight the success of TDD, they also discussed the promise of phenotypic screening to uncover new therapeutic principles and molecular pathways for currently untreatable diseases. They even proposed that phenotypic screening be considered as a new discipline [49].

It is clear that the strengths of PDD and TDD compliment their respective weaknesses. The strengths of TDD include the tools to optimize molecular interactions and translate between genetics and clinical disease markers. TDD is facilitated by complete and accurate knowledge of physiology, chemistry, and pharmacology, which is both a strength and a weakness. Alternatively, the empiricism of PDD can compensate for the often incomplete knowledge available for TDD. However, as both studies noted, PDD requires validated biomarkers and robust physiological relevant assays (Fig. 1.6). As proposed by Moffat and coworkers [50], better integration of the empirical/phenotypic and molecular/target-based approaches is needed with a mind-set to identify an effective molecule. Using this mind-set, the target becomes a tool rather than an outcome.

Clearly, effective integration of drug discovery disciplines is needed, in which different approaches with both strategies and tools are used as appropriate for a given project based on available knowledge. As mentioned previously, the

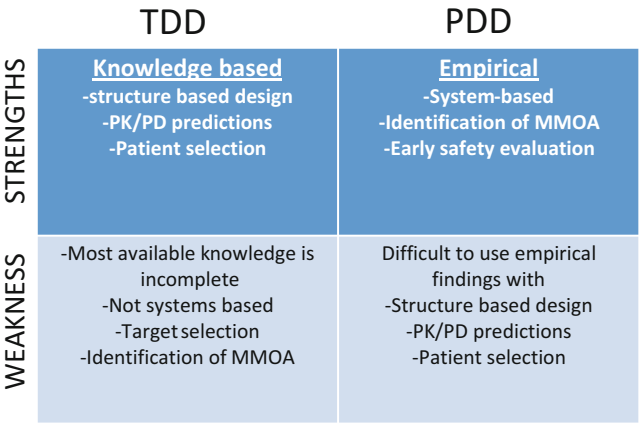

Fig. 1.6 Complementarity of target-based drug discovery (TDD) and phenotypic drug discovery (PDD) strategies

complementary strengths of TDD and PDD should help compensate for their respective weaknesses, with the ultimate goal of improving drug discovery productivity.

1.5 Mechanistic Paradox and Precision Medicine

From the above discussions outlining how medicines work and how they were discovered, it is clear that the relationship between a gene (with a mutation) and pharmacological modulation of the gene product (drug target) to provide a pharmacological response is both complex and unique. The knowledge of how a particular drug works, the patient population in which it works, and how it is best used comes only after it has been discovered and tested clinically. Consequently, this presents a mechanistic paradox in drug discovery:

> while the knowledge of mechanism (e.g. how a drug works) is very helpful to discover and precisely use medicines, paradoxically the knowledge available during drug discovery is rarely sufficiently complete to provide a blueprint for the discovery and initial use of medicines.

Addressing this paradox is an important challenge for pharmaceutical industry performance and translating molecular understanding of the diseases into medicines that effectively and efficiently treat disease in patients. Toward this goal, the Precision Medicine Initiative was implemented in 2015 [6]. The underlying concept of precision medicine is to refine the understanding of an individual illness based on their specific genetic makeup and other personalized medical data. The aim of the initiative, according to US President Obama, is to usher in a new era of medicine that harnesses data to support and advance research, technology, and policies as

well as empowering healthcare providers and patients. The goal of precision medicine is to "get the right treatment to the right patient at the right time," providing more personalized care and ultimately resulting in better outcomes at lower costs.

The initiative has two main components: a near-term focus on cancers and a longer-term aim to generate knowledge applicable to the whole range of ill health and disease. Both components are now within reach because of advances in basic research, including molecular biology, genomics, and bioinformatics. Furthermore, the initiative taps into converging trends of increased connectivity, through social media and mobile devices, and Americans' growing desire to be active partners in medical research [6].

Such a varied array of research activities will propel our understanding of diseases—their origins and mechanisms and opportunities for prevention and treatment—laying a firm, broad foundation for precision medicine. It will also pioneer new models for doing science that emphasize engaged participants and open, responsible data sharing. Moreover, the participants themselves will be able to access their health information and information about research that uses their data [6].

Therapies with safe and effective pharmacological mechanisms will need to be identified in order to realize the full potential value of the Precision Medicine Initiative toward connecting the understanding of the causes of disease to treatment of patients. Unfortunately, what we have learned in the last 20 years is that identification of a gene rarely directly identifies a drug therapy. It will be important to address the knowledge gap of the previously described mechanistic paradox. One approach is to continue acquiring more complete knowledge to provide a drug discovery and development blueprint. However, the magnitude of this challenge is enormous as it involves understanding every dynamic interaction in physiology, as well as how they change with time and between individuals. The cost and time needed to acquire this knowledge and determine its importance will be considerable.

How can drug discovery become more innovative and productive given the inherent knowledge gaps? The drug discovery and development process has evolved over time to better ensure efficacy and safety of medicines in patients. These processes do not however ensure innovation, and it can be argued that they in fact limit innovation. The key challenge is to address the mechanistic paradox: although the pharmaceutical industry is enabled by knowledge and needs this knowledge for precise use of medicines, this knowledge is unfortunately always incomplete.

When successful, the Precision Medicine Initiative will categorize patients more precisely into smaller groups based on the specifics of the disease. In doing so, these diseases may begin to have patient subpopulation numbers similar to rare (orphan) diseases. An orphan disease is categorized by the US FDA as one with less than 200,000 patients. Analysis of drug discovery in orphan diseases provides some insights into the successes and challenges that will need to be addressed including how treatment options can be identified and/or created in a patient relevant time

frame. The most obvious way is to identify an approved medicine that could be repurposed or, if warranted, used off-label. Another more direct option is to discover a medicine specific for that disease. However, currently only a few medicines are discovered each year for rare diseases. A recent analysis by Swinney and Xia in 2014 found that only 46 first-in-class medicines were approved for rare diseases over a 14-year period [46]. In this analysis, the impact of genetic knowledge on successful drug discovery was assessed since over 80% of rare diseases are genetic. It was concluded that genetic contributions (25%) were underrepresented with respect to the number of genetic diseases. This analysis of NMEs approved for orphan rare diseases provided further insights into factors important to bridge the knowledge gap in drug discovery. It was concluded that knowledge of most diseases and the underlying molecular causes is incomplete. An additional challenge is that knowledge of the cause, for instance, a genetic defect or multiple genetic defects, rarely provides a specific molecular solution. Plenge, Scolnick, and Altshuler recently noted most preclinical research programs have incomplete supporting material to accurately inform the drug discovery strategy [42].

The successful genetic approaches, while being fewer than expected based on the number of genetic diseases, were in disease areas with substantial supporting knowledge to facilitate drug discovery. For example, the success of kinases for cancer and enzyme replacement therapy for inborn errors of metabolism was due to significant preexisting research which provided an understanding of both molecular and physiological challenges. Perhaps most interesting were the examples where an understanding of regulatory pathways involved in diseases provided knowledge that led to successful therapeutic strategies [46] (e.g., hereditary angioedema/HAE and cryopyrin-associated periodic syndromes/CAP [51]).

1.6 Opportunities

The challenge of improving pharmaceutical industry performance requires significant innovation. While the complete knowledge needed to draft a blueprint for the discovery and use of an innovative first in class medicines is unlikely be available for many diseases, there are opportunities to innovatively bridge the knowledge gaps. These involve collaborations as well as cross-discipline initiatives and teams. Some examples at different stages of drug discovery and development include:

- The Precision Medicine Initiative (discussed above) which will provide disease biomarkers and help identify underlying genetic causes of diseases [6].
- The European Lead Factory, established in 2013 to find valuable, lead candidates that can be utilized to develop novel treatment options for patients [52].
- Quantitative Systems Pharmacology (QSP) defined as an approach to translational medicine that combines computational and experimental methods to elucidate, validate, and apply new pharmacological concepts to the development and use of small-molecule and biologic drugs [53].

- BioRam, a biopharmaceutics risk assessment roadmap that optimizes drug product development and performance by using therapy-driven target drug delivery profiles as a framework to achieve the desired therapeutic outcome [54].

These initiatives represent a snapshot of the many aspects of drug discovery and development that are not addressed in this introductory chapter but will be discussed in subsequent chapters.

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Chapter 2
New Product Planning and the Drug Discovery-Development Interface

Robin Reagan

Abstract The pharmaceutical industry is under increasing pressure to deliver safe and innovative drugs more quickly without incurring unsustainable R&D costs. Regulatory hurdles have risen in tandem with customer expectations making it essential that the science and commercial functions partner early and effectively throughout a molecule's march from target concept to an optimized, market-ready drug. Unfortunately, numerous examples exist of failures to achieve this key, cross-functional collaboration resulting in well-intentioned drugs paving the road to disappointed hopes. Here we review the key variables and timing of the collaboration between discovery, development, and commercial (new product planning). A successful collaboration ensures that the best molecule is identified and developed resulting in a successful launch, rapid adoption, and broad use over its life with a positive impact on human health.

Keywords New product planning • Pharmaceutical marketing

2.1 Overview and Introduction

The pharmaceutical industry's productivity rate on a background of increasing competition and payer pressures requires optimal teamwork across the pharmaceutical value chain over a long period. While every function brings critical competencies to the drug development journey, one of the most important partnerships in early development is for research and development (R&D) and commercial to co-officiate in marrying science to unmet market needs. When scientists and new product marketers work effectively together, the benefits to customers are far ranging while simultaneously creating a competitive advantage to the firm.

Ironically, new product marketers and early development scientists have much more in common in some respects than new product marketers have with their global and brand management colleagues. Global marketers typically engage when

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a drug is several years prior to launch so expectations for its efficacy, safety, tolerability, formulation, and delivery are fairly set. Local country brand managers usually work from a centrally developed brand plan which they are tasked to execute in their market over 12–24 months. Early new product marketers, like their development colleagues, operate with considerable uncertainty over a significantly longer timeframe, especially when in a therapeutic area new to the company. The high failure rate in early development requires both scientists and early marketers to focus on advancing the science or gaining and reapplying new knowledge.

Marketers involved in early drug development manage uncertainty through a variety of tools and techniques to identify the highest potential drug development ideas from the perspective of the customer. Though these techniques are different from those deployed in the lab, both the marketer and scientist combine statistically validated research with qualitative insight gained from internal and external experts to understand and predict the future. In this chapter we will review how marketers define value by understanding the disease, customer needs, the science, and opportunities and threats concluding with a brief discussion of the development and marketing partnership and ways to make it more productive.

- Marketing's job is to translate customer needs into actionable information for R&D. In turn, R&D must help marketing understand the disease and tools (drugs, formulation, delivery, devices, and packaging) that could meet those needs. Customers win when science and marketing come together with solutions to very specific unmet needs.
- The rise in payer power may be the single most important market change in pharmaceutical marketing over the past 10 years. R&D and marketing ignore this at their peril when embarking upon an early program.
- R&D and marketing, however, must be fully aligned beginning with preclinical data generation on a plan for staggered educational messages that ultimately addresses key unmet customer needs.
- Whether R&D's goals are revolutionary innovation, incremental benefit, or simply a "me-too," marketing should identify and share customer needs to ensure that R&D can translate those needs to possible solutions from which the best molecule is selected.
- From the "inside out," groundbreaking or novel science is exciting and seems valuable. From the "outside in," the question is whether that novel science translates into a benefit or solution the customer doesn't have today.
- Working with marketing early can prompt ideas for assays or preclinical studies to explore hypotheses that increase the probability of identifying and achieving a bundle of benefits in the clinic valued by one or more customer groups and target patients.

(continued)

- R&D input into profile testing in the context of the competitive environment for qualitative and quantitative market research is enormously helpful, especially if the marketer is new to the disease state or early product development.
- The new product planner must anticipate and plan, years in advance, for the impact of regulatory changes, competitor events, and environmental trends with the entire team's expert input.
- It's much better to conduct research prospectively grounded in a firm understanding of unmet customer needs than to be fairly advanced and attempt to retrofit a drug's benefits and risks to a customer.
- Early program scientists and marketers should be natural allies in the pursuit of satisfying customer needs. Each function brings valuable, specialized expertise to the partnership, which can be a highly combustible combination for igniting innovation.

2.2 Understanding the Disease State

Scientists spend years studying a disease, a drug target, or how to translate bench science into an actual drug. Unfortunately, this doesn't always guarantee a commercially successful product. The marketer is R&D's essential link to the customer dealing with the disease, patients, health-care providers (HCPs), and payers, in addition to allied stakeholders such as regulatory authorities, professional organizations, patient advocacy groups, and, in many cases, government agencies. An agile marketer can quickly learn the basics of the science involved in a disease and then focus on understanding the current treatment model and what patient and HCP needs remain unmet. Marketing's job is to translate customer needs into actionable information for R&D. In turn, R&D must help marketing understand the disease and tools (drugs, formulation, delivery, devices, and packaging) that could meet those needs. Customers win when science and marketing come together with solutions to very specific unmet needs.

Unfortunately, not every disease is well understood (e.g., fibrotic disorders) nor does every professional medical society have straightforward guidelines or treatment recommendations. The treatment of many diseases remains a blend of judgment and science based on the best available data, which is then individualized to the patient. Barring a disease with no treatment options, new therapies take time to be evaluated in real-world clinical settings, gain reimbursement, and prove safety in broad use across much more diverse patients than typically seen in clinical trials. Even with older drugs, data may be lacking to prove benefit or risk because the cost of testing a widely held belief is expensive, impractical, or unethical. In other cases, HCPs are reluctant to accept a single trial as sufficient evidence for changing

medical practice for a new or an old drug. Hormone replacement therapy (HRT) is a good example of this. There have been wide ranging opinions regarding the safety, indications for use, and efficacy of HRT products. The clinical trials ranged from the original studies by manufacturers to government-funded studies such as the Women's Health Initiative. Some HCPs found reconciling the different data impossible, so they rely on their own experience informed by reputable sources. This leads to a wide variation in usage. Understanding the human motivations and beliefs behind current treatments and potential future options for both HCPs and patients is a key marketing responsibility.

A basic disease understanding and knowledge of current treatment guidelines is the starting point after which new product marketers conduct exploratory qualitative market research to understand actual practices in a product-agnostic manner, especially in dynamic disease states. Understanding HCP beliefs about the disease and current treatment options is the next step to evaluate whether emerging science will challenge or complement current thinking. Marketers seek to clarify whether, how much and when treatment follows or deviates from current standards. Once marketers have this understanding, they turn their focus to a deeper understanding of customer needs (HCP, patient, caregiver, and payer) using a variety of analytical and statistical tools and methods to gather, organize, and analyze information in a systematic manner.

2.3 Customer Needs

The customer for an ethical pharmaceutical differs from consumer products for several reasons. Most notably, the patient or end user is rarely the drug decision-maker who is typically, though not always, a health-care provider. In addition, other important customers influence the drug decision including payers and, for some diseases, a caregiver. Balancing the many, sometimes conflicting, needs of these multiple customers isn't always easy and in some cases is impossible. A novel drug has a much better chance at success when R&D and marketing work closely together to maximize customer satisfaction within complex boundaries. So while the patient is the ultimate consumer and can choose not to fill a prescription (due to cost) or take a drug (because it's too difficult or the side effects too bothersome), the choice of drug is made by the HCP within the constraints payers and sometimes caregivers raise. Factor in the need to meet regulatory requirements heavily weighted toward safety, and the result is a more complicated matrix of customers than what is involved in motivating a consumer to pick one product vs. another on the store shelf.

Marketers use primary and secondary research to understand these multiple customer needs. Primary research is conducted by the company starting from a blank slate and focuses on very specific objectives. Secondary research is conducted by others using a variety of sources and can range from syndicated research and data mining sold to multiple customers to free sources from

government databases such as the National Health and Nutrition Examination Survey (NHANES) which assesses and tracks changes in health over time. Both primary and secondary research can be qualitative and quantitative. Qualitative market research seeks to understand the "why" behind the customer's response and in early drug development can be conducted as in-depth, individual interviews, focus groups, and expert advisor input gained individually or in an advisory board. Quantitative market research uses validated statistical tools and techniques to get at hard facts such as the likelihood to prescribe a new drug compared to current treatments or the relative value of different benefits.

In early drug development, a key quantitative technique to understand customer needs is segmentation market research. This market research builds on a qualitative disease state understanding to identify and prioritize customer needs in order to group their characteristics, beliefs, and needs for guidance on which customer groups might be best suited for a new drug. In contrast to science, where data are hoped to provide a clear answer, marketers segment, or group, customers to identify unique, identifiable, and actionable differences. There's no single "right" way customers could be grouped, but it's the best way for purposes of understanding the disease or evaluating how novel drugs may solve their needs with an acceptable level of risk. Grouping anti-hypertensive patients by their degree of hypertension is unlikely to be unique. Grouping them by a mix of their comorbidities, coping mechanisms, or support network would likely generate a more actionable segmentation scheme. An, obese middle-aged mother will have different priorities for managing her hypertension than an aged male stroke survivor in assisted living. Segmentation does not look solely at rational, clinical factors (type and stage of disease, family history, age, etc.), but also psychosocial factors and patient-centered (ethnographic) needs.

HCPs similarly and routinely consciously and subconsciously group patients in order to individualize care. In addition to "hard" clinical data, HCPs assess many "soft" factors before changing or intensifying treatment such as a patient's demeanor (upbeat vs. negative), self-care (neat vs. untidy), and motivation (cooperative vs. combative), among others. While no physician will fail to treat a patient appropriately, they avoid pushing a patient to the point where they won't return and may reserve extra effort or innovative drugs for patients who have the resources—physical, mental, financial, and family, to follow the HCP's orders. Understanding this dynamic, how big the different segments are, and consistency across geographies ensures that marketing can propose the best groups of patients for a novel drug and appropriately forecast its market potential.

When scientists or marketers forget the needs of the target patient, it's possible to register a drug and then withdraw it from the market due to commercial failure. For example, eliminating injections is a high priority need for needle-phobic, insulin-dependent patients. However, the number of people with truly debilitating needle-phobia appears to be small. Based on the low, slow adoption of inhaled insulin, the majority of people with diabetes appear to view the safety concerns, hassle, and cost of inhaled insulin as not worth eliminating the injection.

Segmentation market research changes over the course of a drug's development from the brand-agnostic approach outlined above to increasingly specific research framed around the drug in development and its emerging profile. As noted previously, development scientists and new product marketers share the need to assess and plan despite uncertainty around a drug's benefit-to-risk profile. Marketers manage this in early development by testing both ranges and different bundles of effects with physicians, patients, and payers to see the impact on the likelihood to prescribe, take, and reimburse, respectively. Until Phase 3 results are available; however, neither the marketer nor the scientist knows the actual profile which has significant implications for speed of uptake, sales volume, and all the operational requirements necessary to have a drug ready to succeed at launch and beyond.

In some therapeutic areas, HCPs and patients are surprisingly consistent around the world in their unmet clinical and emotional needs. In contrast, payers vary significantly and demand different types and levels of proof to meet their expectations. The most obvious difference is in countries with single payers, typically the government, and those with multiple insurers. However, even among single payer systems, there can be key differences in the type of data required before a novel drug is reimbursed. Payer-specific data needs are not always easily satisfied in Phase 3 clinical trials which must meet regulatory requirements focusing on safety. Yet failure to meet payer requirements can result in patients never receiving an innovative drug due to poor or slow reimbursement. The rise in payer power may be the single most important market change in pharmaceutical marketing over the past 10 years. R&D and marketing ignore this at their peril when embarking upon an early program.

No drug, no matter what level of innovation, sells itself. At a minimum, medical education is necessary simply to make customers aware of a novel drug. Marketing can determine, with R&D's help, what other scientific knowledge needs to be reinforced, created, or changed to prescribe the drug appropriately. If the drug establishes a new class or introduces a different treatment approach, then it is particularly important for R&D and marketing to collaborate on the customer medical education strategy. It's a given that the timing and disclosure of proprietary science require coordination across multiple functions over time. R&D and marketing, however, must be fully aligned beginning with preclinical data generation on a plan for staggered educational messages that ultimately addresses key unmet customer needs.

The more R&D can understand the needs of these different customer groups, then the more their deep expertise and creativity can be unleashed for solutions that a marketer could not conceive. Whether R&D's goals are revolutionary innovation, incremental benefit or simply a "me-too," marketing should identify and share customer needs to ensure that R&D can translate those needs to possible solutions from which the best molecule is selected. Once a chemical structure becomes a candidate, clinical trials become the primary lever to influence its profile. While downstream formulation chemists can try to fix molecular shortcomings through their genius, it's not always possible to solve problems cost-effectively or quickly enough to prevent costly delays. Early, healthy R&D/marketing collaboration enables scientists to design customer needs into the molecule increasing the probability of technical success in the clinic.

2.4 Does Science Matter?

Sometimes it seems like the most fundamental difference between R&D and marketing centers around the value of scientific innovation. This is a perfect example of the difference of an "inside-out" vs. "outside-in" perspective. From the "inside out," groundbreaking or novel science is exciting and seems valuable. From the "outside in," the question is whether that novel science translates into a benefit or solution the customer doesn't have today. It's also a key to avoid the expert's bias against science that seems incremental or "clunky," but is safe and delivers high-value benefits.

So of course science matters. But how it matters is in the clinical value it brings to patients, not simply in being new. Remarkable, elegant science that offers no benefit over current options will be viewed as another, more expensive tool with unproven safety. Working with marketing early can prompt ideas for assays or preclinical studies to explore hypotheses that increase the probability of identifying and achieving a bundle of benefits in the clinic valued by one or more customer groups and target patients.

This is a key area where R&D, working in partnership with marketing, can translate innovative science into satisfying existing and new customer needs both obvious and subtle. One consumer goods example of this is the OXO Good Grips brand of kitchen utensils. Originally conceived as a solution for cooks with manual dexterity issues, the brand has thrived as consumers of all ages realized and valued the utensils' ease of use, quality, and modern design. Importantly, the company understood and met the needs of their primary target customer, before they expanded to broader customer groups [1]. The "science" of designing an easier-to-use tool mattered, but customer insights ensured incorporation of other elements such as avoiding the stigma of a "handicapped" tool that ultimately expanded their sales to many more customers.

Science matters, but we need curious scientists and marketers to collaborate in translating science into customer-centered solutions. Further, this collaboration must continue throughout the R&D value chain and over time as ways to create customer value exist across every function. If you've ever struggled to open plastic clamshell packaging, you know that no matter how great the product, the company lost their customer focus at the final step of packaging and the first point of product experience.

2.5 The SWOT Team or How to Look Critically at Your Program

A SWOT analysis, evaluating your program (strengths and weaknesses) in the context of the general and specific market issues (threats and opportunities), is a common planning tool across companies and functions. In early drug development,

the SWOT analysis forces team members across R&D, clinical, and commercial to look critically at their program and their company's capabilities for positives and negatives. For example, the drug may have the potential for superior efficacy (strength), but must be dosed twice a day (weakness), or research has discovered an exciting drug candidate (strength), but in a therapeutic area where the company has no clinical design experience (weakness). Analyzing strengths and weaknesses focuses on factors that the company can directly affect while evaluating threats and opportunities examines external trends and potential events, e.g., the rise in genomic profiling or a drug class proving a significant benefit such as a cure similar to what hepatitis C drugs have recently achieved.

Market research into the impact of the drug's profile as it advances is necessary to stay current with the market because counterintuitive opportunities and issues can easily arise. For example, if every treatment for a disease has significant, unpleasant side effects and a novel treatment does not, then HCPs may perceive the drug as weak. Market research could establish what trials or data were necessary to prove potent efficacy in addition to tolerability and safety. Further, once one drug has proven a benefit, customers are likely to expect that from subsequent drugs in that class and anything else new unless they offer an offsetting benefit. The bar for customer expectations typically only rises. There are exceptions when a side effect emerges in a leading drug class after widespread use. A well-executed SWOT analysis will capture both "inside-out" factors (program and company strengths and weaknesses you can affect) in addition to "outside-in" factors (threats and opportunities over which you have less control).

Because of differences in expertise, filters, and market knowledge, it's essential that a broad functional group works together to ensure a robust SWOT analysis. The presence of R&D, marketing, medical, and operations in this exercise ensures that the analysis is thorough and diverse. A comprehensive SWOT analysis allows the early development team to work together to monitor the market and account for contingencies while putting in place plans to reinforce strengths and mitigate weaknesses. Late-in-the-game commercial requests can be minimized or at least land on receptive development colleagues prepared to respond positively due to early identification of and planning for that scenario.

2.6 Those Pesky Competitors

Another important, iterative marketing tool throughout drug development is an analysis of the competition. Once the disease state, the unmet needs of groups of patients with that disease, and physician attitudes and beliefs are understood, then it's necessary to understand the attractiveness of current and future treatments and how a novel drug compares. R&D input into profile testing in the context of the competitive environment for qualitative and quantitative market research is enormously helpful, especially if the marketer is new to the disease state or early product development. Moreover, observing qualitative market research, such as

in-depth interviews or advisory board discussions between key thought leaders, can be very valuable for the R&D team. The use of webcams, remote interviews, and virtual meetings makes this much easier and less expensive than just a few years ago when most market research and advisory board meetings were conducted in person. The primary caveat for R&D (or any function) participating in qualitative market research or advisory board discussions is to resist drawing conclusions from a small subset of interviews or opinion leaders who don't reflect "real-world" clinical knowledge or practice. It may be helpful to view qualitative market research and advisory board discussions as proof of concept testing, while quantitative market research, like early clinical trials, addresses key questions based on statistically powered data from a more representative customer sample.

New product marketers evaluate a slew of competitor activities including the impact of new entrants (based on assumptions around their profile), launch order (who is first, second, third, etc.), launch timing (how much of a lead do the first and subsequent market entrants have), approved indications, and price and reimbursement assumptions. Due to its size and pricing freedom, the USA has always been central for long-term product planning, but even in the USA, more uncertainty has entered in some therapeutic areas as the Federal Drug Administration raised higher hurdles than their regulatory counterparts in other countries and regions. The new product planner must anticipate and plan, years in advance, for the impact of regulatory changes, competitor events, and environmental trends with the entire team's expert input. Japan, for example, has modernized their regulatory process in recent years to enable more rapid availability of innovative products. More rapid access to newer, more expensive drugs in a super-aged society, however, may prompt significant changes to the Japanese reimbursement system in the future. The new product marketer in partnership with pricing and reimbursement experts assesses these opportunities and risks to inform early development in order to increase the probability a new drug will be competitive at launch years in the future.

Marketing leads planning for changes in the competitive landscape, but plans will likely be inadequate if R&D is not a strong collaborator throughout the process. Given that regulatory agencies and payers frequently demand remediation in the form of additional clinical work or process changes, it is in the best interest of R&D to engage closely with marketing to ensure frequent, informed market monitoring, analysis, and scenario planning at the research-development interface. Fortunately, the benefit to anticipating competitor moves and market events accrues not just to the most advanced drug but also to those earlier in the pipeline.

2.7 How to Have an R&D and Marketing Marriage Made in Heaven

Perhaps the single most important thing R&D can do to work effectively with new product marketing is to take every opportunity to understand customer needs. The best marketers understand the customer's importance and should respond

enthusiastically to development partners who join them with creative ideas to meet their needs. When everyone has serving the customer as their "true north," then it becomes remarkably easy to navigate across cultures, functions, geographies, personalities, and styles. Even if your company designs and incents R&D and marketing to collaborate, the quality and ease of partnering when everyone is aligned on what's best for the customer will be higher. Your task is much harder if your company has not created processes and rewards for collaboration, but it's still possible to succeed if you seek to understand each other's constraints and work together to overcome them on behalf of the customer [2].

If your firm has structured and funded drug development to enable early, productive collaboration with marketing, then your main effort may be simply to do your job well. Unfortunately, the benefits of early development-marketing partnerships are not immediately obvious to everyone in an organization. Downstream pressures to fund new launches or marketed products can force reduced headcount and resources necessary for development and marketing to work effectively together. If you're in an environment where marketer sightings at team meetings are infrequent or unknown due to organizational design, resources, or culture, then reach out to marketing formally or informally for a consultation to learn more about the customer and whether your target and/or ideas resonate. It's much better to conduct research prospectively grounded in a firm understanding of unmet customer needs than to be fairly advanced and attempt to retrofit drug's benefits and risks to a customer.

Meet with your marketing partners prepared to translate science into layperson terms and the "so what" of novel biology or innovative chemistry. Recognize that your marketer may be fairly new to early drug development and require coaching to recognize when they need to pay attention. Development milestones are obvious points of engagement, but there can be other, subtle moments that ping your marketer's radar such as a dose that is predicted to be large or druggability hurdles that increase active drug ingredient cost. This is an area where marketers and development chemists should jointly challenge the team to improve potency or pursue less difficult synthesis pathways.

Recognize that the majority of marketers are further downstream and may have a very different focus as they work to launch and promote drugs on the market. Most new product marketing groups are smaller and may cut across several therapeutic areas. This can be understandably frustrating to scientists who've spent years to become experts and want an equally experienced and competent marketing partner. Experts can be fairly intimidating for marketers who are trained in transferable skills and are expected to learn quickly how to apply them in new therapeutic areas. As a scientific leader, you can help your marketing colleagues by providing mini-tutorials or easy-to-understand references for their use to level the playing field. What the marketer brings to the partnership is a profound understanding of the customer and the environment in which our drugs will compete. Help your marketer accelerate up the scientific learning curve, and they'll reciprocate by deepening your customer understanding potentially igniting your scientific creativity in ways you may never have considered.

2.8 Should R&D and Marketing Collaborate Early or Late? Yes!

Academic researchers have explored this topic from the perspective of collaboration between different functions and cultures, timing, and even whether the product represents an incremental or breakthrough innovation [3]. Mix all of these important elements with a high dose of variability driven by individual personalities, and there seems to be evidence for a variety of successful approaches. If R&D is well guided directionally by deep customer understanding and strategic guardrails, then one could argue that new product marketing is valuable in early development primarily for occasional consults and updates as the market changes. However, the health-care market is dynamic with constant, rolling changes in customer perceptions and expectations. Combine this with employee turnover and new science offering up novel targets with bundles of benefits previously unavailable, and collaborating early and regularly is wise. In addition, competitive hurdles don't always rise. When unexpected safety issues arise in a marketed drug or HCPs report lower than expected real-world efficacy, then a program with a previously noncompetitive benefit-to-risk profile may be possible to revive.

2.9 R&D and Marketing Are Allies, Not Enemies

Early program scientists and marketers should be natural allies in the pursuit of satisfying customer needs. Each function brings valuable, specialized expertise to the partnership, which can be a highly combustible combination for igniting innovation. Unfortunately, differences in culture, inadequate processes, misaligned incentives, lack of trust, and poor understanding of each other's constraints and timelines can result in an equally explosive recipe for frustration, resentment, and hostility. Scientists are bright, creative, and solution oriented. The best, however, are those able to translate their complex, technical world into benefits the marketer and the customer can understand. Motivated marketers will work hard to learn a new disease state or pathway, but generous R&D colleagues can translate and interpret how their function can create additional benefits. Similarly, if you place the customer first and seek out your marketer to help meet their needs through innovative science, then there's no need for you to become a marketing guru. Your marketer will happily bring you along resulting in rich rewards from your constructive R&D/marketing collaboration for our customers, our companies, and yourself.

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Part II
Druggable Targets, Discovery Technologies and Generation of Lead Molecules

Chapter 3
Target Engagement Measures in Preclinical Drug Discovery: Theory, Methods, and Case Studies

Timothy B. Durham and Michael R. Wiley

Abstract Target engagement (TE) in drug discovery is generally defined as the interaction of ligands with their target biomolecules. Understanding TE allows research teams to design and interpret quality in vivo experiments, providing a more refined assessment of target validation. It can also orient teams toward delivering molecules that better enable clinical studies by focusing SAR efforts on the optimization of projected human performance characteristics. In this chapter, theoretical aspects of TE and its importance for addressing drug discovery issues like selectivity and the relationship of pharmacokinetics to pharmacodynamics are addressed. Methods to measure TE directly are reviewed along with a discussion of how to estimate TE based on pharmacokinetic data. The principles outlined within the chapter are then demonstrated by application to a theoretical drug discovery effort focused on validation of a novel protein target. Finally, two case studies are discussed in which application of these principles was used to optimize compounds toward desired human performance characteristics in one instance and to drive a target de-prioritization decision in another.

Keywords Biological target engagement • Target engagement ratio • Pharmacodynamic response • Target validation

3.1 Introduction

Target engagement (TE) in drug discovery, sometimes expressed as target occupancy, describes the physical interaction of a drug molecule with its corresponding biological target [1]. Having a clear understanding of the time course of TE in vivo is a prerequisite for achieving quality hypothesis testing in the execution of meaningful efficacy studies, both in preclinical models and in patients [1]. Recently,

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much has been written about the often poor translation of preclinical data into clinical outcomes and its impact on the cost and efficiency of the drug discovery process [2–7]. In fact, the failure to achieve efficacy in phase 2/phase 3 clinical trials is now the most common and by far the most costly contributor to the technical failure of clinical candidates. Therefore, it is critical that efficacy experiments are designed to deliver the most useful information possible, to either validate or invalidate specific mechanisms of drug action, given the commitment of resources and time needed to complete them.

To that end, research teams that focus on developing a reliable understanding of TE from their inception are better able to design effectively, learn efficiently, and act decisively in the prosecution of both preclinical and clinical research. For example, this approach impacts a team's ability to make quality decisions regarding the selection of specific tool compounds, along with appropriate doses and dosing regimens, for conducting informative preclinical efficacy studies [8]. Subsequently, those data can be used to more effectively design projected human efficacy experiments, as well as the performance characteristics of drug candidates required to carry them out [9]. Finally, by rigorously focusing SAR efforts to deliver those performance characteristics as early in the program as possible, teams have the opportunity to minimize the number of iterative learning cycles and thus maximize the speed and efficiency of the candidate selection process.

In this chapter, our first objective is to review the basic concepts that drive TE in vitro and in vivo. Then we discuss how research teams can use those concepts to design and analyze studies to explore the relationship of TE with efficacy in more complex biological systems. Finally, we provide a couple of illustrative examples from our own research efforts. Of course unique issues will be encountered with each research team/project, depending on the specific disease area, target family, and mechanism of drug action under investigation. However, this perspective should serve as a useful guide to initiate cross-functional dialogue as teams deliberate on strategies to pursue their targets of interest.

3.2 Basic Concepts

For drugs having reversible, rapidly equilibrating binding interactions with their biological targets, TE can be easily estimated with reliable knowledge of both the drug concentration under study and the affinity constant for the particular drug-target pair. For most drug discovery programs, apparent binding parameters are usually derived from in vitro data which define the concentration-dependent effect of the drug on a relevant biological activity parameter. For example, consider the case represented by the drug-protein complex and corresponding concentration-response curve illustrated in Fig. 3.1. In this simple model system, the protein exists in either a free/active state or a drug-bound/inactive state. Although this example illustrates a small molecule that functions as an inhibitor, the analysis applies equally, regardless of the functional consequences of drug-target binding. Upon

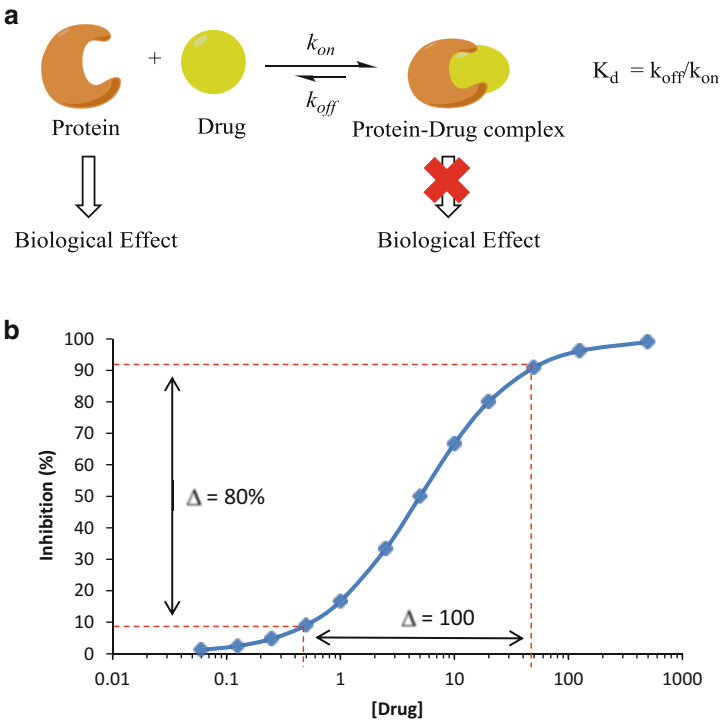


Fig. 3.1 Reversible drug-binding model. (**a**) Reversible drug-binding model. (**b**) Concentration-response curve with Hill coefficient (h) = 1

visual inspection, the theoretical concentration-response curve shown in Fig. 3.1b represents a high-quality data set based on several criteria. At low concentration, several data points across a significant concentration range demonstrate a lack of biological activity and therefore an apparent lack of TE. At the high end, several data points demonstrate saturation of the observed biological activity. In between, the transition from the onset of TE through target saturation occurs over a concentration range of about 100-fold. A more detailed inspection of this concentration-response curve reveals the data points are well represented by the mathematical concentration-response model known as the Hill equation, which describes the relationship as follows:

$$\%\mathrm{TE} = \frac{100}{1 + \left(\frac{\mathrm{EC}_{50}}{[L]}\right)^{h}} \tag{3.1}$$

where h is the Hill coefficient (often referred to as the Hill slope) and $[L]$ is the concentration of the ligand [10]. The Hill coefficient is a term which reflects the stoichiometry of ligand binding. For the vast majority of small molecule drug discovery efforts, desirable mechanisms of drug action rely on a single drug

molecule binding to the biological target, and therefore h values of 1 are viewed as ideal. If a concentration-response curve is observed with $h = 2$, this implies that two molecules of the drug must bind to the protein to produce activity [10]. Thus, discovery scientists often use the Hill coefficient to triage compounds following up on screening campaigns, flagging those whose concentration-response curves appear overly "steep" upon visual inspection [11]. Compounds having values of h outside of the range 0.5–1.5 may be deprioritized based on the perception that they are more likely to act via undesirable or "nuisance" mechanisms [11].

Given access to a quality concentration-response curve, the relative amount of TE can be easily estimated for any given drug concentration, as illustrated by the red dotted lines on the curve shown in Fig. 3.1b. It can be useful to think of TE either in terms of the apparent percentage of target bound (%TE) or as a ratio of the drug concentration relative to the EC_{50}. In this case, a target engagement ratio (TER) may be defined as follows:

$$\text{TER} = \frac{[L]}{\text{EC}_{50}} \tag{3.2}$$

Table 3.1 provides a comparison of these approaches for defining TE, over a wide range of drug concentrations, for an ideal curve with Hill coefficient of 1. The same data is presented in graphical form in Fig. 3.2, which illustrates several key points that will be important in the design of experiments in more complex biological systems.

First, the attributes of a quality plot of TER vs biological activity mirror those of any other quality concentration-response curves, such as the transition from a lack of TE through target saturation over a concentration range and slope consistent with the expected drug-target binding interactions. Such plots offer the additional advantage that multiple compounds, with diverse structures but common mechanisms of action, can be included on the same graph in order to confirm the consistent translation of TE into biological activity (Fig. 3.3).

In further examining the relationship of the TER to %TE, the graph clearly highlights the diminishing returns provided by further increases in drug concentration as the system approaches saturation (Fig. 3.4). For example, the first tenfold

Table 3.1 TER and %TE

TER	%TE
0.01	1
0.03	2
0.1	9
0.3	23
1	50
3	75
10	91
30	97
100	99

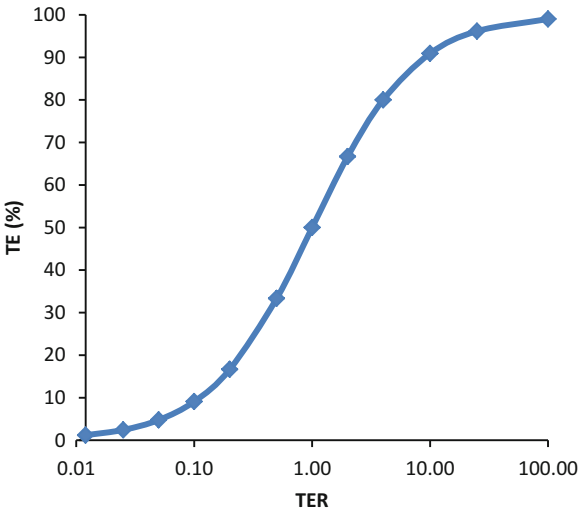


Fig. 3.2 Relationship of % TE to TER for a theoretical drug-target pair where $h = 1$

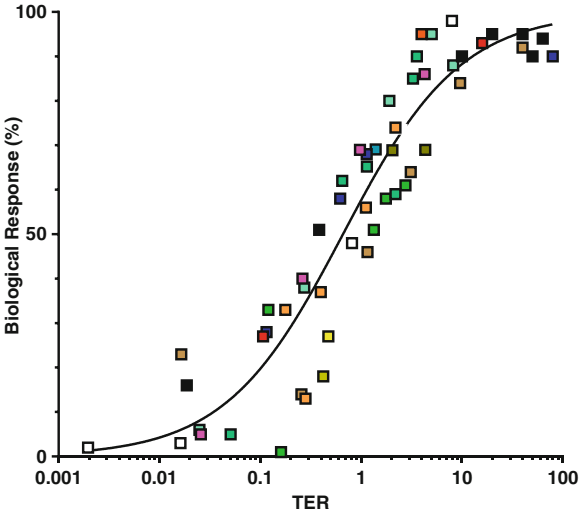


Fig. 3.3 Relationship of % TE to TER for multiple drugs with common MOA. Each color represents a separate compound (with multiple doses) from an in vivo experiment

increase in TER relative to the EC_{50} raises the %TE from 50% to 91%. However, beyond that point, another tenfold increase in TER (total TER of 100) produces only an additional 8% increase in %TE.

Thus, incremental increases in %TE at the high end require a much larger increase in drug concentration. Said another way, at the high end of the TE range, large increases in drug concentration will likely provide little meaningful increase in TE. For this reason, it is important to carefully consider the dose/concentration range used to test mechanistic hypotheses in complex biological systems (e.g., cell-based assays and in vivo). Using a drug concentration much higher than needed

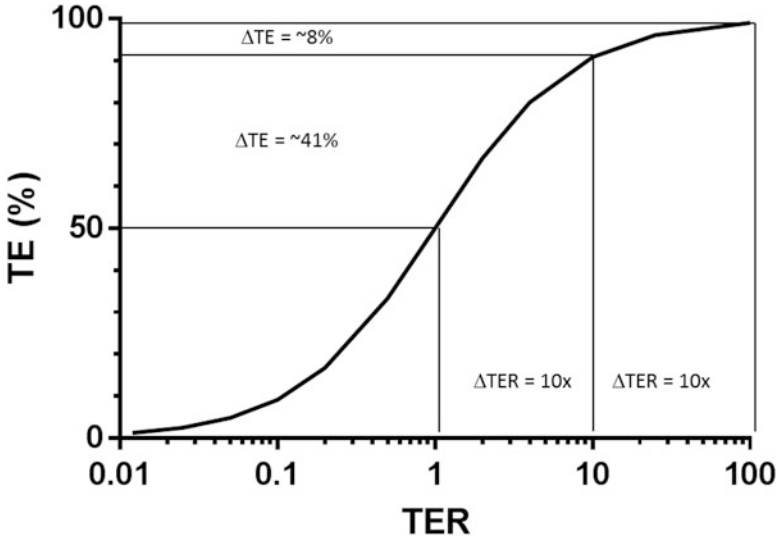


Fig. 3.4 Curve illustrating the relationship of TER to %TE at high vs low TE

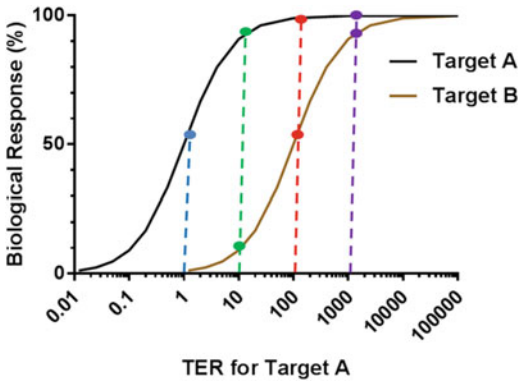


Fig. 3.5 Concentration-response curves for a drug versus two different biological targets with 100-fold affinity difference at the EC_{50} level. The TER values shown on the *X*-axis in the figure represent Target A. For Target B, those TER values should be divided by 100

provides little additional impact on TE, yet may erode the "effective selectivity" for engagement of the desired drug target vs other proteins.

For example, consider the two concentration-response curves for the drug illustrated in Fig. 3.5. As measured at the EC_{50}s, the affinity of the drug for Target A is 100-fold more potent than the corresponding affinity for Target B, seemingly a high-level of selectivity. The dotted lines on the figure highlight the "effective selectivity" for engagement of Target A relative to Target B at several different drug concentrations. The blue line illustrates that at the EC_{50} concentration for Target A, TE for Target B is expected to be insignificant. The highest selectivity would appear to be achieved at a TER of 10 for Target A (green line). At this concentration, ~90% TE is expected for Target A, with only ~10% TE for Target B. Then as the concentration continues to rise, the

effective selectivity erodes. At TERs of 100 (red line) and 1000 (purple line) for Target A, %TEs are not substantially different from each other, saturated at ~99%. However, the %TE for Target B at those concentrations continues to rise, from ~50% to ~90%, respectively. Thus, in spite of the apparent high in vitro selectivity, a lack of attention to detail in the experimental design could significantly erode the actual selectivity of TE for this drug in vivo. This could create ambiguity in the evaluation of the efficacy hypothesis and introduce safety risks, or both, dependent on the impact of engaging Target B in vivo.

3.3 Target Engagement in Vivo

If the assay used to establish the concentration-response relationship in vitro provides a meaningful representation of the drug-target functional interaction, it should also be useful for estimating target engagement in vivo, where the same drug interacts with the same biological target to produce analogous effects, just in a more complex biological environment. If this assumption is true, then the challenge of estimating TE in vivo is reduced to the challenge of defining the concentration of the drug in vivo. Thus, quality estimates of TE in vivo require quality pharmacokinetic data, and toward that end, a number of points are important to remember.

First, the relevant concentration for estimating TE in vivo should be the "effective" drug concentration actually available to interact with the target [12]. Frequently, this will not be well represented by the total drug concentration measured in vivo due to nonspecific interactions that reduce the fraction of "free" drug available to the target. For example, plasma proteins such as albumin can be a significant source of nonspecific protein binding [13–17]. Given the fact that such nonspecific interactions can vary significantly across a related series of compounds, teams need to understand the extent to which nonspecific protein binding may occur and consider adjusting TE estimates accordingly [18–20]. Typically, it is assumed that nonspecific binding to plasma proteins or brain tissue is predictive of nonspecific binding in other tissues. For these proteins, the free fraction (f_u) is easily measured in vitro and can be used as a corrective factor to establish free drug concentration. Thus, using the equation below, one can estimate TER:

$$\mathrm{TER} = \frac{f_u \times [L]_{\mathrm{total}}}{\mathrm{EC}_{50}} \tag{3.3}$$

where $[L]_{\mathrm{total}}$ is the total ligand concentration and EC_{50} is the absolute concentration of ligand in the in vitro assay that delivers 50% of the maximal response.

An alternative to this approach is to divide the total plasma concentration by a functional EC_{50} value determined under conditions in which the nonspecific interactions are already taken into consideration (Eq. 3.4).

$$\mathrm{TER} = \frac{[L]_{\mathrm{total}}}{\mathrm{EC}_{50}\mathrm{functional}} \tag{3.4}$$

Examples may include assays run in whole blood, or in buffers which include physiologically relevant concentrations of albumin as a surrogate. In these cases, the compound is already overcoming the relevant nonspecific interactions in order to produce activity in the functional assay. Thus, incorporation of an additional f_u term into the TE calculation would incorrectly underestimate target engagement.

Another important set of issues to consider derives from species differences that can significantly impact the projection and interpretation of TE in vivo. For most drug discovery teams, the primary assays used for characterizing target affinity as well as for predicting ADME properties logically utilize human proteins. However, species differences in the affinity of drugs for both specific target proteins and nonspecific plasma proteins can be very difficult to predict, even with respect to highly homologous target family proteins [18–20]. In fact, even within the same species, discrepancies due to differences in strains or disease states have been reported [21, 22]. In addition, such selectivity differences can vary with subtle structural changes across a series of related drug molecules. Therefore, it is important for the quality design/interpretation of in vivo experiments (and for the translation of preclinical results to projected human properties) that assays are enabled to characterize both the binding interactions and the effective drug concentration in the species utilized for the in vivo proof-of-concept studies.

It is also important to consider the drug concentration in the compartment in which the biological target resides. This can be particularly challenging when the distribution of the drug is significantly impacted by active transporters [23, 24]. In such a case, the direct measurement of drug concentration in the target tissue may be required. This can be costly, time consuming, and experimentally challenging, thus limiting the team's ability to collect meaningful data points. However, when active transport mechanisms are not involved, the picture can be simplified by the application of the free drug hypothesis [13, 16, 17]. It states that the concentration of *free* drug on either side of a permeable membrane should be the same in the absence of active transport processes. Therefore, the assessment of the *free* drug concentration in plasma is often a useful approximation of the *free* drug concentration in tissues. Given that plasma protein-binding assays (human as well as most of the species routinely used in preclinical research) are readily accessible, experimental assessment of the free drug concentration is relatively straightforward. However, even with compounds in which active transport is not anticipated, it is important to periodically confirm that drug concentrations in the target tissue are as expected before making decisions based on TE using plasma drug levels. Such validation is especially important in the case of target compartments

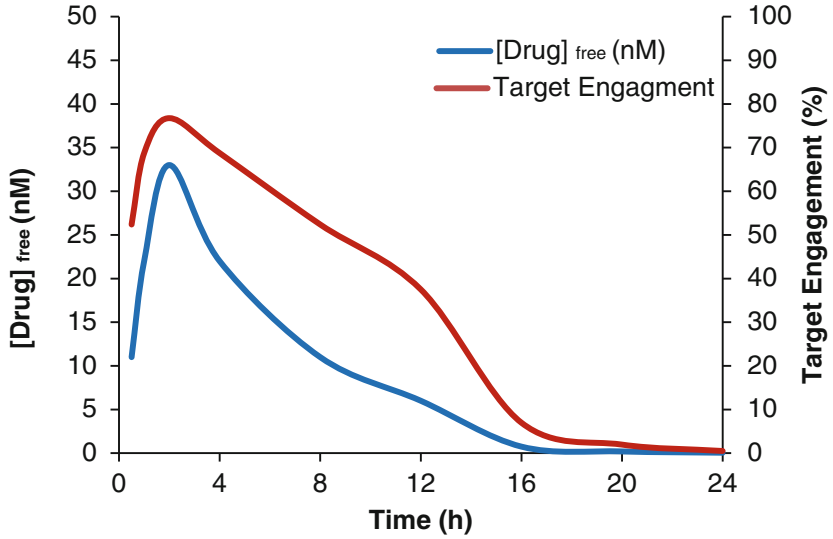


Fig. 3.6 PK/TE vs time for a reversible ligand. In the example shown, the ligand has an $EC_{50} = 10$ nM

where active transporters function most commonly, such as the CNS or the liver [23–25].

A final, very important issue to consider in assessing TE in vivo is its time course [8]. For drugs that have rapid-reversible binding interactions with their biological targets, TE in vivo will change rapidly in response to changing drug levels. Given that a drug's concentration in vivo changes over time, moving through peaks and troughs during each dosing period, it is important to understand how TE changes at meaningful intervals over the full time course explored in any in vivo efficacy experiment. For the example depicted in Fig. 3.6, the %TE is expected to fluctuate between ~80% at the high end and insignificant levels at the low end over the course of the experiment. Assessing the exposure and pharmacology of drugs with this type of profile at single time points would likely have limited applications and could potentially be misleading.

While the time course of TE is tightly linked to the time course of drug exposure for rapid-reversible ligands, this is not the case for drugs that are released slowly from binding interactions with their corresponding targets [26–29]. In the most extreme case, the ligand protein interaction is irreversible, as with the example illustrated in Fig. 3.7 [30–33]. For an irreversible ligand, the parameters that characterize the binding event are K_I and k_{inact}. K_I is the concentration of inhibitor that produces ½k_{inact} (analogous to the term K_M for enzyme substrates), and k_{inact} is the kinetic constant that describes the maximum rate at which the irreversible inactivation occurs (usually through formation of a covalent bond) [34]. [Note: K_I has a different meaning than K_i for competitive inhibitors.]

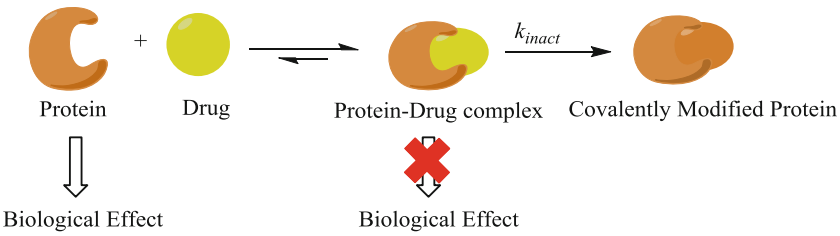


Fig. 3.7 Irreversible drug model

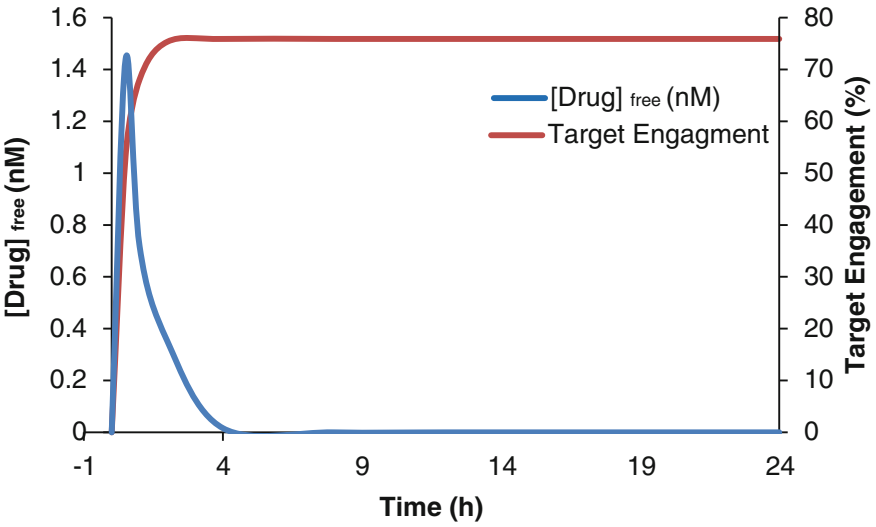


Fig. 3.8 Plot of exposure and target engagement for a theoretical irreversible ligand. For this example, $k_{inact}/K_i = 392{,}000$, $f_u = 0.016$, and the half-life of protein turnover is >24 h

TE for irreversible drugs is controlled by both concentration and time. The percent target engagement can be described by the equation:

$$\%\text{TE} = 100\% \times \left(1 - e^{-\left(\frac{k_{inact}[L]}{K_I + [L]}\right)t}\right) \tag{3.5}$$

(For derivation of Eq. 3.5, see Box 1 [34, 35].) Note that Eq. 3.5 is a reasonable mathematical description when $K_I \gg [L] \gg [T]$ and the rate of target protein resynthesis is slow relative to the experimental window. Figure 3.8 illustrates the PK-TE relationship for a drug with an irreversible mechanism of action as defined by Eq. 3.5. As the graph illustrates, increasing drug concentration at early time points leads to increasing TE. However, as the binding is irreversible, TE continues after the drug is cleared from the system. In this case, recovery of biological activity

in the system depends primarily on the rate at which new target protein is resynthesized. Thus, an apparent disconnect between the time course of drug exposure and the time course of TE is expected for drugs that operate by an irreversible mechanism of action [8, 26–29]. It is important to underscore here that both reversible and irreversible mechanisms are well precedented in medicine with numerous examples of successful, effective drugs. Each approach has different potential advantages and risks, which should be carefully considered in weighing strategic options for the prosecution of each unique project. This discussion highlights the differences expected in translational pharmacology and thus the critical importance of experimentally characterizing the full time course of PK and biological activity to enable a clear understanding of the system under investigation.

Box 1. Derivation of Eq. (3.5) for Irreversible Inhibitors

The amount of target-ligand covalent complex (TL*) can be described by the equation:

$$\mathrm{TL}^* = [T]_{\mathrm{Total}}\left(1 - \mathrm{e}^{-k_{\mathrm{obs}}t}\right) \tag{3.6}$$

where $[T]_{\mathrm{total}}$ is the total concentration of target, k_{obs} is the measured rate, and t is time. The rate k_{obs} can be defined for irreversible inhibitors as

$$k_{\mathrm{obs}} = \frac{k_{\mathrm{inact}}[L]}{K_{\mathrm{I}} + [L]} \tag{3.7}$$

where $[L]$ is equal to the concentration of the ligand. If we substitute Eq. 3.7 into Eq. 3.6, the resulting Eq. 3.8 can then be solved for target engagement ($[\mathrm{TL}^*]/[T]_{\mathrm{total}}$):

$$\mathrm{TL}^* = [T]_{\mathrm{Total}}\left(1 - \mathrm{e}^{-\left(\frac{k_{\mathrm{inact}}[L]}{K_{\mathrm{I}}+[L]}\right)t}\right) \tag{3.8}$$

$$\mathrm{TE} = \frac{\mathrm{TL}^*}{[T]_{\mathrm{Total}}} = \left(1 - \mathrm{e}^{-\left(\frac{k_{\mathrm{inact}}[L]}{K_{\mathrm{I}}+[L]}\right)t}\right) \tag{3.9}$$

Equation 3.9 can then be converted to Eq. 3.5 by multiplying both sides by 100%.

3.4 Application to In Vivo Experimental Design

Target validation (TV) lies at the heart of drug discovery and is particularly important for the exploration of novel targets in which the connection to disease biology is the major point under investigation [36–39]. Given the large number of clinical programs that fail due to lack of efficacy, it is critical that efficacy experiments are designed to deliver the most useful information regarding TV as early as possible. In doing so, precious resources can be efficiently focused to accelerate efforts that have the highest probability of delivering all the way to patients [2, 3, 5, 7]. At a high level, the goal of a TV exercise is to establish the pharmacological consequences of engaging the desired target via an acceptable mechanism, at a useful level, for a suitable duration, and with acceptable selectivity [36–38]. Drug discovery efforts are generally undertaken because there is some level of biological evidence that supports a connection between a target and disease. This can come in many forms, for example, genetic information such as knockout, knockdown, or knock-in experiments in animals or cellular systems. The available evidence in turn supports a hypothesis that selective pharmacological manipulation of that target will produce a desirable outcome. However, this preliminary information is often limited in resolution with respect to both the level and the time course of target manipulation [39]. Therefore, the goal of the study design is often to explore the *full range* of TE over the *full time course* of the efficacy study.

By definition, if such a study were successfully designed and executed, it would either establish the level and duration of TE needed to produce efficacy or it would reliably invalidate the drug target (at least by the mechanism of action for the drug tested) if it failed to deliver the required pharmacological results. In our experience, a sustained TE of $\geq$90% (or a TER $\geq$ 10) should be sufficient to test the translational pharmacological hypothesis in most systems. In the event that $\geq$90% TE is sustained in an efficacy experiment and no pharmacological signal is detected, it is likely that the TV hypothesis is disproven. Further, by *demonstrating* that the expected TE was achieved in such an experiment, teams can feel confident that the hypothesis was successfully tested, and the lack of efficacy was not due to any inadequacy in the performance characteristics of the tool molecule (e.g., the compound wasn't "potent" enough). As a result, a crisp termination decision can confidently be made, saving the time and resources required to identify "better" tool molecules. Such termination decisions can sometimes be viewed negatively within the culture of some research teams. However, driving TE informed TV decisions as quickly as possible ensures technical resources are efficiently applied to the most valuable target opportunities, which is clearly in the best interest of both patients and research organizations.

To illustrate the application of these principles, consider the following example of experimental design to explore the pharmacological validation of a typical early drug discovery target.

Target X is a hypothetical peripheral protein reported to have significant potential impact on a human disease. The published data supporting this hypothesis

consists of embryonic knockout of Target X in rodents showing protection in preclinical efficacy models used to simulate the disease state. For this example, we assume those efficacy models will require multiple days of drug treatment, and thus the relevant time course of TE for design purposes will be 24 h periods of time. The research team's goal is to identify a compound that can be used to demonstrate that modulation of Target X in vivo (in this case through inhibition or inactivation) can produce a similar protective effect in the efficacy models.

In pursuit of this goal, the research team established a recombinant protein biochemical assay and a functional assay for Target X. The functional assay utilizes rodent albumin at a quantity which should be sufficient to account for nonspecific protein binding. Using compound screening, followed by some SAR optimization, a potential tool compound with encouraging potency (see Table 3.2) and drug-like properties has been identified. Kinetic assessment shows that the compound demonstrates rapid-reversible binding kinetics. The drug also has measurable activity against the related Target Y. Based on the biological function of Target Y, significant modulation could produce effects that confound the efficacy readout for Target X. Therefore, the potential for erosion of selectivity in vivo must be taken into account in any pharmacological validation experiment. Accordingly, rodent in vitro assays have been enabled to ensure that the level of in vivo TE for both Target X and Y can be reliably projected and interpreted in the efficacy studies. Interestingly, the rodent in vitro data demonstrate that the affinity of the drug for rodent Target X is five-fold weaker than human. However, the selectivity for rodent Target X vs Y is ten-fold greater than for human Target X vs Y, suggesting a greater potential for selective engagement of Target X in the preclinical efficacy POC experiment.

As an initial step in the process of in vivo characterization of the tool compound, a PK study was conducted in the rodent species to be used in the preclinical model. As shown in Fig. 3.9, a reasonable oral dose (10 mg/kg PO) produced an oral exposure curve which showed encouraging absorption but almost complete clearance from the plasma by 24 h. Combining this PK data with the EC_{50}s from either the biochemical assay (corrected by f_u) or the functional assay, TER values for Target X and Y at several time points can be projected (Table 3.3). Again, it is important to re-emphasize that for the purposes of the preclinical proof of concept, the f_u and assay values from the appropriate efficacy species should be used for projecting TERs in the design of the efficacy experiments. If the program is successful and continues forward, the human values will of course be critical for projecting human properties, prioritizing compounds, and ultimately selecting the best clinical candidate for patients.

Table 3.2 In vitro data for potential in vivo tool compound

Rodent $f_u = 0.18$	Biochemical EC_{50}	Functional EC_{50}
Target X	Human = 4 nM	Human = 20 nM
	Rodent = 20 nM	Rodent = 100 nM
Target Y	Human = 40 nM	Human = 200 nM
	Rodent = 2000 nM	Rodent = 10,000 nM

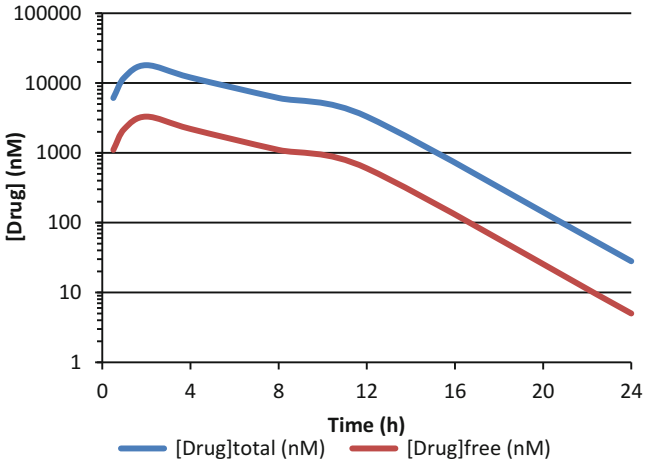


Fig. 3.9 Rodent PK for a potential tool molecule following a 10 mg/kg, PO dose

Based on the biological validation data (embryonic KO) and the duration of the in vivo experiment, evaluation of full sustained target engagement (TER $\geq$10) throughout the experiment is needed to drive a definitive decision on the pharmacological validation of Target X. However, to maximize the likelihood that any observed efficacy signal is coming primarily from modulation of Target X, a design that minimizes concomitant engagement of Target Y during the efficacy study is highly desirable. As shown in Table 3.3, the equations discussed previously were applied to project TERs based on the corresponding plasma concentrations from the PK experiment using measured rodent f_u and the rodent functional assay. In our research programs, we have found it advantageous to develop simple computational tools to facilitate the evaluation of potential tool molecules to explore the relationships between TE and biological activity in vivo. Software such as Microsoft Excel allows one to build spreadsheets from Eq. (3.4) to allow data from PK experiments, in vitro assays, and protein-binding assessments to be instantly converted to TERs. These same tools can also be used prospectively to determine what dose one expects will deliver the desired levels of target engagement. Simple computational tools like these allow chemists and pharmacologists to readily determine the suitability of tool experiments for specific studies and to identify what performance parameters might need to be improved. Similarly, we have built tools in our organization that can be used to determine the suitability of compounds for use in osmotic pump experiments from Eq. (3.12) (vide infra). Note that the functional and biochemical TER values closely agree. Therefore, to simplify the remainder of the example, we will only show functional TER values.

In assessing the data, it is clear that the potential tool compound could not sustain a sufficient TER at Target X with a single daily oral dose 10 mg/kg, as the 24 h TER is much less than 1. However, using the assumption that exposure will vary in a linear, dose-dependent fashion, the team can project other doses and

Table 3.3 Estimation of TERs for tool molecule vs Targets X and Y from rodent PK data

	10 mg/kg, PO		Target X		Target Y	
Time	$[Drug]_{total}$ (nM)	$[Drug]_{free}$ (nM)	$TER_{Functional}$	$TER_{Biochemical}$	$TER_{Functional}$	$TER_{Biochemical}$
0.5	6100	1100	61	55	0.61	0.55
1	12,000	2200	120	110	1.2	1.1
2	18,000	3300	180	170	1.8	1.7
4	12,000	2200	120	110	1.2	1.1
8	6100	1100	61	55	0.61	0.55
12	3300	600	33	30	0.33	0.30
24	28	5	0.25	0.25	<0.01	<0.001

Table 3.4 Projected PK and TER values (Targets X and Y) for dosing 100 mg/kg PO, QD, and 3 mg/kg PO, BID

	Projected exposure/TER: 100 mg/kg PO, QD			Projected exposure/TER: 3 mg/kg PO, BID		
Time	Total (nM)	Target X $TER_{Functional}$	Target Y $TER_{Functional}$	Total (nM)	Target X $TER_{Functional}$	Target Y $TER_{Functional}$
0.5	61,000	610	6	1833	18	0.2
1	120,000	**1200**	**12**	3667	37	0.4
2	180,000	**1800**	**18**	5500	**55**	**0.6**
4	120,000	**1200**	**12**	3667	37	0.4
8	61,000	610	6	1833	18	0.2
12	33,000	330	3	1000	10	0.1
24	280	3	0.03	2000	**20**	**0.2**

dosing paradigms for design purposes. Table 3.4 illustrates such a projection for the larger QD dose of 100 mg/kg. At this dose, the compound sustains a minimum TER of ~3 for 24 h; however, it comes at the cost of very high drug exposure at C_{max}, resulting in >50% engagement of Target Y for >12 h. Further, Target Y is inhibited by >90% for at least 4 h (see red boxes in Table 3.4).

To address excessive engagement of Target Y, BID dosing was evaluated. Inspection of the 12 h values in Table 3.3 reveals a TER of ~30 with the 10 mg/kg dose. This suggests that with BID dosing, the dose could be lowered by a factor of ~3 relative to 10 mg/kg and still sustain a TER ≥10. Accordingly, the dose of 3 mg/kg BID was projected. As the data in Table 3.4 illustrate, this dose/regimen is not only expected to sustain the desired TER for Target X over 24 h, but the %TE for Target Y should not rise to even 30% during the full course of the efficacy experiment (see green boxes in Table 3.4).

Figure 3.10 shows plots for all three of these experimental designs overlaid in one graphical representation. Such illustrations are often quite useful for comparing the relative strengths and weaknesses of design options prior to making the final selection of dosing conditions. It is important to note that this type of analysis is generally useful for choosing doses/regimens to explore the upper end of the in vivo dose response. However, if efficacy is observed, *it is equally important to explore the lower limits of efficacy detection as well*. Just as with in vitro concentration-response curves, evaluating the full range of the in vivo concentration response adds confidence that the observed pharmacology derives from the expected mechanism of drug action. Establishing the minimum effective level and duration of target engagement may also have important implications for the appropriate definition of safety margins should the team advance molecules on to toxicology studies [8]. Dose fractionation is one useful strategy to define the minimum level and duration of exposure needed to drive efficacy in vivo. Reference 8 (Tuntland et al.) has an overview of dose fractionation methodology with some additional leading references. PK/PD experts can help teams determine when and how to conduct such studies.

The preceding vignette illustrates that by using these design principles, the team was able to design a quality efficacy experiment to achieve their objectives. By

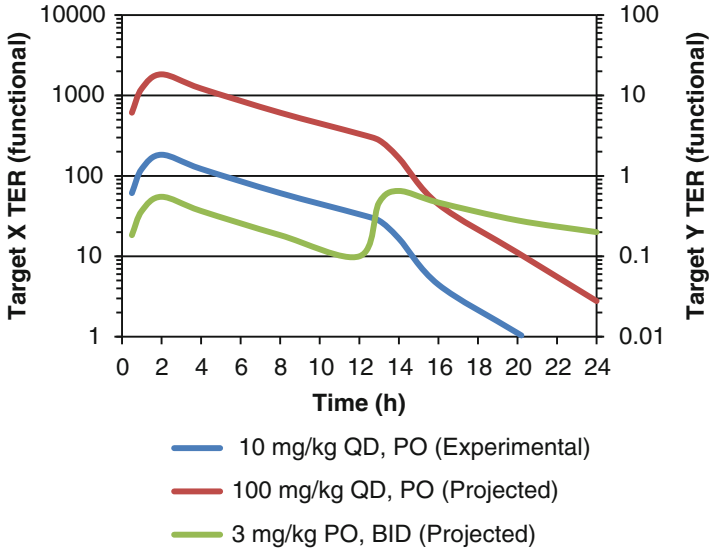


Fig. 3.10 Overlay of experimental design options: the time course of $TER_{functional}$ using different doses and dosing regimens

carefully considering the in vivo selectivity issues associated with Target Y, they minimized the risk of generating misleading efficacy data by using a too high dose. By exploring multiple dosing paradigms, they avoided spending additional time and resources in search of improved tool molecules with better selectivity versus Target Y, or with PK profiles having smaller differences in peak-trough ratio. Frequently, drug hunting teams face the challenge of needing to establish pharmacological validation for targets armed with tool molecules that may not be fully optimized in terms of potency and/or pharmacokinetic properties. In the next section, we address one approach that can help to expedite TV experiments using technology to achieve TE in vivo and overcome tool molecule limitations.

3.4.1 Compound Delivery via Pump as a Means to Facilitate Target Validation

Conducting in vivo studies to establish target engagement is a resource-intensive effort. In the best scenarios, compounds with a combination of suitable potency and pharmacokinetics are readily available. However, for many drug discovery efforts, available tool molecules are insufficient in terms of either potency or PK performance. Resolving those issues through SAR development requires the application of medicinal chemistry, ADME, and biology resources to identify suitable tools through iterative rounds of optimization. The resource requirements and timelines for these efforts can be significant. Therefore, any strategy a team can devise to

allow the use of "suboptimal" tool compounds can facilitate significant time/cost savings, especially if the TV exercise ultimately fails to validate the target.

Pump delivery presents a powerful tool for enabling interrogation of the relationship between PK and efficacy [9, 40–43]. There are multiple types of implantable infusion pumps that can be used. Some newer electronic pumps allow the control of compound delivery via a user input program. These programs can be designed to vary the rate of compound dosing over the course of the study, such as with cases in which dosing alignment with circadian cycles is desirable. Some pump systems can even be refilled during the course of an in vivo experiment. This can drastically extend accessible duration of infusion, eliminating the need for multiple surgeries which can compromise efficacy studies. Currently, these systems are just emerging, and we would anticipate that their use will continue to increase over time.

Nonelectronic osmotic pumps provide a less expensive but still powerful tool for conducting sustained delivery. Figure 3.11 shows a simplified diagram of how a mechanical osmotic pump works [40–43]. The pump consists of a drug reservoir that is made of an impermeable membrane. This is encased within a semipermeable membrane housing. Between the two membranes is an osmotic engine that acts to push fluid out of the outlet at one end of the pump. For use in vivo, the pump reservoir is filled with drug solution, and the pump is implanted into the test animal, under the skin in the subcutaneous space. Water from the animal tissues slowly diffuses through the semipermeable membrane into the osmotic engine. This causes the volume of the engine to increase which in turn applies force against the drug reservoir. This pushes the drug solution through the outlet. Usually, after a short induction period, this reaches a steady rate that continues until all the pumping capacity has been exploited. Time ranges for infusion are dependent on the type and size of the pump used but can last from days to up to 4 weeks.

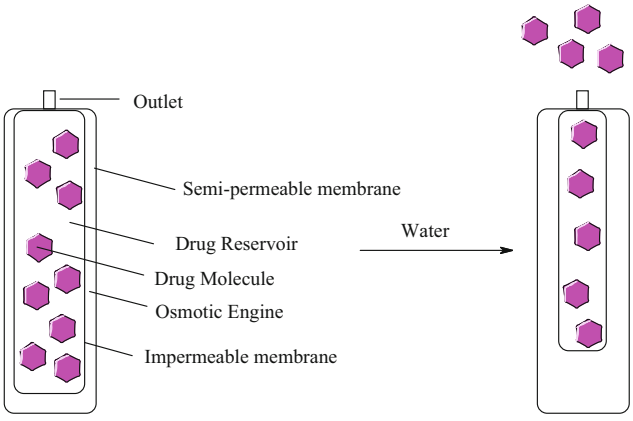


Fig. 3.11 Diagram of an osmotic pump

3.4.2 Designing an Osmotic Pump Study

To conduct a continuous infusion study using an osmotic pump, several design factors need to be considered. Most importantly, the team needs to define the concentration of drug in plasma needed to achieve the desired TE in the target compartment. Once the desired plasma level is defined, studies can be designed with a few simple pieces of experimental data. Steady-state plasma concentrations can be projected with the following information:

1. The IV clearance of the drug in the efficacy species.
2. The maximum solubility of the drug in an acceptable vehicle.
3. The manufacturer's rating information for the available pumps, which includes the infusion rates, reservoir volume, and maximum duration of use.

From this information, a researcher can determine if any available osmotic pump will be acceptable for the study design. Using the desired steady-state plasma levels of drug and the clearance, one can determine the necessary level of solubility using the following equation:

$$\frac{C_{ss} \times \mathrm{Cl} \times W}{R_i} = S \tag{3.10}$$

where C_{ss} is the desired plasma steady-state level, Cl is the IV clearance, W is the body weight of the animal, R_i is the rate of infusion of the pump, and S is the concentration of the drug solution in the pump [9]. If we replace L in Eq. (3.3) with C_{ss}, we can then solve for C_{ss} and substitute this expression into Eq. (3.10) to give the following modified equation:

$$\frac{\left[\frac{\mathrm{TER} \times \mathrm{EC}_{50}}{f_u}\right] \times \mathrm{Cl} \times W}{R_{\mathrm{infusion}}} = S \tag{3.11}$$

Note that as discussed above, in cases where the in vitro assay incorporates nonspecific protein binding in the experiment, application of f_u is not appropriate, and the equation simplifies to

$$\frac{\mathrm{TER} \times \mathrm{EC}_{50\mathrm{functional}} \times \mathrm{Cl} \times W}{R_{\mathrm{infusion}}} = S \tag{3.12}$$

Using the above approach, tool compounds and experimental designs can be evaluated prior to costly in vivo efficacy experiments.

Thus, if a potential in vivo tool compound is identified, the team needs to measure the in vitro potency and the IV clearance (both in the efficacy species) as well as the solubility limit in the vehicle anticipated for pump use. With this information, an in vivo concentration-response study such as the one illustrated in Fig. 3.12a can be designed. First, Eq. (3.10) can be applied to project the upper limit of accessible steady-state exposure and thus the upper limit of the TER that can be

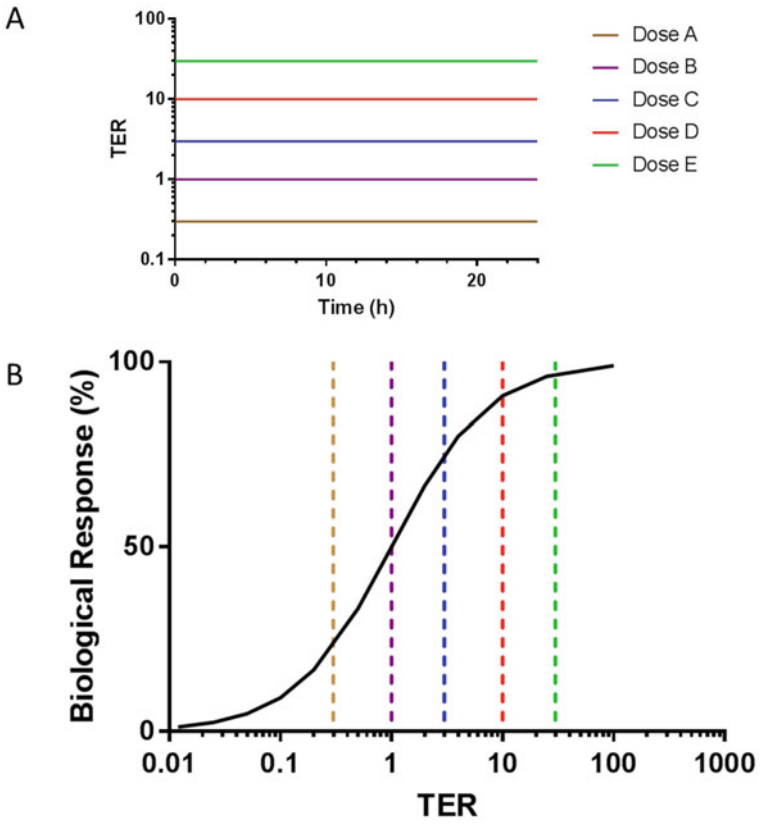


Fig. 3.12 Biological response vs target engagement ratio experimental design. (**A**) TER vs time for an infusion pump multidose study. (**B**) Plot of Biological Response (%) vs TER overlaid with the theoretical TERs from the doses illustrated in panel **A**. A dose-response experiment achieving these TER values would provide a sufficient number of points to enable estimation of the concentration-response curve in vivo

sustained with the tool compound being considered. If this step is encouraging, the design can be completed by adding lower doses in half log units to explore the lower levels of the in vivo concentration-response curve. Figure 3.12b illustrates the application of the strategy to a hypothetical efficacy experiment and shows how the PK might translate in vivo.

Clearly the use of pump technology has a number of practical advantages for the design and execution of efficacy studies. By eliminating the variation of exposure levels during the course of the study, it enables a very clear definition of the relationship between steady-state drug concentration and the associated biological response. Further, this method minimizes the potential for erosion of selectivity in vivo which can come from large increases in peak vs trough drug exposure with oral administration. For chronic (multiple days) efficacy models, pumps also eliminate the need for oral dosing. This can be particularly valuable for some

models in which repeat oral dosing can result in significant stress for the study animals and compromise experimental results.

However, the use of pump technology is not ideal for every application, as there are caveats. For example, severe solubility limitations of drug molecules may eliminate them as candidates for pump studies, or may cause pumps to fail during efficacy experiments. Also, some in vivo efficacy protocols are not compatible with the need for implant surgery. In such cases, administering drug with in-food or in-water formulations can also be a useful, practical alternative [44]. However, the potential benefits of both accelerating proof-of-concept studies and achieving high-quality in vivo concentration-response relationships suggest this technology is an important strategic option that teams should aggressively consider in the early prosecution of their drug discovery programs.

3.4.3 Approaches to Measuring Target Engagement In Vivo

The previous sections describe methods for estimating in vivo TE based on the combination of quality in vitro and PK data. Often, such methods are the only practical options available for research teams. However, when feasible, the experimental demonstration of TE is a powerful method for building confidence in the analysis and interpretation of efficacy experiments. In this section, we highlight a few of the emerging methods for biophysical characterization of drug-target complexes in vivo.

Cellular thermal shift assay (CETSA) is a relatively new biophysical technique that allows assessment of TE in both cells and animals [45–50]. This approach relies on the same thermal stability assay used broadly in structural biology to assess compound binding to proteins. In a CETSA experiment, cells or animals are dosed with compound or vehicle. Cells/tissues are then harvested for analysis from each group. Cell aliquots from these harvested pools are then incubated at various temperatures for a fixed time. Under heating, native proteins not associated with the compound denature and precipitate at lower temperatures than those bound to compound. The cells are lysed and the amount of soluble target protein remaining is quantified by immunoblotting. The quantity of soluble target protein then provides an indication of TE. Measurement of TE in a dose-responsive fashion, referred to as $ITDRF_{CETSA}$, has also been achieved.

The developers of CETSA demonstrated its value by comparing the clinical PARP-1 inhibitors iniparib and olaparib [48]. Iniparib failed to meet phase 3 efficacy endpoints, while olaparib was recently approved by the FDA. Using CETSA the authors demonstrated that iniparib did not engage PARP-1. Conversely, olaparib did show positive TE of PARP-1 in the CETSA assay. These results suggest that iniparib may act through an alternative mechanism.

Recent coupling of the CETSA approach to tandem MS capabilities has resulted in a technique dubbed thermal proteome profiling (TPP) (Fig. 3.13) [51–53]. This variation leverages tandem mass tag (TMT) labeling. The power of this approach is

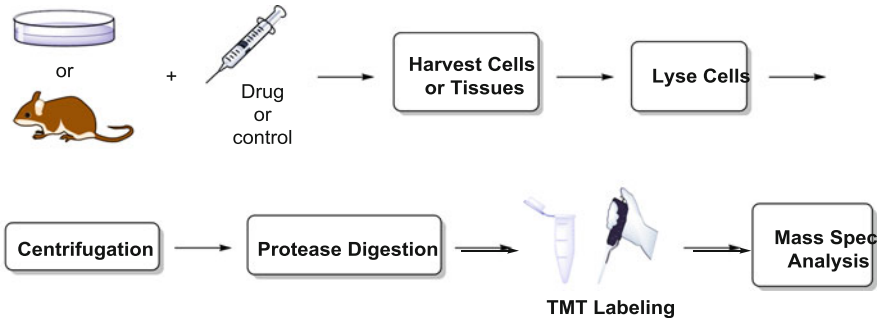


Fig. 3.13 Process diagram for thermal protein profiling

it allows assessment of TE for a given compound within the entire measurable proteome. Briefly, cells are treated with compound or vehicle and then heated and lysed, and soluble protein fractions are separated as in the previous CETSA approach. The difference is that rather than immune quantifying the protein using a target-specific antibody, the lysates are digested with a protease and labeled with TMT reagents. Mass spec analysis of the TMT-labeled peptides from treated versus control groups then allows identification of the proteins interacting with the target using standard proteomics methods.

An alternative to direct biophysical characterization is to measure target occupancy by competition with a tracer ligand of known affinity for the drug target. Positron emission tomography (PET) imaging and LC MS/MS techniques have proven to be highly valuable examples of this approach. PET is a particularly powerful technique because it enables direct assessment of target occupancy in vivo [54–62]. For example, PET has been a powerful tool for the clinical evaluation of TE in patients, particularly in the CNS. However, it requires the development of radioligands (with the radiolabel introduced at a very late stage in the synthesis) and access to specialized equipment, which increases the cost of enablement. During a PET experiment, the study drug is administered to the subject followed by a radiolabeled ligand which then competes for the target. The level of target occupancy can then be assessed by radiographic imaging. PET ligands need to meet specific pharmacokinetic and potency criteria. This often requires investment of medicinal chemistry resources to develop and characterize suitable ligand candidates.

LC MS/MS techniques provide a lower-cost option to assess target engagement that might be more attractive for early preclinical work, especially when target validation has not been confirmed [54]. These methods have advanced significantly in recent years. LC MS/MS works similarly to PET but does not require the synthesis of radioligands. This allows it to be easily incorporated into standard medicinal chemistry programs as a parallel activity. This technique has been used in many drug discovery efforts for both GPCRs and enzymes [54, 63–65].

Approaches to measuring target engagement using specially designed chemical agents have also been developed [1]. This can be a powerful method to assess TE,

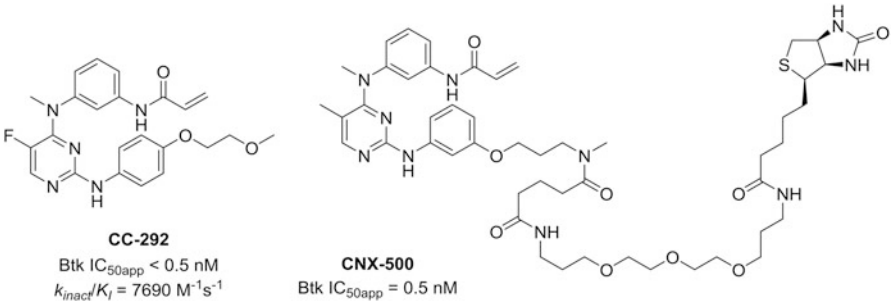


Fig. 3.14 Btk inhibitor **CC-292** and tool compound **CNX-500**

and recent examples of the use of this approach with covalent irreversible kinase inhibitors have been described. These approaches can be applied at any point in a project life cycle, from early discovery efforts into the clinic. However, the methods require the development of chemical tools.

As a recent example, Bruton's tyrosine kinase (Btk) is a target of interest for autoimmune and B-cell malignancies [66]. Because cysteine 481 in Btk is not conserved in many other kinases, inhibitors which can react with this cysteine have been pursued as an alternative to reversible ATP competitive inhibitors. Celgene has disclosed a clinical candidate, **CC-292**, which uses this approach [66]. To measure TE with **CC-292**, Celgene developed **CNX-500** which is a biotinylated inhibitor capable of competing with CC-292 for Btk (Fig. 3.14) [66]. **CNX-500** has been used to assess target engagement in both mice and human subjects. Plasma B-cells of treated animals/patients are isolated. Treatment of the lysate of these B-cells with **CNX-500** inactivates any Btk not already covalently bound to **CC-292**. The **CNX-500** ligated protein is then captured and quantified using ELISA. Using this approach Celgene was able to establish timelines for Btk protein turnover, **CC-292** PK-PD relationships, and **CC-292** target engagement efficacy relationships in a collagen-induced arthritis model in mice.

3.4.4 The Relationship of TE to Pharmacodynamics

As discussed previously, the characterization of TE in vivo links the time course of drug concentration (PK) with the time course of target binding. Although methods for the direct measurement of in vivo TE continue to advance, most frequently TE is inferred based on the measurement of some resulting biological activity, or pharmacodynamic activity (PD) [8]. The characterization of PK/PD relationships and the construction of mathematical models to describe them represent a challenge of sufficient detail and scope that it comprises an independent discipline unto itself. Numerous sources are available to provide a thorough and effective treatment of the fundamental principles of PK/PD and their application to drug discovery, and it is

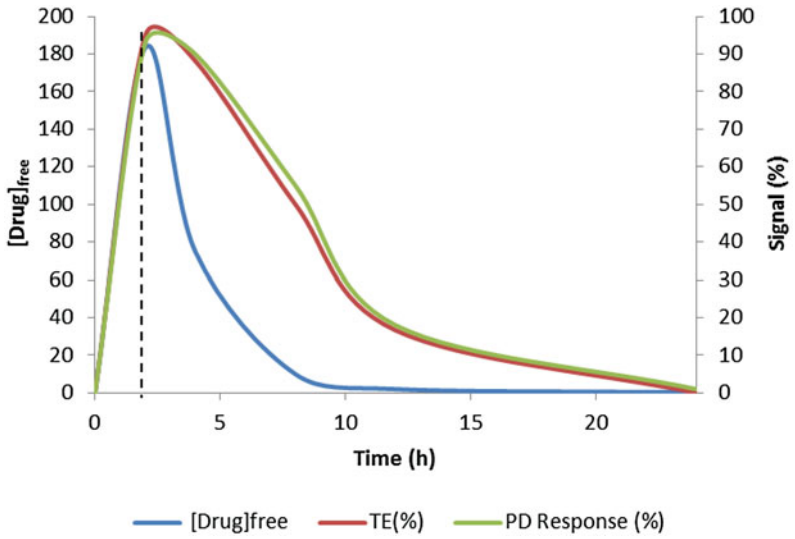


Fig. 3.15 Time course of drug exposure and TE and PD response for a direct concentration-response system

certainly not our intention to try to exhaustively review that work [8, 64, 67–69]. Actually, the practical purpose of learning to understand the time course of TE is to gain greater control over the modulation of biological activity via drug action. So to some, distinctions between the time course of TE and PD may seem rather arbitrary. However, the terms are sometimes used interchangeably (mistakenly), and there are some subtle yet important differences between the two that can impact study design, interpretation, and iteration. Therefore, we highlight here a few key terms and principles to consider in the design of efficacy experiments.

Several factors combine to determine how the time course of an in vivo biomarker readout may change in response to changes in drug concentration. Two examples include (1) the time required for signaling between the point of TE on the biological pathway and the biomarker being measured and (2) the rate at which that biomarker is cleared from the compartment of measurement. If the transmission of the biological signal being measured in vivo occurs quickly, relative to changes in drug exposure, a so-called direct concentration-response relationship between PK and PD will be observed [8]. Figure 3.15 shows a hypothetical example of such a profile, illustrating the relationship between a PK curve (blue) and both a TE curve (red) and a resulting PD response curve (green). In this case, the interaction of the drug with its biological target is characterized by rapid-reversible binding kinetics. Thus, as shown previously in Fig. 3.6, changes in the time course of TE respond rapidly to changes in the time course of drug exposure. Subsequently, due to the rapid transmission of TE into a biological signal, changes in PD activity for this system basically mirror changes in TE. Direct concentration-response systems have the practical advantage that experimentally they are more straightforward to characterize in vivo relative to corresponding indirect systems.

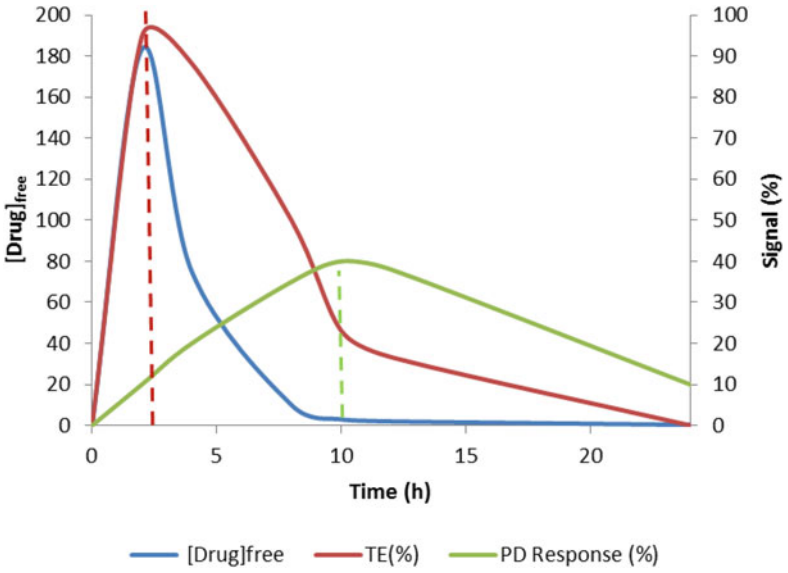


Fig. 3.16 Time course of drug exposure, TE and PD response for an indirect concentration-response system. In this example, the drug and target are the same as those represented in Fig. 3.15; however, the PD biomarker has a reduced clearance rate

On the other hand, if transmission of the biological signal being measured is slow relative to changes in drug levels, an apparent disconnect or hysteresis is observed, between the time course of drug exposure (PK) and the time course of the corresponding PD readout [8]. One example of such an "indirect" PK/PD relationship is shown in Fig. 3.16. In this example, both the drug molecule and the biological target are the same as from the example in Fig. 3.15 (note that the time course of both drug exposure and TE are identical in the two figures). In fact the *only* difference in the example depicted in Fig. 3.16 is a reduction in the clearance rate of the biomarker generated in the process. The figure clearly illustrates the difference between the time course of drug exposure (also TE) and the time course of PD that results from this subtle change in the system.

Figure 3.16 also highlights the significant risks inherent in evaluating PK/PD at single time points in such a system. The red dotted line illustrates the time point associated with the C_{max} for drug exposure. The C_{max} is frequently selected as the time point to evaluate in the design of single time point efficacy screens. However, in this case, due to the slow equilibration of the biomarker, the amount of change in the biological signal observed at C_{max} would appear insignificant, in spite of the fact that TE in this system is effectively saturated at that specific time point. Conversely, if the team evaluated the PK and PD at the 10 h time point (green dotted line), in this system it would appear that a significant biological effect was observed, but with an insignificant drug concentration (and actually at an insignificant level of TE).

Of course there are numerous different experimental conditions that could produce indirect concentration-response relationships. As discussed previously, the effect of slow dissociation of the drug from the target protein (Fig. 3.8) is such an example. Thus, this section underscores again the importance of understanding the full time course of both PK and PD, through the full range of TE, as early in projects as possible. If the system under investigation has a direct PK/PD relationship, it should be relatively straightforward to build in vivo relationships linking TE with efficacy. If not, the team should be sure to seek collaboration (at the very least consultation) from a PK/PD professional.

3.4.5 Case Studies in Using TE

In this section we highlight two examples of drug discovery projects in which TE analysis is used to address key questions regarding pharmacological target validation. In one example, efficacy experiments are designed to provide insight into the in vivo function of insulin-degrading enzyme (IDE), a target of interest for diabetes. In the second example, an in vivo concentration-response relationship is developed for an osteoarthritis (OA) drug program, and that data is used to refine the desired performance characteristics to focus SAR efforts toward the selection of a clinical candidate.

3.4.5.1 Application of TE in a Program Exploring Insulin-Degrading Enzyme as a Potential Target for Insulin Sensitization [70]

Insulin-degrading enzyme (IDE or insulysin) is an evolutionarily conserved zinc metalloprotease belonging to the cryptidase family [71]. Members of this protease family contain a large active site (~15,700 $Å^3$) referred to as a crypt, which can fully enclose substrates. In vitro, IDE binds and cleaves a diverse array of substrates, including insulin, glucagon, amyloid beta-peptide (Aβ1–40 and Aβ1–42), ubiquitin, amylin, insulin-like growth factor II, atrial natriuretic peptide, and transforming growth factor alpha [71–79]. Of its many substrates, IDE is exceptionally effective at degrading insulin ($K_m = 85$ nM and $k_{cat}/K_m = 2.42$ min^{-1} μM^{-1}) [74].

Although significant biochemical characterization of IDE had been completed, the role of IDE in the physiological regulation and action of insulin had not been clearly defined at the time we became interested in the target. The majority of prior efforts exploring the in vivo role of IDE came from gene deletion studies. Several research groups have evaluated $IDE^{-/-}$ mice, but the phenotypes reported for the knockouts have varied. In some $IDE^{-/-}$ mouse cohorts, increased insulin levels were observed, but in other cohorts no change in insulin levels occurred [80–82].

Because IDE had been claimed to be the primary regulator of insulin clearance in vivo, we hypothesized that inhibiting IDE activity could reduce insulin

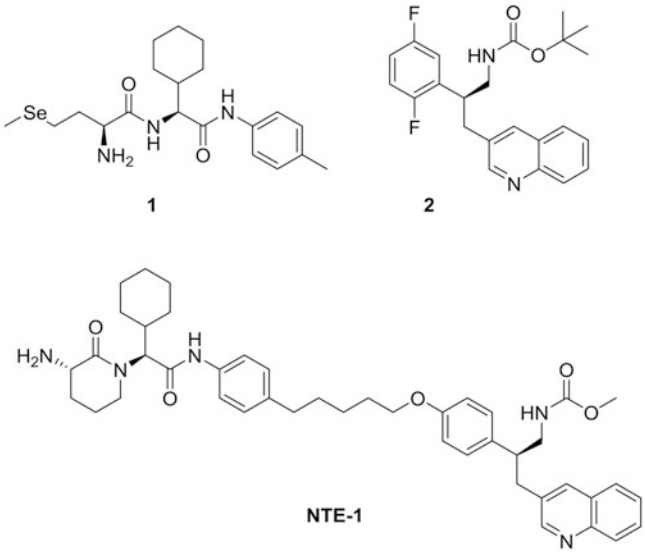


Fig. 3.17 IDE inhibitors

breakdown and prolong insulin action (i.e., IDE inhibitors would act as insulin sensitizers) [70]. At the time our work was initiated, suitable pharmacological inhibitors were not available for in vivo studies, and thus tool molecules needed to be identified. In support of the program, a biochemical assay and a cell lysate assay of insulin degradation were developed. This cell lysate assay served as a functional assay and contained large amounts of protein, allowing assessment of inhibitor activity within an environment where an opportunity for substantial nonspecific protein binding existed.

To identify small molecule inhibitors, a medium throughput screen was conducted, which yielded two weak partial inhibitors (compounds **1** and **2**) that were successfully co-crystallized with IDE (Fig. 3.17). Based on the binding location of the two molecules in proximal exosites on the protein, compound **NTE-1**, a potent, full inhibitor of insulin degradation was designed (Fig. 3.17). **NTE-1** is a reversible tight-binding inhibitor (Table 3.5) and fortunately has suitable PK properties in rodents to support in vivo studies using SC dosing. This compound also had equivalent potency in mouse, rat, and human enzyme systems.

Treatment of DIO mice with **NTE-1** followed by oral glucose challenge resulted in lower glucose excursion, increased plasma amylin, and slightly increased insulin (Fig. 3.18). Plasma TER levels >10 (based on the lysate assay) were sustained over the time course of the experiment, suggesting ≥90% inhibition of IDE function in vivo.

These initial results were encouraging because glucose excursion in the treatment arm was lower in the oral glucose tolerance test (OGTT), as would be expected with an insulin sensitizer. However, the fact that insulin changes were

Table 3.5 IDE inhibitor characterization

Compound	1	2	NTE-1
hIDE insulin IC_{50} (nM)	2000	2000	4 ± 2
Rat hepatocyte lysate insulin degradation IC_{50} (nM)	ND	ND	18
k_{off} (min^{-1})	ND	ND	0.0047
$t_{1/2}$ (h)	ND	ND	2.45

ND = not determined

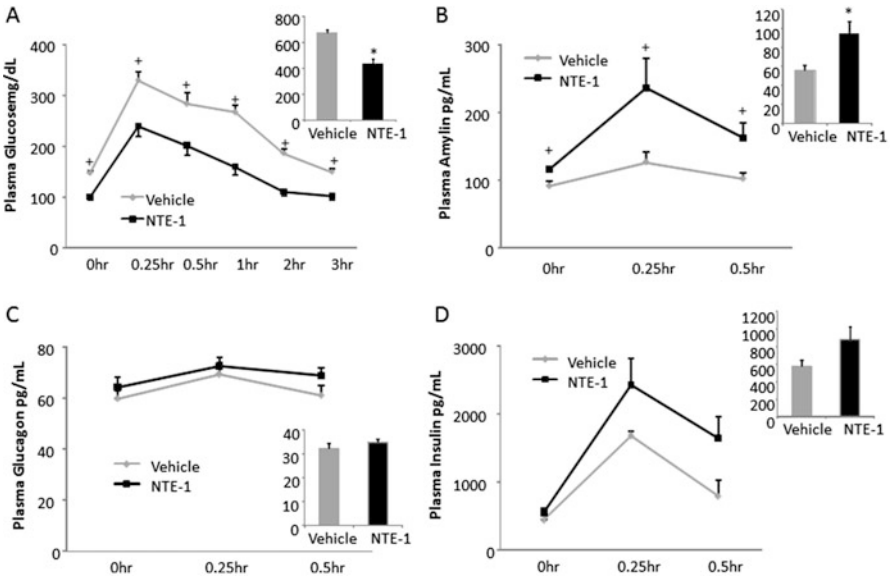


Fig. 3.18 Effects of **NTE-1** treatment on plasma glucose, amylin, insulin, and glucagon in DIO mice after an oral glucose challenge. Fasted mice received a 15 mg/kg dose of **NTE-1** SC followed by an oral glucose load. **NTE-1** treatment produced statistically significant increases in glucose clearance and plasma amylin levels. Plasma insulin was elevated but did not reach statistical significance. Inset graphs represent analyte AUC. (**A**) Whole blood glucose; (**B**) Amylin; (**C**) Glucagon; (**D**) Insulin. + = $p < 0.05$ vs vehicle by RM-ANOVA and * = $p < 0.05$ relative to vehicle by Student's *t*-test

not statistically significant was concerning. Additionally, amylin (which is also an IDE substrate) is known to slow gastric emptying which could suppress glucose excursion [72, 74, 83]. Thus, while we had evidence of TE, the relative contributions of IDE inhibition to glucose lowering via amylin and insulin could not be determined in this experiment.

Therefore, we conducted a euglycemic clamp study in SD rats to allow us to focus solely on the impact on insulin action of IDE inhibition. The design of this clamp study is shown in Fig. 3.19. In this experiment, SD rats were surgically fitted with catheters to allow infusion of multiple substrates. After recovery from surgery, the rats were connected to infusion pumps that delivered a sustained level of drug or

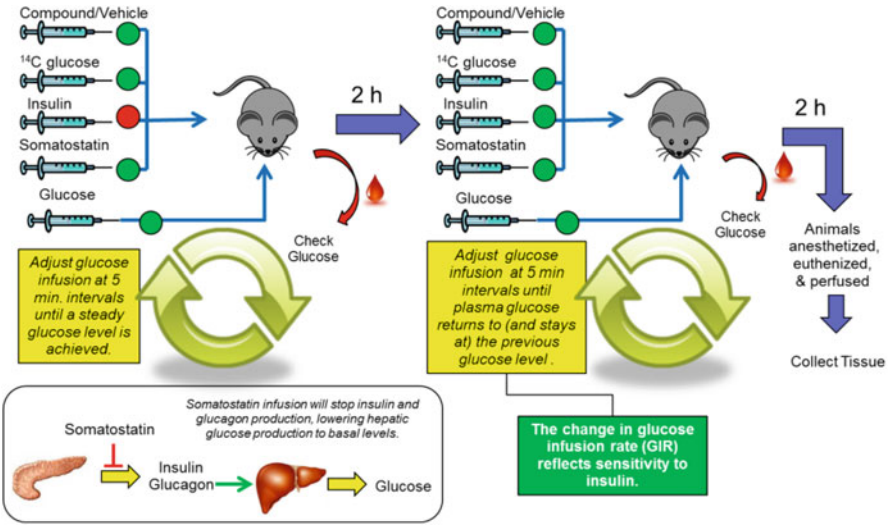


Fig. 3.19 Euglycemic clamp study

vehicle in addition to radiolabeled glucose (to allow measurement of hepatic glucose production (HGP)) and somatostatin (to suppress endogenous insulin and glucagon production). The dose of **NTE-1** was chosen to sustain a TER of $\geq$130 and 110 in the biochemical and cell lysate insulin degradation assays, respectively. In this case, we selected this high target engagement goal because the likelihood of confounding pharmacology due to inhibition of other cryptidases was anticipated to be very low [71].

Once the fixed infusion was started, a second adjustable pump was used to deliver glucose. The glucose infusion rate (GIR) was adjusted until the animals' plasma glucose was constant at 105 mg/dL. At this point, a fixed dose of insulin was added to the steady infusion pump, causing the animals' plasma glucose to drop. The glucose infusion rate was then increased until the animals once again had a steady plasma glucose level of 105 mg/dL. By comparing the difference in GIR and/or HGP in the stabilized/clamped animals before and after insulin challenge, any effect of **NTE-1** on reducing insulin clearance or enhancing insulin action can be quantified. To ensure that the compound had inhibited IDE, we measured **NTE-1** concentration in the liver at the termination of the experiment and found that the liver concentration was ~70 mg/g (approximately 95 mM). TE was further supported by strong inhibition of insulin degradation in liver lysates made from tissues of the treated animals (Fig. 3.20b).

Notably, **NTE-1** did not have any impact on insulin action or clearance in this experiment as revealed by assessment of the GIR or HGP (Fig. 3.20a and b). Because of our confidence in the TE we achieved in this experiment, we concluded that the majority of the impact on glucose we observed in our OGTT studies were driven by inhibiting IDE-mediated amylin clearance. Based on these data, IDE

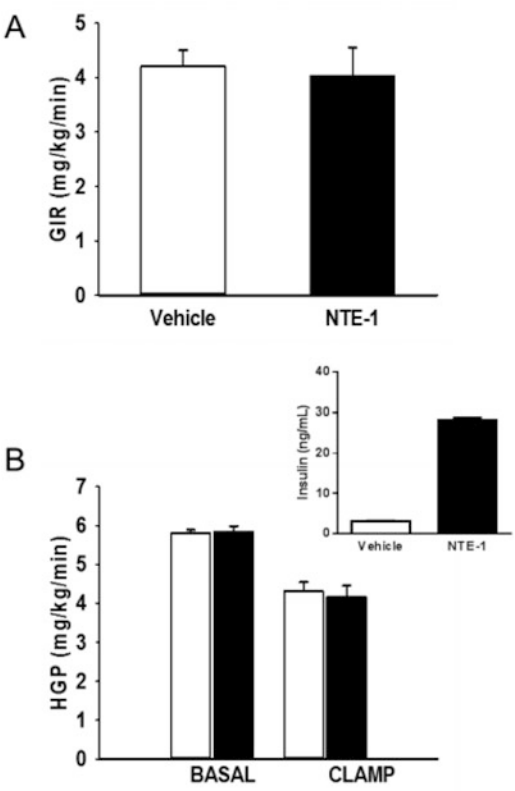


Fig. 3.20 **NTE-1** treatment effects in a euglycemic clamp in Sprague Dawley rats. (**A**) Average glucose infusion rate (GIR) during the last hour of a euglycemic clamp. (**B**) Hepatic glucose production (HGP) during basal and euglycemic clamp is shown. Basal and clamp measurements were obtained after 2 or 4 h infusions of vehicle or **NTE-1**, respectively; *Inset*: Insulin degradation in lysates from perfused livers harvested from animals after completion of the euglycemic clamp. **NTE-1** treatment resulted in significant preservation of exogenously added insulin relative to vehicle

inhibitors would *not* be anticipated to be effective insulin sensitizers [70]. Thus, a quality data package was generated from a tool molecule with poor ADME properties relative to what would be expected for a candidate for diabetes therapy. This enabled a decision to deprioritize further investment in the development of this target in favor of efforts with a higher probability of technical success.

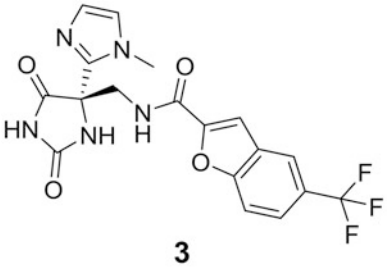
3.4.5.2 Use of TE to Establish Clinical Candidate Performance Characteristics for Aggrecanase Inhibitors as Disease-Modifying Treatments for Osteoarthritis [9, 84]

Osteoarthritis (OA) is a disease characterized by the degradation of joint cartilage leading to pain and loss of function. The societal impact of this disease is significant, including a major economic burden on healthcare systems and compromised quality of life for patients. Current treatments for OA include symptom relief (NSAIDs) and surgical joint replacement. Unfortunately, no treatment regimen which can directly affect the progression of OA has yet been approved.

At the structural level, the joint contains both cartilage and synovial fluid, a non-Newtonian fluid composed of water, hyaluronic acid, and lubricin. The process by which nutrients and waste products are exchanged between plasma and the joint space is believed to be diffusion controlled [85]. Cartilage is a nonvascularized tissue composed of type II collagen and aggrecan. One hypothesis for OA progression is that the rate of extracellular matrix (ECM) synthesis versus degradation becomes unbalanced. Therefore, it has been hypothesized that ECM protease inhibitors could halt or reverse the progression of OA.

ADAMTS-4 and ADAMTS-5 (aggrecanase 1 and aggrecanase 2) are zinc metalloproteases that are known to have a specific and primary role in aggrecan degradation [86–91]. The hypothesis that inhibition of ADAMTS-4 and ADAMTS-5 in humans could impact OA is supported by data generated in genetically modified mice (animals with either ADAMTS KO or stabilized aggrecan substrate) as well as in human chondrocytes [91–94]. Notably, the active sites of ADAMTS-4 and ADAMTS-5 share significant active site similarity with the matrix metalloproteases (MMPs) [96]. There are 28 MMPs known, all of which have a high level of homology at the catalytic site. In fact, several MMPs have been described as ECM proteases and have been nominated as potential drug targets for OA. However, due to the significant selectivity challenges associated with developing small molecule inhibitors of this class, off-target toxicity has been a barrier to clinical success to date. Therefore, a drug discovery effort to develop inhibitors of any member of this enzyme class would need to have high MMP selectivity, to facilitate a quality test of the efficacy hypothesis, and to avoid potential off-target toxicology.

Our efforts to discover aggrecanase inhibitors led to a series of hydantoins found to be potent, dual inhibitors of ADAMTS-4 and ADAMTS-5 with high MMP selectivity (Fig. 3.21). Compounds such as **3** were found to be efficacious in an acute PD model (mono-iodoacetate, MIA) and in a more resource-intensive surgical efficacy model of OA, with joint damage induced by meniscal tear [84]. Having



3

hADAMTS-4 IC_{50} = 4 nM
hADAMTS-5 IC_{50} = 4 nM
rat Plasma hADAMTS-5
IC_{50} = 35 nM
hMMP IC_{50}s > 10000 nM

Fig. 3.21 Aggrecanase inhibitor 3

established a qualitative linkage in vivo between PD activity in the MIA model and efficacy in the surgical model of disease, we wanted to develop a more quantitative relationship between TE parameters and PD activity in the MIA in order to more effectively define performance characteristics for compounds that would be desirable for clinical study [9].

To that end, rat oral exposure data were collected for compound **3**, as well as several analogs, in order to enable the TE-based design of MIA experiments. As the representative data in Fig. 3.21 illustrate, in vitro potency was measured in the presence of rat plasma in order to obtain a functional assessment of the drug concentration needed to overcome the relevant nonspecific protein binding in vivo. To ensure that species selectivity of target affinity was accounted for, rat ADAMTS4 potency was evaluated for all compounds studied in vivo and was found to be indistinguishable from the corresponding human potency. Because synovial fluid is generally known to present low barriers to the passive permeation of small molecules and is not under the influence of active transporters, the free drug hypothesis was applied [85]. Thus, plasma levels from the rat PK studies were used in combination with rat plasma IC_{50}s to approximate TERs in the target compartment. Finally, due to the fact that the MIA model requires several days of drug treatment, 24 h time intervals were considered as the relevant time course for TE in the experimental design.

As shown in Table 3.6, at a dose of 10 mpk/PO compound **3** produces a TER of >200 at C_{max}, but by 8 h the TER dropped to ~6. Thus, in order to ensure that TE would be sustained over each 24 h period during the time course of the efficacy study, BID dosing was indicated. Using this design strategy, compound **3** and several similar analogs were evaluated in the rat MIA model of cartilage degradation. In this assay, animals are injected with MIA which causes protease release into the synovium. After an incubation period, animals are treated with compound BID, PO for 4 days. Sacrifice of the animals 4 h post final dose and lavage of the knee allows assessment of aggrecan degradation by NITEGE fragment quantification using ELISA. As can be seen in Fig. 3.22, this study design yielded a range of PD activities which gave reasonable correlation to the TE in plasma.

Based on the evaluation of this data set, the MIA EC_{50} appears to correspond to a TER of ~150, when measured at a single time point of 4 h. However, inspection of the full PK curves for the compounds in Fig. 3.22 shows that the plasma concentrations of the inhibitors are expected to differ by >2 orders of magnitude between C_{max} and C_{min} over the course of the efficacy experiment [9]. Thus, in order to eliminate the significant variation in exposure levels and to minimize any potential

Table 3.6 Rat pharmacokinetics[a]

Compound	Rat plasma hADAMTS-5 IC50 (nM)	AUC (nM h)	C_{max} (nM)	Target engagement ratio at C_{max}	8 h plasma (nM)	8 h target engagement ratio
3	35	20,000	7600	220	220	6.3

[a]Data based on a 10 mg/kg PO dose in Lewis rats

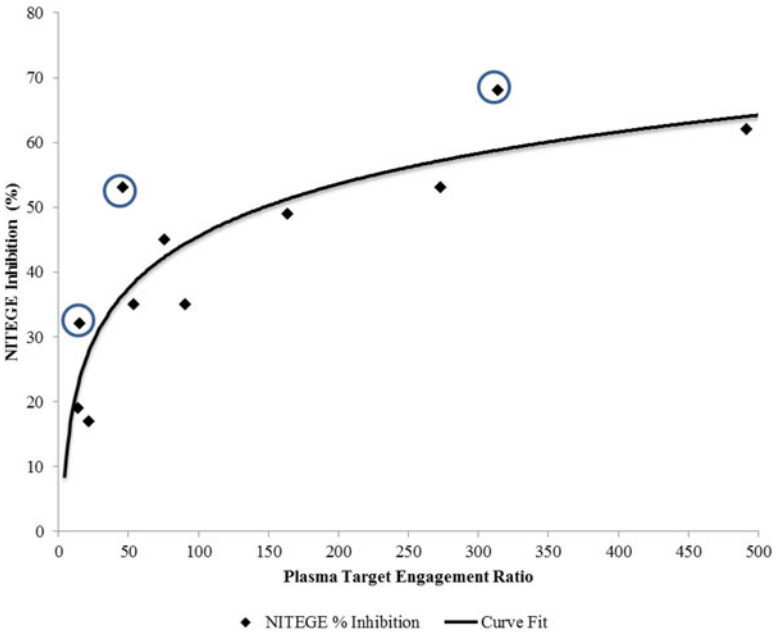


Fig. 3.22 MIA NITEGE inhibition versus target engagement ratio at 4 h. Data shown is pooled from dose-response experiments using compound **3** (highlighted with *blue circles*) and several analogous inhibitors of similar structure, potency, selectivity, and PK properties

for erosion of selectivity vs MMPs in vivo, an infusion pump design was evaluated. Compound **3** was found to have solubility (30 mg/mL in PEG300) and rat IV clearance properties making it ideal for use in such an experiment. It also showed in lab simulation that it maintained consistent release from the osmotic pump over the required dosing period. Thus, for the TE/PD study design, four dose groups were utilized, with a high dose of 30 mg/mL followed by half log reductions down to a low dose of 1.44 mg/mL. This dose range was estimated to provide a range of steady-state TERs from ~100 down to ~3, respectively.

As shown in Fig. 3.23, osmotic pump infusion of compound **3** produced consistent plasma levels throughout the time course of the MIA experiment. A plot of the PD activity versus the steady-state TER of compound **3** produces a logarithmic curve (Fig. 3.24, $R^2 = 0.8$). Thus, from these experiments we concluded that the high TERs achieved at C_{max} in the previous oral experiments were not required to drive PD efficacy. Further, the role of in vivo selectivity erosion was eliminated as contributing factor as well, as estimates of in vivo TERs for all other MMPs measured never rose above ~0.01 over the course of the study using the pump design. In fact, a compound capable of sustaining a minimum TER in plasma $\geq$10 will produce an ED_{50} effect in the MIA model. This is particularly noteworthy since ED_{50}s in the MIA model were found to correlate with statistically significant improvement in total joint score in the challenging surgical model [84].

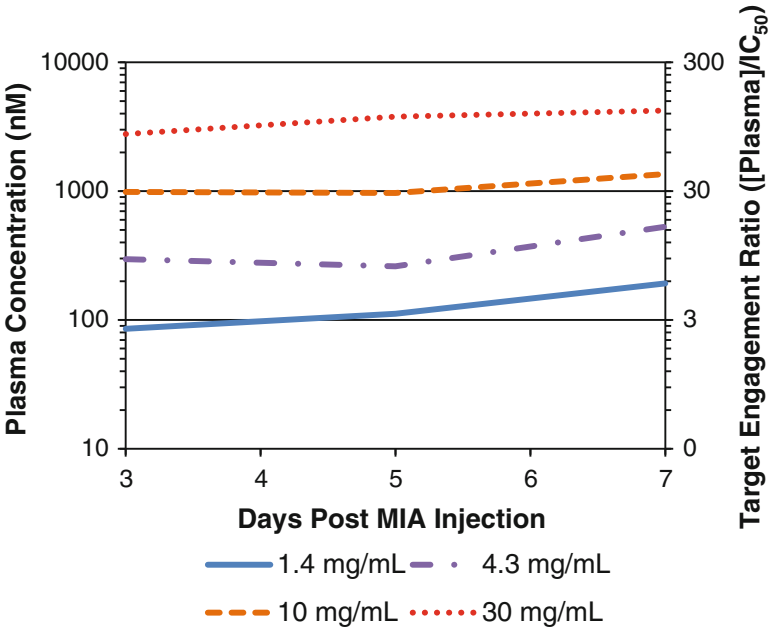


Fig. 3.23 Plasma levels of compound **3** versus time using infusion pump dosing

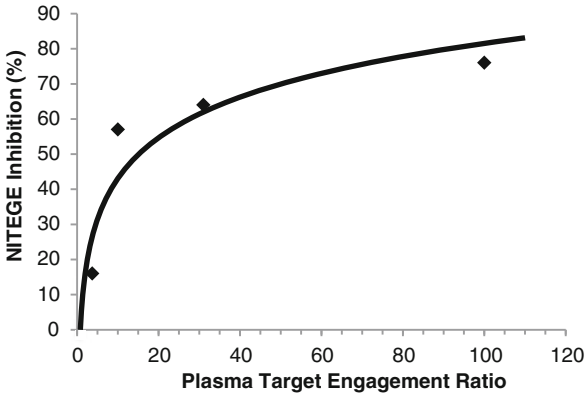
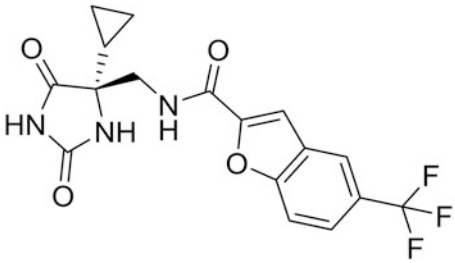


Fig. 3.24 Compound **3** infusion pump MIA study

Based on this analysis, further SAR efforts were focused on the development of an inhibitor with a combination of human potency and projected human ADME properties sufficient to sustain a free drug concentration $\geq$ 10 times above the human biochemical ADAMTS-4 and ADAMTS-5 IC_{50}, with an attractive projected oral dose QD in man. This strategy led to the identification of compound **4** (Fig. 3.25), which showed an attractive combination of potency, selectivity, MIA efficacy, and projected human PK properties supporting the prediction that it would deliver the desired level of human TE at a dose of 45 mg/PO, QD. By



4

hADAMTS-4 IC_{50} = 6 nM
hADAMTS-5 IC_{50} = 5 nM
rat Plasma hADAMTS-5
IC_{50} = 63 nM
hMMP IC_{50}s > 7600 nM

Fig. 3.25 Aggrecanase inhibitor **4**

comparison, compound **3** was projected to require an oral dose of 600 mg QD in man to achieve the same level of sustained TE [9].

This example illustrates the utility of osmotic pumps to enable the efficient development of a TE/PD relationship in our preclinical model, with nonoptimal compounds. Subsequently, that information was used to guide our efforts to identify a molecule with performance characteristics supportive of human clinical study. This stands in contrast to previous efforts in which the same TE concepts described in this chapter were used to invalidate several of the MMPs which had been nominated as potential therapeutic targets for OA. In these cases, potent but poorly selective tool compounds demonstrated efficacy in both PD and surgical efficacy models. However, unlike our aggrecanase inhibitor program, as target engagement with high selectivity was achieved for the MMP targets of interest (to improve toxicology profiles), efficacy in preclinical models was lost. Like the IDE program, in these cases the disconnection of selective TE from the desired efficacy facilitated prioritization decisions to focus resources on higher potential efforts.

3.5 Conclusion

In summary, the proactive development of a reliable understanding for the time course of in vivo TE represents a powerful strategy for driving timely/quality decision-making across the value chain for cross-functional drug discovery teams. At the front end, TE analysis can dovetail with lead generation technologies to accelerate the identification of useful tool compounds for the design of quality in vivo studies that enable preclinical target validation. In this phase, the use of alternative dosing methods such as infusion pumps can be particularly impactful for accelerating data acquisition without the need for costly compound optimization

cycles. On the back end, TE analysis can be employed to refine desired performance characteristics for candidate selection, to improve the patient focus and efficiency of lead optimization efforts and more rapidly enable clinical experiments. Thus, the application of TE-based drug discovery strategies represents a critical link in the collaborative process of efficiently translating molecules into medicines.

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Chapter 4
In Silico ADME Techniques Used in Early-Phase Drug Discovery

Matthew L. Danielson, Bingjie Hu, Jie Shen, and Prashant V. Desai

Abstract The process of drug discovery and development is time consuming and expensive. In silico tools, in combination with in vitro and in vivo models, provide a valuable resource to improve the efficiency of this process. In this chapter, we provide an overview of various in silico tools and models used to identify and resolve absorption, distribution, metabolism, and excretion (ADME) challenges in drug discovery. In general, structure-based in silico techniques such as docking and molecular dynamics simulations have limited applicability in the ADME space due to the promiscuity of many ADME targets and the limited availability of high-resolution 3-D structures. Pharmacophore models, a ligand-based in silico method, can be used to identify key structural features responsible for the interaction with the target of interest. However, due to broad ligand specificity and the probability of multiple binding sites in many ADME targets, pharmacophore models have limited prospective applicability across structurally diverse chemical scaffolds. Conversely, quantitative structure-property relationship (QSPR) models are capable of extracting knowledge from a wide variety of chemical scaffolds and have prospectively shown utility as predictive models for many ADME endpoints measured in the pharmaceutical industry. QSPR models, especially those based on machine learning techniques, are known to have limited interpretability. To address this challenge, the use of QSPR models is typically coupled with information derived from trends between ADME endpoints and physicochemical properties (e.g., lipophilicity, polar surface area, number of hydrogen bond donors, etc.) during drug discovery. Furthermore, knowledge extracted by the matched molecular pair analysis (MMPA) of ADME data provides insight that is used to identify fragment replacements to improve the ADME characteristics of compounds. In conclusion, an effective amalgamation of in silico tools is necessary to influence the design of compounds that will possess favorable ADME properties. Finally, in silico tools should never be used in isolation; they make up one arm of the

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integrated and iterative learning cycle that is comprised of in silico, in vitro, and in vivo models that we recommend using to effectively drive a drug discovery project.

Keywords In silico ADME • Quantitative structure-property relationship models • Matched-molecular pair analysis • Predictive models • Physico-chemical properties

The drug discovery and development process is time consuming and expensive, encompassing approximately 15 years and over two billion dollars to bring a drug to market [1]. Stage-appropriate use of models is an integral part of the drug discovery process. Early-phase drug discovery uses various in silico and in vitro models to explore potency, ADME properties, and safety. As drug discovery progresses, preclinical in vivo animal models are used to estimate how a compound will behave in humans, and ultimately model situations are created in a controlled clinical environment (clinical models) before the compound is approved for use in the general population.

In an attempt to reduce the time and cost associated with the drug discovery process, in silico tools are one class of models employed throughout this process. In silico tools have a direct impact on how drug discovery progresses and are especially useful in the early-phase of drug discovery where a clinical candidate is being pursued and optimized. These tools are used to design and prioritize the synthesis of compounds with desirable affinity, specificity, a multitude of ADME properties, and safety with the goal of delivering the best possible compound to test in the clinical setting.

In this chapter, we provide an overview of various in silico models and tools employed to identify and resolve ADME challenges during the process of drug discovery. Generally speaking, in silico ADME tools are classified into two major categories, structure-based and ligand-based. Each class of in silico tools are addressed in subsequent sections.

4.1 Structure-Based In Silico Models

When sufficient structural information exists on the protein of interest, generally in the form of a nuclear magnetic resonance or crystallographic X-ray structure, structure-based drug design techniques are used in early-phase drug discovery. In structure-based drug design, interactions between the protein and the ligand are the focus of the study, and this is commonly referred to as rational drug design. Novel ligands can be designed de novo, meaning the interactions between a hypothetical ligand and the protein are optimized with the goal of creating a compound with high affinity and selectivity. Molecular docking can be used to orient a ligand within the active site of the protein to provide an estimate of the protein-ligand interaction. However, molecular recognition between a protein and a ligand is a complex process

that does not occur in a static structure. Molecular dynamics (MD) and Monte Carlo (MC) simulations are computational techniques used to create trajectories that model the protein-ligand fluctuations and dynamics in atomic detail [2, 3].

4.1.1 Molecular Docking

The goal of molecular docking is to model the potential interaction between a protein and a ligand [4]. Although several docking programs exist [4–11], each docking program can be broken down into two general parts: the search function used to orient and place the ligand inside the binding pocket (binding pose generation) and the scoring function used to quantify the protein-ligand interaction and predict the binding affinity (binding affinity prediction). This chapter provides an overview of the current status of molecular docking but does not go into detail on search algorithms or scoring functions, both areas of active research.

For certain protein targets, the search algorithm may generate bioactive binding poses (root-mean-square deviation <2 Å) during the search process for 90% of compounds, but this percentage can be as low as 40% for other protein systems [12]. This is especially challenging for ADME targets that are known to bind a diverse array of compounds and are promiscuous in nature. For many ADME targets, factors such as the size of the binding pocket (relatively large and hydrophobic), the water network within the active site, and protein flexibility lead to significant challenges while utilizing molecular docking. Figure 4.1 illustrates this point on one class of ADME targets, the cytochrome P450 (CYP) family of enzymes. CYPs are estimated to be involved in the metabolism of approximately 75% of drugs currently on the market with CYP3A4 known to metabolize approximately 50% of such compounds [20]. While several publications exist on CYP3A4 docking [21–26], the abovementioned problems limit its use in early-phase drug discovery programs outside of qualitative idea generation.

In instances where the docking search algorithm identifies a bioactive binding pose, current scoring functions are not accurate enough to reliably predict the binding affinity [27–29]. The correlation between the experimentally measured and predicted binding affinities for a series of compounds binding to the same protein target is usually weak and often influenced by the size of the ligand rather than the underlying physicochemical contributions to the binding affinity [30, 31]. Therefore, bioactive binding poses are not always ranked as the most energetically favorable (or top ranked) during the docking procedure [12]. In addition, the lack of accuracy and separation in binding affinity prediction makes it challenging to predict the binding affinities of compounds within a structure-activity relationship (SAR) series let alone in silico de novo-designed compounds. A recent review by Lill [32] discusses many of the current problems and challenges of molecular docking and goes into greater depth on techniques used to overcome such obstacles.

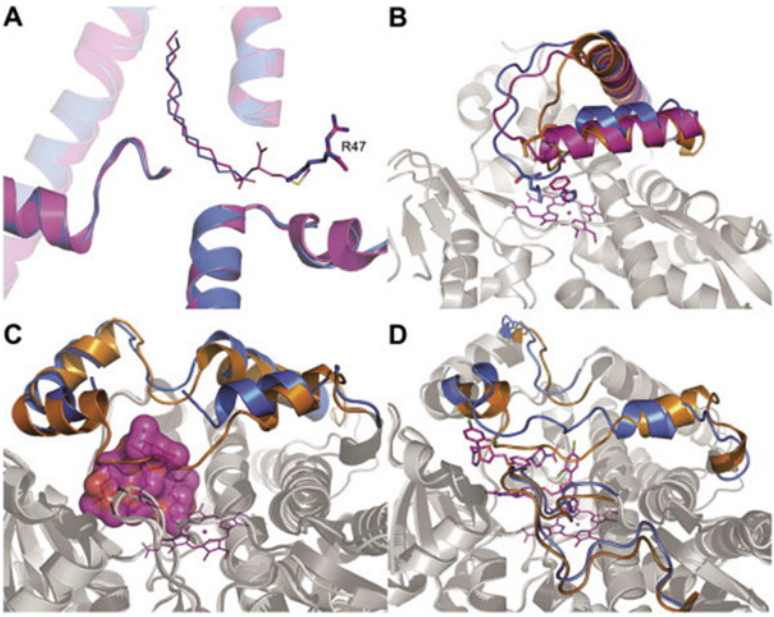


Fig. 4.1 Reproduced from Danielson et al. Potentially increasing the metabolic stability of drug candidates via computational site of metabolism prediction by CYP2C9: The utility of incorporating protein flexibility via an ensemble of structures. Eur J Med Chem 2011 Sep.;46(9):3953–63. Copyright © 2001 published by Elsevier Masson SAS. All rights reserved [13]. Examples of protein flexibility in cytochrome P450 enzymes: (**a**) Changes in Arg47 side-chain rotamer in P450 BM-3 depending on the bound ligand (palmitoleic acid and corresponding protein in *blue*, PDB-code: 1FAG [14]; N-palmitoylmethionine and corresponding protein in magenta: 1ZO9 [15]). (**b**) Alternative loop conformations are observed in CYP119 when different ligands are bound. Compared to the apo structure of CYP119 (F/G loop in orange: 1IO7 [16]), the F/G loop adapts distinct configurations when 4-phenylimidazole (ligand and loop in magenta: 1F4T [17]) or imidazole (blue: 1F4U [17]) is bound. (**c**) In CYP3A4 significant protein flexibility occurs in the F/G portion of the protein (apo: *orange*, 1TQN [18]; erythromycin bound: *blue*, 2J0D [19]) to accommodate erythromycin and part of the F–F′ loop becomes disordered. This motion causes the solvent-accessible volume of the binding site to significantly increase and can dramatically affect ligand binding. (**d**) CYP3A4 exhibits a protein breathing motion increasing the size of the binding pocket to accommodate two ketoconazole (ligands in *magenta*, protein in *blue*: 2V0M [19]) compounds without significant conformational changes of the helices or loop regions composing the binding pocket (apo: *orange*: 1TQN [18])

Post-processing is one such technique designed to overcome the problem of using simplistic scoring functions in docking and can significantly improve the successful prediction of binding affinities [33, 34]. Post-processing techniques incorporate dynamic information of the protein-ligand system after the docking process has been completed. The top-scored binding pose, or several favorably scored poses, is used as input to subsequent MD simulations. In combination with free-energy methods such as free-energy perturbation [35], thermodynamic integration [36], molecular-mechanics Poisson-Boltzmann or generalized Born surface

area [37], or linear interaction energy analysis [38], a more accurate estimation of the free energy of binding is possible [33]. However, this process is relatively time consuming and requires that the bioactive binding pose is within the top-ranked binding poses in order to limit computational time, a criterion that is not always evident when carrying out molecular docking studies on large and rather promiscuous ADME targets.

4.1.2 Molecular Dynamics

Molecular dynamics (MD) is a computational technique used to study the physical movement of atoms. The first MD simulation of a biomolecular system was done in 1977 on bovine pancreatic trypsin inhibitor using a simplistic molecular mechanics potential to describe the properties of the system [39]. Although this simulation was only performed for 9.2 ps, it was a groundbreaking study that showed that integrating Newton's equations of motion over a series of very short-time steps (usually one or two femtoseconds) could transform a once static X-ray structure into a dynamic trajectory from which time-averaged properties could be calculated. Underlying any MD simulation is a physics-based force field that defines all parameters of the system. Several force fields and MD programs exist [40–46], and the parameters are usually defined by high-level quantum chemical calculations or empirically fit to experimental properties. In addition to the force field parameters, a potential function, or mathematical relationship, is needed to describe how the individual atoms of a system interact during the MD simulation. Most force field potentials describe the interactions between atoms in the system in terms of a five-component description of intra- and intermolecular forces. The AMBER force field potential is shown in Eq. (4.1) and consists of bonded (bonds, angles, and dihedral terms) and nonbonded (van der Waals and electrostatic terms) components [42].

$$
\begin{aligned}
V(r^N) = {} & \textstyle\sum_{\text{bonds}} K_r \left(r - r_{\text{eq}}\right)^2 && \text{bond term} \\
& + \textstyle\sum_{\text{angles}} K_\theta \left(\theta - \theta_{\text{eq}}\right)^2 && \text{angle term} \\
& + \textstyle\sum_{\text{dihedrals}} \frac{V_n}{2}(1 + \cos\left[n\phi - \gamma\right])^2 && \text{dihedral term} \\
& + \textstyle\sum_{i<j}^{\text{atoms}} \left(\frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^{6}}\right) && \text{van der Walls term} \\
& + \textstyle\sum_{i<j}^{\text{atoms}} \frac{q_i q_j}{\varepsilon R_{ij}} && \text{electrostatic term}
\end{aligned}
\tag{4.1}
$$

In this type of potential, intermolecular bonds are treated as a simple Hooke's law springs with a characteristic force constant K_r and equilibrium bond length r_{eq}.

The angular term accounts for bond angle bending in the system, and the dihedral term represents the intrinsic torsional energy due to twisting about bonds. The van der Waals term accounts for the attractive London dispersion and repulsive van der Waals nonbonded forces and is calculated by a 12-6 Lennard-Jones potential. Force field assigned atomic partial charges are used to calculate the nonbonded electrostatic interaction between two atoms by solving Coulomb's law. Summing over all pairs, triplets, and quartets of atoms in the system, the force field potential provides an estimate of the energy of the system at a particular configuration. A more detailed description of MD and the algorithms associated with this technique can be found elsewhere in the literature [3, 41–43, 47–49].

Currently, MD simulations are performed on macromolecular systems comprised of thousands of atoms, and several different explicit and implicit water models exist to solvate the system [47–53]. The nanosecond time scale is routinely reached in MD simulations, and in specialized instances protein systems have even been simulated up to the millisecond time scale [54, 55]. With increasing computer power and advances in technologies and methods, millisecond time scale simulations may become routine in the near future. However, this also brings with it additional challenges such as storing, analyzing, and interpreting such a vast array of data. Despite the previously mentioned problems, MD simulations are routinely used to turn a static X-ray crystallographic structure into a dynamic system. Snapshots taken from the MD simulation provide some estimate of protein flexibility and can be used as alternative templates for molecular docking, and this technique has been utilized in several CYP isoforms [13, 56–61]. While MD simulations have become routine in the computational chemistry field, their application in early-phase drug discovery has not. This is especially true for ADME targets due to very limited number of high-resolution X-ray crystallographic structures and their promiscuous nature. Additionally, the time and resource intensive nature of MD simulations and the rather fast-paced movement of chemistry SAR on project teams further limit the application of MD simulations during this phase.

4.2 Ligand-Based In Silico Models and Tools

4.2.1 Quantitative Structure-Property Relationship (QSPR) Models

Quantitative structure-activity relationship (QSAR) models are one of the commonly employed ligand-based techniques to predict the activity of compounds. The field of modern QSAR can be traced back more than 50 years to a model produced by Hansch [62]. QSAR sophistication has grown from its early application on a small congeneric series of compounds using simple linear regression to now being applied to data sets comprised of thousands of diverse compounds utilizing a wide variety of statistical and machine learning algorithms.

When such models are used to predict various properties, including ADME endpoints, they are referred to as quantitative structure-property relationship (QSPR) models. Given the promiscuity and limited structural knowledge of ADME targets, QSPR models are commonly used in the pharmaceutical industry to address ADME-related challenges. The basic premise of QSPR methodology is to develop a relationship between an observed property and structural features of a compound. Considering a set of compounds with observed experimental data (training set), a model is developed that can be used to predict the activity of other compounds (test set) not included in the initial training set. Compounds are represented using a variety of molecular descriptors that describe the chemical structure and properties of the compound. A relationship between the molecular descriptors and the observed response is computed using mathematical techniques such as linear regression, artificial neural network, support vector machine (SVM), and random forest (RF). A general description of such algorithms is summarized in Sect. 4.2.1.4. Figure 4.2 illustrates the general process of building and applying QSPR models to a group of compounds, and each step of the process is further explained below.

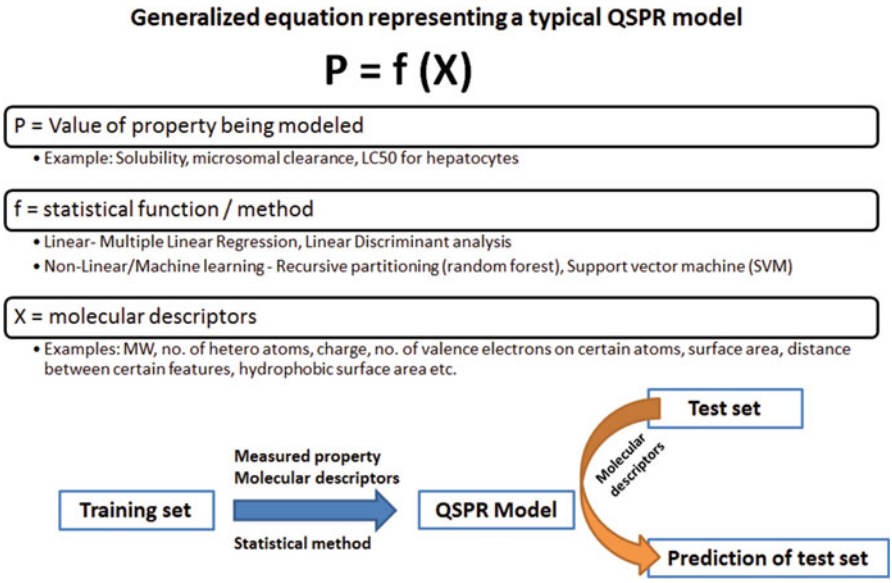


Fig. 4.2 Schematic representation of key components when building and applying QSPR models. The *top section* shows the generalized equation representing a typical QSPR model and lists key components required to derive such an equation for a given data set. The *bottom section* depicts a typical workflow used to build and use a QSPR model

4.2.1.1 Data Set Selection and Curation

The first step to create any QSPR model is the selection of the data set that the model will be built upon. A key consideration when choosing any data set to create a model upon is that the data should be accurate, reliable, reproducible, and measured using identical experimental conditions for all compounds. This can be a significant challenge when building QSPR models based on public databases compiled by collating data from multiple labs spanning a variety of experimental protocols. Stouch et al. demonstrated that models based on data sourced from multiple labs showed poor predictive capabilities for compounds tested in a rigorous and consistent manner [63]. For example, in the case of a hERG inhibition model provided by an external vendor, the data were collated from several different laboratories using a variety of assay conditions: different cell types expressing the hERG channel and different activation potentials for the channel, along with combining binding and inhibition data. The predictions from the vendor model had a poor correlation coefficient of 0.01 and a high root-mean-square error (RMSE) of 1.3 log units for the test set evaluated by the authors.

Following the selection of data, the importance of data curation cannot be overemphasized. In order to create the best possible QSPR model, it is critical to minimize the inclusion of potentially erroneous data. The potential sources of erroneous data include false positives/false negatives, under-/overestimated responses, spurious results (e.g., microsomal stability $>100\%$), incorrect structural representation of compounds, data below the analytical detection limits, and impure material. For example, while building a classification model for P-glycoprotein (P-gp) efflux, Desai et al. excluded compounds reported as non-substrates displaying $>60\%$ inhibition of a fluorescent P-gp substrate, very slow passive permeability, and very low cell partitioning (all cases suggesting potential false negatives) in addition to compounds with poor mass recovery (potentially spurious data) [64]. When feasible, it is good practice to find and utilize analytical data related to identity and purity of compounds. Such information is commonly available in an industrial setting but not easily found for data compiled from multiple sources and available in public databases like ChEMBL. In a previous study, several public and commercial databases were investigated, and error rates in chemical structure annotation ranged from 0.1% to 3.4% [65].

In order to properly curate the assay data that will be used to build a model, it is critical to understand the experimental protocol and potential caveats associated with that given measurement. One of the common issues leading to potentially erroneous results is poor solubility of the compound in the medium used for the assay (e.g., none or very little of the compound is in solution giving an incorrect assay value). This can potentially be addressed by running a parallel experiment to measure the solubility of the compound in the buffer used for the ADME assay. For example, at Eli Lilly and Company, aqueous kinetic solubility in pH 7.4 phosphate buffer is measured for all compounds tested in high-throughput ADME assays. This information is used to curate the data for various ADME endpoints wherein compounds that are not in solution at the concentration used for the given ADME

assay are not included in the QSPR model. To summarize this section, while it is often an overlooked and underappreciated step, data curation based on detailed understanding of the experimental measurement is a critical step in building high-quality QSPR models.

4.2.1.2 Training Set Selection

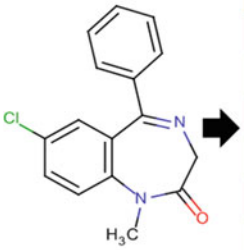
Following data curation, the next logical step of creating a QSPR model is selecting compounds to construct and train the model. What size or how many compounds needed to be in the training set is a precarious question that is sometimes asked. No easy answer to the question exists, and the size of the training set needed to build a useful model depends on the complexity of the endpoint and the intended use of the model. For example, for models intended to be applied prospectively to compounds spanning a wide range of structural diversity, the training set should reflect similar structural diversity and perhaps as much diversity as possible. Prospective model performance, meaning how well the model predicts compounds not in the training set, also depends on whether the training set encompasses the entire range of the assay response. For models such as microsomal metabolic stability that are based on a continuous response (assay range from 0% to 100%), the ideal situation is to have a training set containing compounds spanning the entire 0–100% range and uniformly distributed if possible. For categorical response such as low or high, an even or close to even distribution of compounds between the categories is desired.

Models constructed with training sets that span a narrow spectrum of the entire assay response (e.g., a training set containing 95% of compounds that have microsomal metabolic stability of >90% when the assay range spans 0–100%) or with a highly skewed distribution of the categorical response (e.g., 95% of compounds in the training set belong to the "high" class) are likely to result in QSPR models with limited utility when used prospectively.

4.2.1.3 Molecular Descriptors

Following data curation and training set selection, molecular descriptors must be calculated in order to derive the mathematical relationship between chemical structure and assay activity. Molecular descriptors are numerical parameters derived from chemical structures, and a wide variety of descriptors are used to build QSPR models. Physicochemical (e.g., log P, pK_a, MW), topological (e.g., atom connectivity), constitutional (e.g., number of nitrogen), and quantum chemical (dipole moment, atomic charges) are few examples of common types of descriptors. To gain a deeper understanding and comprehension of molecular descriptors, the reader is referred to a publication by Todeschini and Consonni [66].

In addition to molecular descriptors, molecular fingerprints are often used to represent chemical structures [67, 68]. A molecular fingerprint is comprised of a series of substructures, and the presence/absence of such substructures determines



Diazepam

Key position	Fragment description	Fingerprint code
11	4M ring	0
14	S-S	0
19	7M ring	1
45	C=CN	0
78	C=N	1
92	OC(N)C	1
163	6M ring	1

Final fingerprint

19 78 92 163

Fig. 4.3 Snippet of MACCS fingerprint of diazepam

the numerical code for the molecular fingerprint [69–71]. For example, Molecular Access System (MACCS) fingerprint uses a set of structural features to code the compound into a binary representation [72]. Figure 4.3 shows an example snippet of the MACCS fingerprint representation for the drug diazepam. The column titled "key positions" in the figure assigns a number to a particular chemical feature, listed under "fragment description." The "fingerprint code" is a binary value associated to the absence (assigned zero)/presence (assigned one) of the chemical feature. Using the "key positions" and "fingerprint code," one can derive the final fingerprint shown in Fig. 4.3. Only "fingerprint codes" that are present in the compound are kept in order to keep the fingerprint code vector sparse.

Typically, when constructing a QSPR model, a large collection of molecular descriptors and a variety of fingerprints are calculated. The descriptors and fingerprints are subsequently evaluated using statistical approaches to select the optimal combination to relate chemical structure to the activity of the endpoint. When constructing a model for the first time, several versions of the QSPR model may be built using various combinations of descriptors or fingerprints followed by several iterations of prospective model evaluation (Sect. 4.2.1.5) to identify the optimal collection of descriptors or single best fingerprint [73].

4.2.1.4 QSPR Model Training/Building

After data curation, training set preparation, and descriptor/fingerprint selection, the QSPR model is ready to be built. Mathematic algorithms such as linear regression, artificial neural network, SVM, and RF are routinely used to train and build QSPR models [74]. Linear regression (for continuous response) or discriminant (for categorical response) models assume that the measured property value is an additive response to the underlying molecular descriptors. For example, in the QSPR model for solubility shown in Eq. (4.2) [75], it is assumed that solubility is linearly dependent on lipophilicity (log *P*) and topological polar surface area (TPSA).

$$\log S = -1.0377 \log P - 0.0210 \mathrm{TPSA} + 0.4488 \tag{4.2}$$

Besides prediction, linear models may provide mechanistic insight and can be interpretable in nature as long as the molecular descriptors are "simple" and intuitive. Thus, in case of the solubility model in Eq. (4.2), the negative coefficient for log *P* suggests that an increase in the lipophilicity of compounds is expected to decrease solubility.

Given the complexity of most ADME-related responses, linear models appear to only be applicable over a relatively narrow spectrum of compounds that contain conserved structural motifs. In practice, such models are rarely useful prospectively due to their inability to extrapolate and predict compounds outside their immediate domain of applicability. Machine learning methods such as RF [76, 77] and SVM [78, 79] have been applied to QSPR models to combat the abovementioned limitations and are capable of elucidating more complex relationships between structural descriptors and the observed response.

In general terms, RF models are based on several iterations of the recursive partition approach, and SVM models identify a hyperplane in the high-dimension descriptor space to enable maximum separation of observed responses. Within the pharmaceutical industry, a large amount of ADME data are generated in a consistent manner, and therefore such machine learning methods are preferred to build "global" QSPR models that are designed to be applicable across multiple drug discovery projects that cover a broad spectrum of chemical space [80]. In our experience, such models typically outperform linear QSPR models in extracting structure-property relationship knowledge from large sets of diverse compounds. However, given the complexity of RF and SVM models, they are relatively less interpretable compared to linear models and often offer limited mechanistic insight to go along with predictions. Although generally less interpretable, it should be noted that it is possible to get an estimation of the most influential descriptors for RF models, in turn providing some understanding of key molecular characteristics influencing a given endpoint. For example, in case of an RF model for P-gp efflux, Desai et al. identified that molecular features related to the number of hydrogen bond donors (HBD), TPSA, and hydrogen bond strength were most influential in terms of P-gp efflux of compounds [64].

4.2.1.5 QSPR Model Evaluation

The performance of a QSPR model is evaluated using a variety of parameters depending on the type (continuous vs. categorical) and the intended use of the model. Performance parameters are typically calculated at three stages of the model building process. For example, after building a continuous response model, the first stage is to assess the ability of the model to fit the training set compounds. This metric is commonly referred to as r^2 in the QSAR/QSPR literature. The second stage evaluates the ability of the model to predict the set of compounds left out of

the model building process in an iterative manner (called cross-validation, leave-one-out, or leave-some-out) is referred to as q^2. The third stage is known as external or prospective validation, and the model's ability to predict compounds that were not used during any stages of the model building process is evaluated.

The ability of the model to fit the training set simply serves as a feasibility assessment. It does not provide an assessment of the model's ability to predict compounds outside the training set and therefore isn't particularly useful [81]. Cross-validation is based on prediction of compounds left out of the model but is still an internal validation as it derives the test set from the existing pool of compounds. Depending on the modeling method employed, the cross-validation test set can bias the choice of descriptors and other model-related parameters [82]. Many experts in the QSAR community believe that this type of validation often overestimates a model's ability to predict a true external or prospective test set. Therefore, in order to comprehensively evaluate the utility of a QSPR model, it is critical to assess its predictive ability against an external prospective test set [64, 83–85].

For QSPR models based on continuous data, the square of the correlation coefficient (r^2) between the observed and predicted value (referred to as q^2 when used in the context of cross-validation) is the most common performance parameter reported. RMSE between the observed and predicted values is another key parameter used to assess continuous response model performance. Higher values of r^2 (maximum 1 for a perfect model) and smaller values of RMSE are desirable [86]. In many cases, Spearman's rank correlation coefficient (ρ) is also reported as an indicator of model performance [87]. Depending on the intended use of the QSPR model, one or more of these parameters may be utilized to determine how well a particular model is preforming. For example, if the goal is to identify a model wherein predictions are *correlated* with the observations (not necessarily to predict the absolute value of the property), the r^2 of a prospective test set would serve as a useful parameter. On the other hand, to simply rank order the prospective compounds, a model with high ρ value would be sufficient. If the goal is to accurately predict the absolute value of the property, a model with low RMSE would be necessary. The ideal QSPR model would have favorable performance values for all of the abovementioned metrics.

Classification QSPR models have a different set of performance metrics compared to regression models. Commonly reported performance parameters for classification models are based on the fraction/percent of correct predictions (overall accuracy), the accuracy of each experimental class (sensitivity and specificity), and the accuracy of each predicted classes (PPV and NPV). Table 4.1 provides details to calculate the abovementioned parameters and is referred to as a contingency table or confusion matrix. In addition to these widely used metrics, parameters such as the kappa index are often reported to assess the agreement between prediction and the experimentally determined category. A kappa value of 1 indicates perfect agreement between predictions and experimental values, -1 suggests complete disagreement, and 0 indicates the prediction is no better than random chance. In general, a kappa value >0.4 is considered an indicator of reasonable model performance with useful predictive power [88, 89].

Table 4.1 Contingency table with equations for a classification QSPR model

	Experiments			
Predictions	Positive	Negative		
Positive	TP	FP	Positive predictive value (PPV)	TP/(TP + FP)
Negative	FN	TN	Negative predictive value (NPV)	TN/ (TN + FN)
	Sensitivity	Specificity	Overall accuracy = (TP + TN)/N	
	TP/ (TP + FN)	TN/ (FP + TN)		

TP, TN = true positive, true negative; FP, FN = false positive, false negative; N = total number of compounds

4.2.1.6 Interpretation of Model Prediction

In addition to the abovementioned parameters for model evaluation, several other factors should be considered when assessing the utility and/or applying a QSPR model to a given drug discovery project. In the case of a continuous response model, an applicability domain-related parameter should also be considered in addition to the predicted value if available. Meaning a parameter that indicates if the QSPR model can, or should, predict a compound of interest based on what the model was trained on. If the compound of interest is vastly different than all compounds in the training set, it is expected that such an applicability domain parameter would be unfavorable. Several methods to estimate the applicability domain for a QSPR model have been described in the literature, and they generally provide a qualitative indicator of the confidence for each prediction or a quantitative estimation of the confidence interval around the predicted value [90–93].

In addition to the standard contingency table metrics commonly reported (see Table 4.1), if one is evaluating a classification QSPR model built with a machine learning method (e.g., RF or SVM), the predicted scores of each compound give an estimation of the relative confidence or reliability of prediction [64, 77, 94]. For example, for two compounds predicted to be in the same category, the compound associated with higher score is assumed to be a more reliable prediction compared to the other.

In addition to the abovementioned numerical parameters reported to determine QSPR model applicability/reliability, in order to conduct a thorough assessment of the utility of a model for a given chemical scaffold or drug discovery project, one should always consider:

- The inherent experimental variability in the measurement, especially in case of the high-throughput ADME assays. Model performance has been shown to be directly related to the inherent variability in the measurement of the given assay parameter [95]. For regression QSPR models built on continuous data, one should evaluate the performance of the model based on the proportion of predicted values that falls within the experimental variability of the measured

response and not just rely on an r^2 value. For example, if the inherent variability of an assay is threefold, a model built on these data should be evaluated with this variability in mind. One should check the proportion of the prospective test set that are predicted within threefold of the experimental values. A regression model may not have an r^2 value of 0.9 for this model, but if 90% of the predicted compounds are within threefold of the experimental values, then that model will still be useful.

- Due to the variability in ADME high-throughput assays, we build and advise the use of categorical QSPR models for such data.
- The QSPR model should be evaluated on a prospective test set that spans the entire spectrum of the response, or in the case of a categorical model, the test set should have a balanced distribution of compounds from each category or one that mirrors the training set distribution.
- The assessment of a QSPR model should not be based on a small fraction of compounds, only the most recent compounds, or only the potent compounds from a given chemical scaffold or drug discovery project.
- A QSPR model should not be evaluated based on its performance against a second experimental endpoint not directly predicted by the model. For example, comparing predictions from a QSPR model built on in vitro microsomal metabolic stability data against an in vivo clearance outcome should not be done without establishing if this is permissible. The compound and scaffold of interest may be cleared by mechanisms other than microsomal metabolism, and an in silico microsomal clearance QSPR model should not be expected to accurately predict the in vivo clearance value for such cases.

4.2.2 ADME QSPR Models Used at Eli Lilly and Company

Over the past couple of decades, many publications pertaining to the application of QSPR models for ADME-related physicochemical properties and in vitro/in vivo endpoints have been published. In an attempt at brevity, the reader is referred to review articles that summarize this area of research [96–98]. Table 4.2 provides a brief summary of ADME QSPR models developed and used at Eli Lilly and Company. The data set for each individual model was generated by/for Eli Lilly and Company using consistent experimental conditions for each individual ADME in vitro or in vivo assay. Total data set size ranges from 2,000 to 80,000 depending on the throughput of the particular assay. All ADME QSPR models are built using an SVM algorithm with an optimum molecular fingerprint selected for each assay endpoint.

Table 4.2 Representative list of ADME QSPR models used at Eli Lilly and Company

Endpoint	Data source	Training set size (in thousands)	Model type: Classification (C) Continuous (R)
Kinetic aqueous solubility	Solubility of DMSO stock diluted at various concentrations in phosphate buffer (pH 7.4)	80	C
High-throughput solubility	Solubility of DMSO-dried sample in buffers at pH 2, 6, and 7.4	30–32	C
Passive permeability	Passive permeability across MDCK cells	15	C and R
Hepatic microsomal stability (human/mouse/rat/dog/monkey)	Stability in hepatic microsomes	20–80	C and R
Cytochrome P450 competitive inhibition (CYP3A4/CYP2D6/CYP2C9)	% inhibition of CYPs at 10 μM	65	C
CYP3A4 time-dependent inhibition	Time-dependent inhibition of CYP3A4 at 10 μM	10	C
P-glycoprotein substrate recognition	Efflux by human P-glycoprotein overexpressed in MDCK cells	4	C
In vivo mouse brain unbound concentration	Unbound concentration of compound 5 min post-IV dose	2	R
In vivo mouse brain: plasma partition coefficient of unbound compound (Kpuu)	brain:plasma partition coefficient of unbound drug (Kpuu) in mouse 5 min post-IV dose	2	C and R
Fraction unbound (plasma, brain, microsomes)	Equilibrium dialysis at 1 μM incubation	6–8	R

4.2.3 Prospective Validation of ADME QSPR Models at Eli Lilly and Company

In an industrial drug discovery paradigm where new pharmacological targets are constantly explored, it is important to update global QSPR models to ensure their applicability and prospective prediction performance. Figure 4.4 highlights the outcome of this chronological process at Eli Lilly and Company where prospective performance of ADME QSPR models was maintained for several classification models used over the past several years.

As drug discovery project teams synthesize and test new compounds in various ADME in vitro assays, the global models are updated by curating and adding the new data to their respective training sets. Before updating any particular model, the existing model is prospectively evaluated to measure its predictive performance

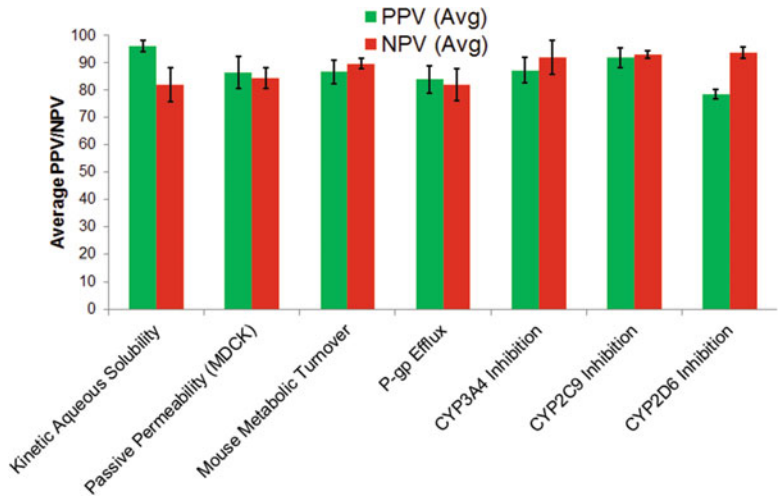


Fig. 4.4 Prospective validation of ADME QSPR classification models used at Eli Lilly and Company. Average PPV and NPV over the last 8–10 versions are shown. Error bars represent the standard deviation. All models were applicable for ~80% of prospective test sets when score cutoffs were used to "accept" a prediction

against data generated *after* the model was built. The result of this assessment for a set of seven Eli Lilly and Company ADME models is shown in Fig. 4.4. The training set for these models range from ~4,000 to 75,000 and increases in number with every model update cycle. Focusing on the mouse metabolic turnover model, the oldest version of the QSPR model in Fig. 4.4 was built using ~40,000 compounds. Before updating the model, it was prospectively evaluated against an additional ~4,000 compounds, and after showing suitable performance, the new data were added to the training set of the existing model to build the next version containing ~44,000 compounds.

All models in Fig. 4.4 are SVM models using fingerprints as descriptors and provide categorical predictions, along with a score representing the reliability of such a prediction. As explained in Sect. 4.2.1.6, predictions associated with higher scores are expected to have greater likelihood of aligning with the measured response. Based on the prospective validation results, suitable score cutoffs (typically 0.7 on a scale of 0–1.0 for both prediction categories) are assigned to "accept" a given prediction, while predictions with scores below the cutoffs for a given category are labeled as "indeterminate." The PPVs/NPVs shown in Fig. 4.4 are calculated for compounds with "acceptable" scores. For all models listed in Fig. 4.4, >80% of the test set compounds had "acceptable" scores, and thus the models were applicable for >80% of the test sets. As shown in Fig. 4.4, the average PPV/NPV for the ADME models ranged from 75% to 85% in prospective testing. Given such consistent prospective performance, the ADME QSPR models are routinely used to design and prioritize compounds for synthesis and testing during early-stage drug discovery. The performance of various versions of the global P-gp

efflux model and its application in identifying and addressing challenges related to central nervous system (CNS) drug discovery projects is described in detail by Desai et al. [64].

4.2.4 Trends Between Calculated Physicochemical Properties and ADME Parameters

To complement the usefulness of ADME QSPR models, the physicochemical properties of compounds influencing ADME properties is well documented. One of the earliest analysis of ADME properties was performed by Lipinski leading to the "rule of five" suggesting that poor absorption and permeability are more likely if the molecular weight (MW) is >500, the number of NH and OH hydrogen bond donors is >5, the calculated log *P* (i.e., *c*log *P*) is >5, and the number of N and O atoms is >10 [99]. The goal of this guideline was not necessarily to rule out certain synthetic ideas but rather steer the synthetic chemistry effort toward chemical space that is more likely to yield compounds with superior ADME properties. Subsequently, several analyses describing the trends between calculated physicochemical properties and in vitro/in vivo ADME parameters have been reported [100–103]. In an exhaustive analysis of a large and structurally diverse set of preclinical compounds profiled at GlaxoSmithKline, Gleeson reported relationships between several ADME assays and calculated physicochemical descriptors [100]. This included in vitro ADME endpoints like solubility, permeability, rat brain tissue and plasma protein binding, P-gp efflux, and inhibition of the CYP isozymes. Several in vivo ADME parameters like oral bioavailability, clearance, volume of distribution, and CNS penetration in the rat were also analyzed. Some of the calculated physicochemical descriptors used in this analyses were *c*log *P*, *c*log *D*, the number of hydrogen bond acceptors (HBA) and donors (HBD) (typically counted as number of N + O for HBA and NH + OH for HBD), positive and negative ionization states, molecular flexibility, molar refractivity, MW, TPSA [104], and the number of rotatable bonds. From this descriptor list, ionization state, *c*log *P*, and MW were identified as the most influential physicochemical properties for ADME properties. The paper suggested that compounds with a MW of <400 and a *c*log *P* of <4 were preferred with regard to maintaining a favorable ADME profile. In another report by Varma et al. [102], ionization state, lipophilicity, and polar descriptors were found to be the physicochemical determinants of renal clearance in human based on a compiled data set of ~400 marketed drugs. It is important to keep in mind that the conclusions about correlations between physicochemical and ADME properties can be strongly influenced by the size and nature of the database employed. Moreover, many of the physicochemical parameters are not independent of each other. For example, an increase in MW is likely to be associated with increase in the number of heteroatoms like N and O, which in turn are associated with TPSA.

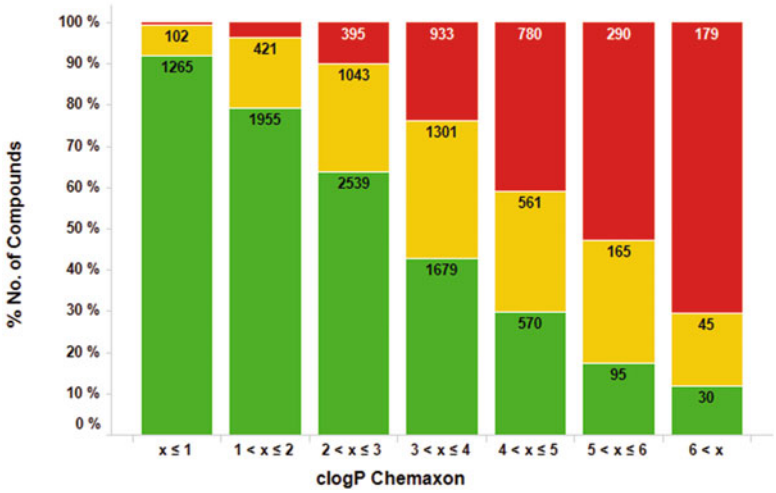


Fig. 4.5 Experimental rat microsomal $Cl_{int,u}$ vs *c*log *P*. *Green* = slow, *yellow* = moderate, *red* = rapid $Cl_{int,u}$. Global data analysis suggests compounds with *c*log *P* of <4 are less likely to have rapid $Cl_{int,u}$

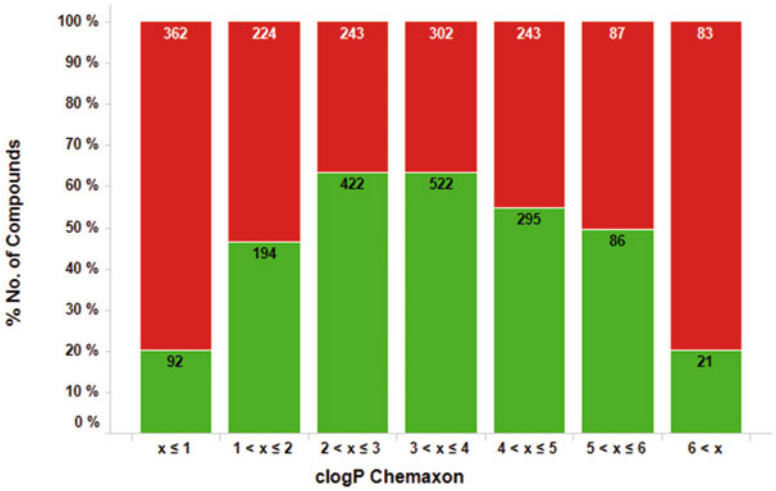


Fig. 4.6 Experimental MDCK permeability vs *c*log *P*. *Green* = rapid permeability, *red* = slow. Global data analysis suggests that compounds with *c*log *P* between 2 and 4 are more likely to have rapid permeability

Figures 4.5, 4.6, 4.7, and 4.8 along with summary Table 4.3 detail Eli Lilly and Company's ADME in vitro data in relation to key physicochemical properties over the past 2 years. Figure 4.5 shows the trend that as *c*log *P* increases so does microsomal unbound intrinsic clearance ($Cl_{int,u}$) [105]. This analysis indicates

Fig. 4.7 Experimental MDCK permeability vs TPSA. *Green* = rapid permeability, *red* = slow. Global data analysis suggests compounds with TPSA of <100 Å^2 are more likely to have rapid permeability

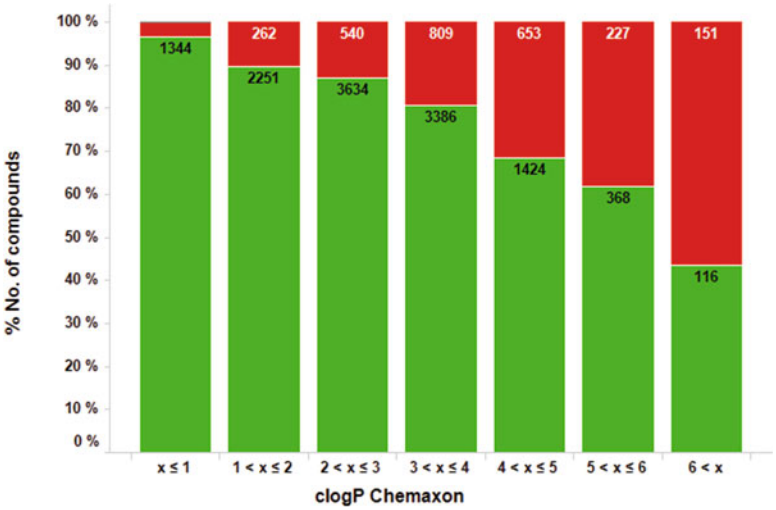


Fig. 4.8 CYP3A4 inhibition vs *c*log *P*. *Green* = low inhibition, *red* = high inhibition. Global data analysis suggests compounds with *c*log *P* of <4 are more likely to have low inhibition of CYP3A4

that compounds with *c*log *P* value <4 are more likely to have slow unbound intrinsic clearance (Fig. 4.5) and a low CYP3A4 inhibition potential (Fig. 4.8). Similarly, compounds with *c*log *P* between 2 and 4 (Fig. 4.6) and TPSA <100 Å^2 (Fig. 4.7) are more likely to have rapid permeability across MDCK cells. Desai

Table 4.3 Trends between calculated physicochemical properties and ADME endpoints from Eli Lilly and Company data

Physicochemical parameter	Desirable range	Trends with ADMET properties
*c*log *D* at pH = 7.4 (Chemaxon)	<3	Higher kinetic aqueous solubility, slow microsomal metabolism and unbound intrinsic clearance ($Cl_{int,u}$), low CYP inhibition potential, high unbound CNS exposure
	1–3	Rapid passive permeability
*c*log *P* (Chemaxon)	<4	Slow microsomal metabolism and unbound intrinsic clearance (Clint,u), low CYP inhibition potential, high unbound CNS exposure
	2–4	Rapid passive permeability
Most basic pK_a	<7.4	Lower risk of P-gp efflux
Molecular weight	<400 Da	Higher unbound CNS exposure
TPSA	60–90 Å^2	Rapid passive permeability, lower risk of P-gp efflux, higher unbound CNS exposure
Number of NH + OH groups	<3	Rapid passive permeability, lower risk of P-gp efflux, higher unbound CNS exposure
Number of N + O atoms	<8	Rapid passive permeability, lower risk of P-gp efflux, higher unbound CNS exposure
Number of negatively charged atoms	0	Higher unbound CNS exposure

et al. have previously published physicochemical trends for efflux by the P-gp transporter and reported having the most basic pK_a < 8.0 and TPSA <60 Å^2 as key physicochemical properties of P-gp non-substrates [64].

4.2.5 Pharmacophore Modeling

Another ligand-based modeling technique that is used in drug discovery is pharmacophore modeling. The word pharmacophore has several definitions associated with it despite the concept being around for over 40 years. A medicinal chemist may define a pharmacophore as a structural fragment or functional group related to a chemical compound or series of compounds. Computational chemists often define a pharmacophore as a collection of hydrogen bond acceptors, hydrogen bond donors, aromatic rings, charged atoms, and hydrophobic regions of compounds that provide affinity and specificity to a particular target. The official IUPAC definition states, "A pharmacophore is the ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target structure and to trigger (or to block) its biological response" [106].

No matter the definition, the concept of pharmacophore modeling is simple and even intuitive to medicinal chemists working in early drug discovery. The

technique models the interaction between a ligand and a binding site, thereby producing a model of the spatial arrangement of molecular features essential for biological activity. The central premise of a pharmacophore model states that if a compound contains the needed molecular features in a spatial orientation that matches the model, the compound should bind to the target of interest. Pharmacophore models have been created for several ADME targets along with being used to predict activity, selectivity, toxicity, and enrichment in high-throughput screening experiments [20, 74, 107–110].

The scope of this chapter provides an overview of pharmacophore modeling and will only briefly introduce the two general parts of any pharmacophore modeling program. However, extensive literature has been published that describes pharmacophore models in greater detail [111–113]. In general, pharmacophore modeling can be broken down into two general steps: (1) molecular super positioning of ligands and (2) scoring how well a ligand matches the pharmacophore features.

The molecular super positioning (also known as alignment) of ligands is time consuming and represents a significant challenge to creating any pharmacophore model. This step inherently involves the alignment of flexible compounds that have multiple possible conformations. Precomputing ligand conformers is common in many of the pharmacophore program available today [111–113]. When conformers are pre-generated, pattern-matching techniques are then used to create the ligand alignment. Many pharmacophore programs use a rigid-body alignment technique that is some type of a maximum common substructure search [114] implemented with the Bron-Kerbosh clique detection algorithm [115] that accounts for the spatial arrangement of pharmacophore features. Scoring functions differ between software, but they generally account for things such as number of matching pharmacophore points along with the spatial orientation and the internal energy of the matching ligand conformer along with some sort of volume or binding site matching term. Throughout the pharmacophore building process, several parameters must be set and optimized, thereby complicating the process of creating an optimal pharmacophore model or one that the entire community uses or accepts for that matter. The reference ligand, or set of ligands, used to create the pharmacophore alignment is often subjective and requires the skill and knowledge of a computational expert.

However, it can be especially challenging to create useful pharmacophore models for targets that are known to be flexible and promiscuous in binding many compounds. Most ADME targets fall into this class, but there is no lack of pharmacophore models published for such targets [107, 109, 116–118]. For example, pharmacophore models have been published for several CYP enzymes, including CYP3A4, that are known to be extremely flexible and recognize diverse compounds. Figure 4.9 displays a pharmacophore model for the organic anion-transporting polypeptide 1B1 (OATP1B1), a liver-specific uptake transporter that lacks high-resolution structural information.

While many pharmacophore publications exist, in many instances pharmacophore models are created using a small subset of compounds known to bind to such targets (10–15 compounds maximum). Such models may perform well on very similar compounds (meaning if the alignment was done with a statin

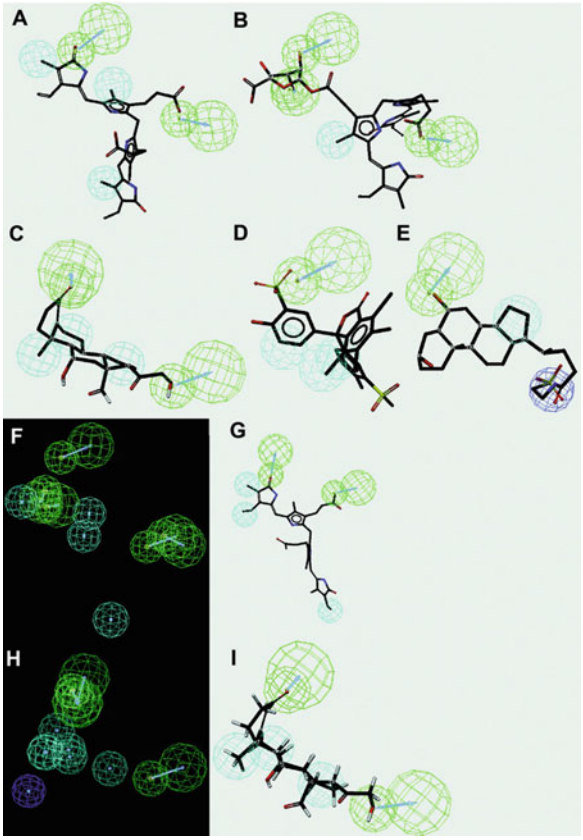


Fig. 4.9 Reproduced from Ekins et al. Comparative pharmacophore modeling of organic anion-transporting polypeptides: a meta-analysis of rat Oatp1a1 and human OATP1B1. J Pharmacol Exp Therap 2005, 314(2):533–541 [116]. Pharmacophores generated from substrate data for human OATP1B1 expressed in oocytes (showing bilirubin mapped to features) (**a**), human embryonic kidney cells (showing bilirubin monoglucuronide mapped to features) (**b**), rat Oatp1a1 expressed in oocytes (showing aldosterone mapped to features) (**c**), CHO cells (showing BSP mapped to features) (**d**), HeLa cells (showing taurohyodeoxycholate mapped to features) (**e**), merged OATP1B1 model using pharmacophores described in **a** and **b** (**f**), meta-analysis model using all cell type compound data for human OATP1B1 (showing bilirubin mapped to features) (**g**), and merged Oatp1a1 model using pharmacophores described in **c**, **d**, and **e** (**h**), showing aldosterone mapped to features (**i**). Pharmacophore features include hydrophobes (*cyan*), negative ionizable (*blue*), and hydrogen bond acceptors (*green*)

compound, the pharmacophore model more than likely will predict other statin-like compounds as likely to interact with the target), but they are not particularly useful in a drug discovery setting where diverse chemistry is being explored on many projects.

The other extreme also is problematic for ADME targets, meaning creating a pharmacophore model based on hundreds of compounds. This is due to the fact that

generating a "unique" pharmacophore pattern for ligand binding is extremely challenging given the diversity of compounds. More often than not, the number of unique matching pharmacophores for several hundred diverse structures will be very few and limited. For example, a pharmacophore model constructed on 500 OATP1B1 inhibitors may only have three pharmacophore points that match the majority of the 500 compounds. When this occurs, the pharmacophore model is not useful as it is incapable of differentiating between active and inactive compounds in the data set. In order for any pharmacophore model to be useful, it has to be shown to not only differentiate active vs inactive compounds but additionally it must have predictive power that informs the design of de novo compounds. This validation criterion is not examined in many published ADME pharmacophore models, and it is essential to evaluate before making the claim that a useful model has been created.

4.2.6 Site of Metabolism Prediction

Understanding and modulating drug metabolism is one of the fundamental concepts of ADME. Several computational techniques exist to predict the site of metabolism (SOM) on compounds. It should be noted that publications and research on SOM prediction exist for metabolizing enzymes other than CYPs [119–122]. However, due to their significance in metabolizing compounds, SOM predictions by CYP enzymes dominate the published literature and will be the focus of this section.

Prior studies predicting SOM of compounds interacting with CYPs have utilized a variety of computational methods such as quantum chemical calculations, pharmacophore models, QSAR, molecular docking, MD simulations, and basic empirical/chemical rules [13, 121, 123–138]. Recent reviews published on CYP SOM prediction provide a good summary of prior studies and techniques used [139, 140]. Although previous studies have been performed to predict SOM, there is no consensus about which method performs "best." In general, the top performing methods claim to accurately predict the experimental SOM 80% of the time or greater.

Recent thinking suggests that the SOM of a compound is influenced by two factors: (1) the intrinsic reactivity of each site in the compound to oxidation and (2) the accessibility of individual atoms to the CYP heme group, the site where oxidation occurs in the enzyme. The intrinsic reactivity is normally estimated using Hartree-Fock, semiempirical methods such as the Austin Model 1, or density functional theory quantum mechanical calculations of the chemical reaction. Accessibility to the CYP heme group is routinely estimated with solvent-accessible surface area calculations, molecular docking, and other structural features.

Several commercial SOM prediction programs exist that allow users to profile compounds to overcome metabolic liabilities. While this may be possible, caution should be used when proposing such a strategy using SOM tools in isolation. In a publication by Vaz et al. [141], they address problems associated with the metabolic

"blocking" strategy. Metabolic "blocking" occurs when a halogen atom, typically a fluorine atom, is attached to the atom/region of the compound susceptible to metabolism in order to reduce the metabolic turnover. Despite literature examples where this strategy was shown to be successful, the general strategy of "blocking" typically shifts the SOM to another atom or region of the compound due to the promiscuous nature of CYPs. In many instances, halogenating a site, typically an aromatic ring, makes the compound more lipophilic. This ultimately can lead to no change, or even increase, in affinity for CYPs and thus expose other sites on the compound to oxidation. In addition, the more lipophilic compound could potentially fit the CYP pocket better and hence become potential CYP inhibitors. By possibly fixing one ADME problem (metabolism) by introducing additional lipophilicity through "blocking," another problem may also arise in the form of solubility limitations.

When trying to mediate metabolic ADME problems, we suggest that multiple in silico tools and methods are used to provide a balanced ADME profile of a compound. In addition to SOM prediction software, in silico models of unbound intrinsic clearance, metabolic stability, log *P*, and solubility should be monitored with any proposed structural change to mediate a metabolic liability. Besides altering the reactivity of a particular site, we suggest evaluating options to reduce the affinity of a compound for CYPs as well. A reduction in log *P* by modifying hydrophobic groups into polar moieties and/or removing hydrophobic fragments from the compound is more likely to provide the reduction in metabolic turnover needed for a particular project.

4.2.7 SPR/STR Knowledge Extraction Using Matched Molecular Pair Analysis

Knowledge-driven modification of compounds is desirable to achieve the optimal potency and ADME properties. For each drug discovery project, a useful QSAR/QSPR model is able to accurately predict the activity of a compound. However, the model provides limited information pertaining to what modifications should be made to the compound in the next cycle of drug design. The matched molecular pair analysis (MMPA) technique is a promising approach to address this issue. MMPA was first coined by Kenny and Sadowski [142] to describe any systematic method of identifying structural matched molecular pairs (MMPs) from a set of compounds and associated property change. In this context, MMPs are generally defined as pairs of compounds that differ only by a single, localized structural transformation, and Fig. 4.10 shows an example [144].

The basic premise of MMPA is essentially an extraction of information within a chemical series featuring a common core. The property of interest can be plotted against the substituents at a given position of the core in order to identify the effects of the structural transformation on the property [145]. Various automated methods,

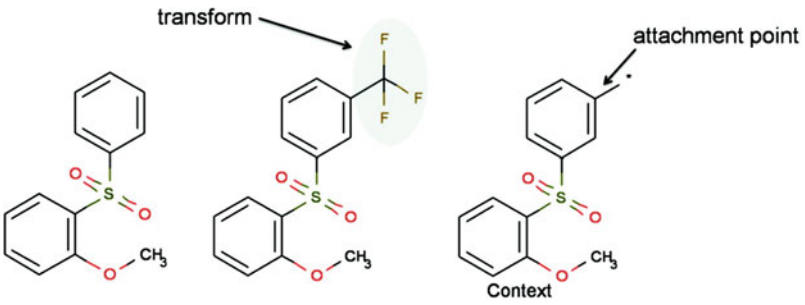


Fig. 4.10 Permission to use from Papadatos et al. [143]. Example of a matched molecular pair. The transformation is H to CF_3 (a single-point change) and is highlighted in *blue*. The *asterisk* in the context denotes the attachment point

including supervised and unsupervised methods, have been developed to identify MMPs and quantify the associated biological changes on large data sets. Supervised methods require predefined molecular transformations to identify the MMPs in the data set [144, 146]. However, any possible MMPs that are outside the predefined structural transformation dictionary cannot be identified. Unsupervised methods have the potential to identify all MMPs within a compound data set without a predefined molecular transformation dictionary [147–151]. It decomposes the compounds into fragments first and then indexes the fragments for rapid sorting and identifies the core scaffolds and R-group substituents. For a more detailed summarization of current MMPA methods, the reader is referred to a review by Griffen et al. [145].

After the MMPA algorithm identifies all possible MMPs, the results are tabulated to show differences between MMPs for a measured endpoint. The effect of a specific chemical substitution is typically summarized by the mean response change, the sample standard deviation of the response change, and the standard error of the mean for each endpoint. The total number of pairs identified for each substituent is also reported to assess the significance of the effects. Leach et al. recommended at least 20 MMPs should be identified for a useful molecular transformation [144]. More recently, Kramer et al. have recommended the use of paired *t*-test to calculate the number of pairs necessary to achieve statistical significance with a given average activity difference. They also demonstrated the importance of building pairs from identical assays measured in the same laboratory [152].

To provide quick and easy understandable guidance, the effects of a molecular transformation on different endpoints can be summarized by a simple symbolic colored arrow or circle that informs the medicinal chemists what compounds to be synthesized [153]. In addition, the structural transformations information can be summarized as rules in a knowledge database. By querying a compound of interest against the knowledge database with MMP rules in place, virtual compounds can be proposed to determine if the property of interest is likely to improve with the associated structural modification.

MMPA methods have been used to assess the mean effect of different substituents on various ADME parameters such as solubility [143, 144, 154], permeability [147, 149], clearance [149], and CYP inhibition [147]. Not surprisingly, common structural modifications, such as replacing hydrogen with a methyl group or changing a methyl to an ethyl substituent, were the most frequently observed MMPs [149].

In general, the structural changes that displayed favorable changes for an endpoint could also be explained by the associated change in physicochemical properties. For example, Gleeson et al. reported that replacing an aliphatic hydrogen atom with a hydroxyl, ethyl, or benzyl group leads to a decrease in CYP3A4 pIC50 $>$ 0.2 log unit in 55%, 15%, and 10% of MMPs. This finding correlates well with the change in *c*log *D* (pH 7.4) of the substituents [147], meaning that as the compound becomes less lipophilic, it is less likely to be an inhibitor of CYP3A4. This observation is aligned with our internal analysis of trends between lipophilicity and CYP3A4 inhibition (Fig. 4.8).

Leach et al. also found that the addition of heavy halogens on aromatic rings was detrimental to solubility and a numerical estimate for such effects was also calculated. For instance, adding bromine to an aromatic ring led to over an order of magnitude reduction of aqueous solubility [144]. Therefore, if a drug discovery team is trying to increase the solubility of their scaffold, they should avoid adding heavier halogens, such as bromine, to their compounds.

While molecular substitutions that track closely with the molecular properties can be useful in guiding the design of new compounds, they may not be overly insightful to a well-versed medical chemist. It is more interesting to identify the substituents that display changes not associated with their physicochemical property changes. For example, despite the considerable increase in lipophilicity caused by phenyl substitutions of an aliphatic hydrogen (Δclog *D* at pH 7.4 of +1.8 log units), the average change in pIC_{50} of CYP1A2 inhibition for 147 pairs of compounds was quite insignificant (ΔpIC_{50} of 0.11) [147].

Another type of MMP is called "switch" transformations, which acts to turn on or turn off the activity. Regardless of the starting value of the endpoint, such MMP transformation results in approximately the same ending value. For example, it was reported that the replacement of a hydrogen by a 4-piperidine group resulted in a microsomal clearance value of ~20 μL/min/mg for all the studied compounds regardless of the starting microsomal clearance values [149].

One should be aware that MMPA results depend on both the transformation and the chemical context. This is manifested by the observation that although many of the molecular transformations are statistically significant with large mean activity changes, most of them also have high variability [149]. Therefore, making conclusions based on the average activity change across the entire MMPA data may be misleading for the chemical series of interest [143, 147]. For example, global context independent MMPA indicated that substituting a pyrimidine for a hydrogen atom increased CYP2C9 inhibition [147]. However, when the same substitution occurred for an aliphatic hydrogen (context dependent), a decrease in CYP2C9 inhibition was observed [147].

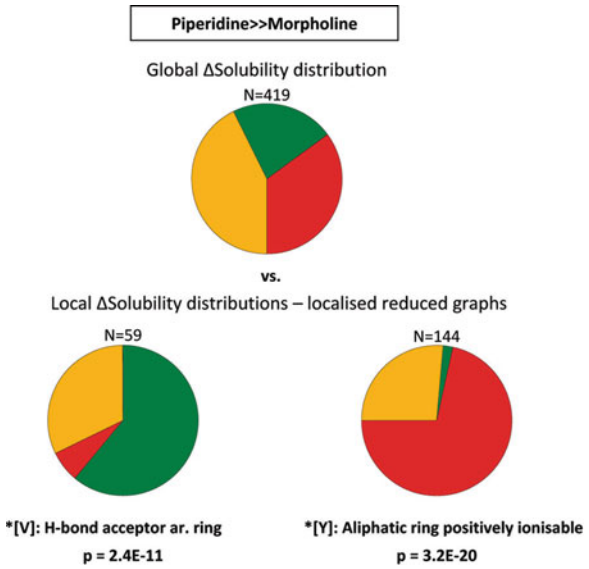


Fig. 4.11 Permission to use from Papadatos et al. [143]. Global and local MMPA distributions for the piperidine to morpholine transformation for a solubility data set. The *colors* reflect the effect of each transformation with *red*, *amber*, and *green* denoting unfavorable (decrease), zero, and favorable (increase) changes in solubility. Different outcomes are observed depending on the context of the compound; if the attachment point is a polar aromatic ring [V], then there is an increase in solubility, while if the attachment point is a positively ionizable aliphatic ring [Y], then solubility decreases

Another example also showed the importance of the chemical context for the MMP transformation. It was observed that transforming a piperidine ring into a morpholine ring has conflicting effects on solubility depending on whether the transformation was added to a polar aromatic ring or a positively ionizable aliphatic ring (Fig. 4.11) [143]. Several recent publications have proposed adding two dimensional contextual information about the compound or three dimensional (3-D) information pertaining to binding environment into the MMPA analysis to address the issue of context dependency in MMPA [155, 156].

4.3 Integrated and Iterative Use of Models in Early Drug Discovery

As mentioned in the introduction to this chapter, the application of in silico, in vitro, and in vivo models is inherent to the drug discovery process. It should be noted that the use of such models in isolation is unlikely to be fruitful and may even be misleading. Therefore, models should be applied in an integrated and iterative

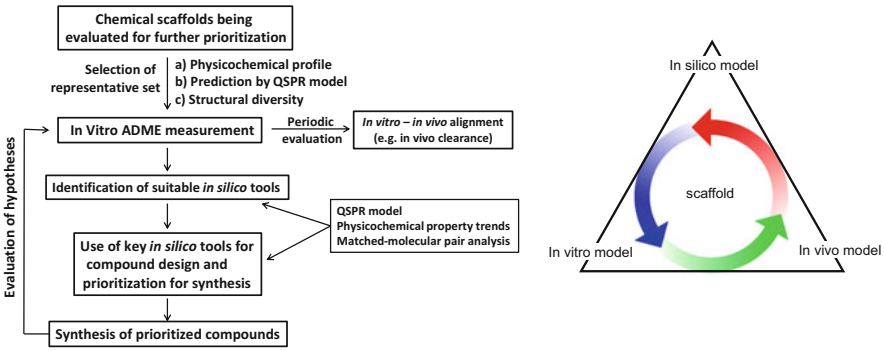


Fig. 4.12 Integrated and iterative use of models in early-phase drug discovery. The left schematic shows the recommended process to identify and integrate in silico, in vitro, and in vivo models. The schematic on the right illustrates the importance of the iterative learning cycle

fashion to build structure-activity and structure-property knowledge toward identifying the best clinical candidate possible for any given drug discovery project.

Once a scaffold has been identified that interacts with the desired pharmacological target, to assess the applicability of in silico ADME models for that particular scaffold, one needs to select a set of compounds that will be tested in vitro. As depicted in Fig. 4.12, this representative set should span the range of predicted in silico values, include various physicochemical characteristics, and include as much structural diversity as possible in order to systematically evaluate in silico model(s). While it would be preferred to select "active" compounds against the biological target for this assessment, this is not a requirement. It is more important to focus on including diversity as mentioned above. The in silico-in vitro analyses will help assess whether the in silico model(s) are applicable for a particular scaffold or along with predicted physicochemical properties can be used to guide and prioritize the synthesis of compounds. In an analogous manner, it is equally important to explore the relationship between in vitro ADME models and the in vivo profile of compounds in order to select an appropriate suite of in vitro tools to prioritize the selection of compounds for in vivo assessment. This iterative learning cycle (shown in Fig. 4.12) provides an efficient strategy to identify and resolve various challenges related to optimizing compound potency and ADME properties rather than using a filtration approach where only the active compounds progress for in vitro and in vivo ADME measurements.

To detail how this integrated and iterative process unfolds in the pharmaceutical industry, consider this example. The typical goal of most small compound drug discovery project is to identify compounds that can attain, and maintain, sufficient in vivo unbound concentration to engage the pharmacological target following oral dosing. To that end, it is important to balance compound potency with key ADME parameters like solubility, permeability, and clearance from the body. For this example, let us assume that the discovery project team has access to global QSPR models for solubility, permeability, and microsomal stability.

The first step to establish the in silico-in vitro connectivity is to select a set of compounds from the scaffold and subsequently compare the outcome from corresponding in vitro measurements. This set of compounds should represent a range of predicted property (solubility, permeability, and microsomal stability), calculated phys-chem properties (e.g., *c*log *P*, TPSA), and be structurally diverse. This step will determine if the global ADME QSPR models are applicable for the scaffold in question and if they provide reasonable predictive performance to enable the prioritization and design of compounds predicted to have a balanced ADME profile in terms of the three ADME endpoints mentioned above.

Before implementing this strategy, it is important to test a small set of compounds spanning a range of measured solubility, permeability, and microsomal stability in the in vivo models to determine whether the oral exposure of these compounds is aligned with their in vitro profile. For example, if the in vivo clearance is rapid for compounds with low microsomal turnover in vitro, it would suggest that the primary clearance mechanism for such compounds is likely to involve non-oxidative pathways and/or excretion via renal or biliary route. Typically, elimination routes outside the oxidation pathway would not be identified using a microsomal stability assessment (in silico or in vitro). In such cases, one might consider testing the compounds in an in vitro hepatocyte clearance model (that will account for various non-CYP metabolic enzymes) to see if better alignment is observed with in vivo clearance. Once a suitable suite of in silico and in vitro tools have been identified that align with key in vivo characteristics, an efficient and robust strategy to integrate these models in an iterative manner can be implemented.

4.4 Summary

In this chapter, a variety of structure- and ligand-based in silico methods used to identify and resolve challenges related to the optimization of key ADME properties have been described. Given the promiscuity of many ADME targets and the limited availability of high-resolution 3-D structures, structure-based in silico techniques like docking and MD simulation have significant challenges and therefore have limited applicability for this purpose. Ligand-based in silico methods such as pharmacophore models can be useful to identify key structural features responsible for the interaction with the target of interest. However, due to broad ligand specificity and likelihood of multiple binding sites (e.g., P-glycoprotein) for many ADME targets, pharmacophore models also have limited prospective applicability across structurally diverse chemical scaffolds.

QSPR models, especially machine learning models, can extract knowledge from a wide variety of chemical scaffolds and a large number of compounds enabling their utility as predictive models for many ADME endpoints. Not surprisingly, QSPR models are one of the most commonly employed in silico tools for ADME optimization during the drug discovery process, especially in an industrial setting

where a large number of structurally diverse compounds are routinely measured in a variety of ADME assays. At the same time, QSPR models have limited interpretability and thus typically don't provide direct clues to design new compounds to address ADME challenges.

To address that limitation of QSPR models, trends with calculated physicochemical properties like molecular weight, *c*log *P*, TPSA, and others are effectively utilized during the design process to optimize the ADME characteristics of a given chemical scaffold. Similarly, knowledge extracted by the MMPA of existing ADME data also provides clues that identify fragment replacements toward improving the ADME characteristics.

To summarize, an effective amalgamation of in silico tools is valuable in guiding the design of compounds with favorable ADME properties on a drug discovery project. These models must be verified to show they provide valid predictions or the integrated in silico-in vitro-in vivo cycle breaks down. Finally, in silico tools should never be used in isolation. They make up one arm of the integrated and iterative learning cycle that we recommend using in order to effectively drive a drug discovery project.

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Chapter 5
Discover Toxicology: An Early Safety Assessment Approach

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Abstract Early safety assessment efforts from target identification to lead development have undergone rapid growth and evolution over the last 10 years. In this chapter, we will discuss the current development trends driving the need for early safety assessment practices. We will discuss the key areas of focus which include target-related, off-target-related, and chemical property-related toxicities. We will offer an overview of the various scientific approaches being utilized in each of these focus areas along with an organizational framework that has proven effective in de-risking the early portfolio. We will conclude with some perspectives on application within the project team setting and traps associated with data over interpretation.

Keywords In silico safety pharmacology • In vitro toxicology endpoint • In vivo toxicology prediction • Livery injury • Toxicogenomics • Gene editing • Microphysiological culture systems • Heart injury cell models • Injection site irritation • Skeletal muscle injury cell models • Gastrointestinal injury cell models

5.1 Introduction

Drug safety is an integral part of the drug development process and represents a key set of experiments that enable investigational new drug (IND) and new drug application (NDA) submissions (The Federal Food, Drug and Cosmetic Act). Ideally, the drug discovery process should culminate with the delivery of a candidate drug with the widest possible margin of safety. To achieve this key deliverable, toxicology organizations within the pharmaceutical industry are constantly evolving to deliver candidate drugs with an optimized safety profile. As a result, many toxicology organizations are integrating with discovery efforts once a validated

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target enters the portfolio. This chapter will discuss discovery phase toxicology activities that can be applied to facilitate the delivery of optimized drug candidates into the drug development process. Key focus areas for this chapter will be target-based toxicology risk, structure-based toxicology risk, early safety pharmacology assessments, in silico and in vitro safety screening, and application of early in vivo biomarker screens.

5.2 Toxicology Target Evaluation and Assessment

Potential drug targets are estimated to be approximately 8000 with 482 molecular targets hit by known marketed drugs [1]. Target classification, based on approval, includes enzymes, receptors, ion channels, transporter proteins, metabolites, nucleic acids, and chromatin binding proteins. The target can be defined as a molecular structure that will undergo specific chemical interactions with candidate drug molecules that result in a desired clinical effect—ultimately for the treatment of a disease. Unfortunately, pharmacological interaction of some targets may result in unacceptable side effects for the given disease indication. Building awareness and understanding of potential safety liabilities of targets are important for the selection of targets with a higher probability of technical success.

Each of the target classes has unique biological and pathway consequences that pose a challenge when assessing on-target or target-related toxicological risk. A thorough literature characterization of the target is the primary line of understanding toxicity risk associated with molecular interactions with the target. At the same time, a complete understanding of the biology associated with modulation of the target could include thorough analysis with data mining tools inclusive of the following areas: genomic, phenotypic, preclinical, and clinical data. Description of the target and understanding mechanism of action related to a particular disease indication can be explored through search of scientific publications and exploration of biomedical and genomic information sources like the National Center for Biotechnology Information (NCBI). Table 5.1 describes some useful publically available tools for exploring potential drug targets, biological pathways, and identification of potential animal models to characterize risks associated with target modulation. Stepwise characterization of the target can be simplified in the following linear path: description of the target gene or protein → mechanism of action/indication → tissue level distribution → toxicological effects associated with manipulation → selectivity of subtypes or closely related targets (kinases, receptors, etc.) → prior experience or previously published information. This stepwise linear strategy as depicted in Fig. 5.1 offers a strategic approach for conducting a thorough risk assessment of the target.

After research of the target, various studies are utilized to elucidate predicted and experimental effects of target modulation. Studies utilizing organ- or tissue-specific in vitro models confirm any on-target risk and identify tool molecules that can be used to test hypotheses in preclinical in vivo studies utilizing wild-type and

Table 5.1 Assessment of target and building a risk assessment and mitigation strategy in early safety development are possible by utilizing web-based tools for complete characterization of pathway, disease, and genomic models

Target	URL	Utility
Biological, pathway, and disease information		
	OMIM	Online Mendelian Inheritance in Man® (An Online Catalog of Human Genes and Genetic Disorders)
	NCBI	The National Center for Biotechnology Information advances science and health by providing access to biomedical and genomic information
	RefSeq	Provides annotated summaries based on function
	PubMed	PubMed comprises more than 23 million citations for biomedical literature from MEDLINE, life science journals, and online books. Citations may include links to full-text content from PubMed Central and publisher web sites
	The Human Protein Atlas	Good mRNA/protein expression data (please review Ab validation with caution)
	Mouse Genome Informatics	Mouse knockout phenotype and animal model description
	PubCHEM Project	Chemistry and structural focused search
Genomic and molecular information		
	Ensembl Genome Browser	Sequence alignments and orthology searches
	NCBI Homology Guide	A list of all NCBI databases and tools for sequence alignment and comparison
	NCBI HomoloGene	Useful tool for rapid % homology comparison of the target protein/RNA sequences against all species (NB after inputting your target of interest)
	NCBI BLAST	Finds regions of local similarity between biological sequences, compares nucleotide or protein sequences to sequence databases, and calculates the statistical significance of matches. BLAST can be used to infer functional and evolutionary relationships between sequences as well as to help identify members of gene families
	UniProt	The mission of UniProt is to provide the scientific community with a comprehensive, high-quality, and freely accessible resource of protein sequence and functional information
Selectivity, target subtype, prior experience, regulatory	PharmaPendium	A collection of regulatory documents, preclinical and clinical data that encompasses safety ADME, and drug interactions

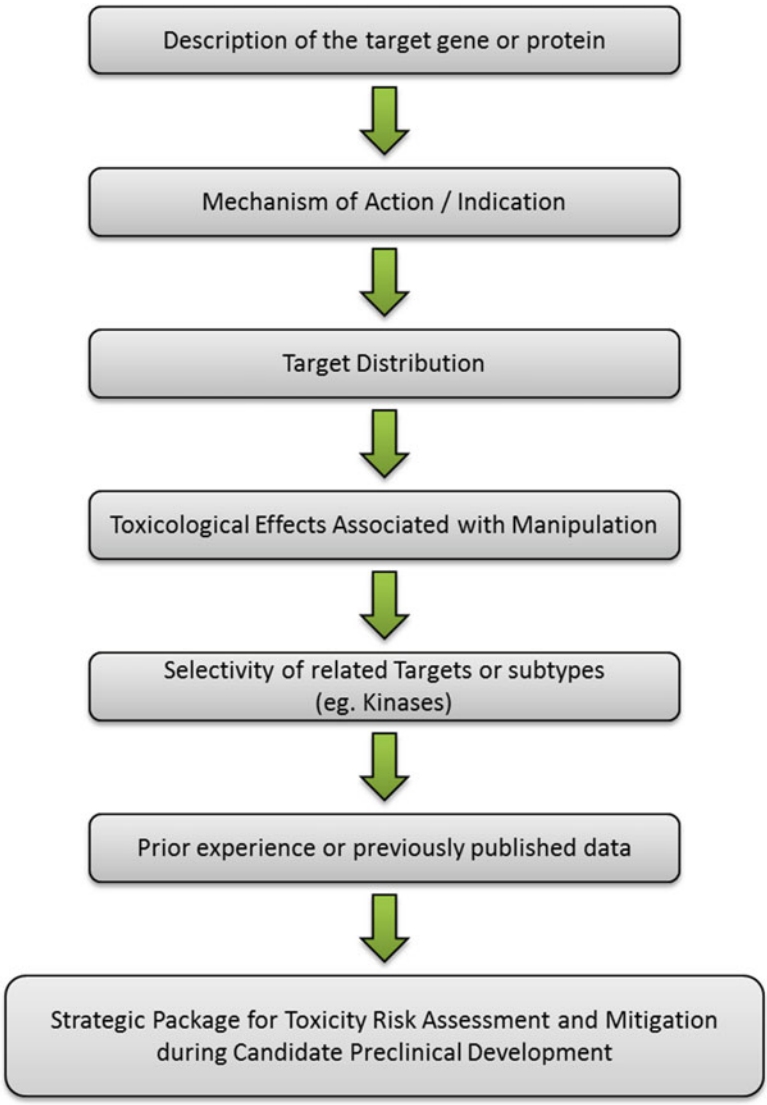

Fig. 5.1 A linear, stepwise approach to the characterization of on- and off-target safety in Target to Lead Development

knockout (KO) animals. Genetic editing and deletion of target genes of cell-based or whole animal systems are essential for identification of candidate development risk and strategy building. Gene KO and knockdown (KD) animal models are an excellent tool to exploit toxicological effects associated with target manipulation or direct "on-target" consequences of pharmacological modulation of the target of interest (step 4, Fig. 5.1). At the extreme, these knockout or knockdown

experiments help define embryonic lethality and organ or tissue development failure. Alternatively, these systems help understand how to balance target modulation or "titer" pharmacologic activity to balance the potential "on-target" safety profile. Understanding target modulation with KO or KD technology is both helpful for gauging overall toxicity risk with target modulation and critical to building a strategy for candidate development. Additional detail of gene editing technology is discussed in Sect. 5.7.1.3, Technologies. Taken together, these studies are conducted to better understand the risks associated with a given target activity. With this knowledge, a strategy can be developed to establish the margin of safety early in the discovery process and where appropriate define opportunities such as dosing frequency or modified absorption and distribution properties that can be utilized to optimize the margin of safety [2].

5.3 Off-Target Assessment

While modulation of an intended target may have unintended consequences, toxicological effects can also result from modulation of unintended pharmacology. It is estimated that adverse drug reactions cause 100,000 fatalities annually in the USA at a cost of $177 billion per year [3]. Therefore, in silico and in vitro profiling of adverse drug receptors (ADRs), pharmacological modulation that results in adverse effects, can help scientists identify and avoid detrimental adverse drug reactions. ADRs can come from isoforms of the target protein, proteins within the same target class, proteins with similar binding sites, or general promiscuity across target classes. Here we will present examples of anti-targets, in silico and in vitro screening options of ADRs, and considerations on how to apply these data to molecules in drug discovery.

5.3.1 In Silico Safety Pharmacology

It has been estimated that there are approximately 21,000 protein-coding genes in the human genome with just over 17,000 encoded proteins currently identified [4, 5]. It is unknown what percentage of proteins result in adverse effects when modulated with an agonist or antagonist, and it is not feasible to run a potential therapeutic in enzymatic or biochemical assays for all known proteins. Even prioritizing in vitro pharmacology profiling to the list of known ADRs can be a costly endeavor, so screening molecules through in silico pharmacology models can inform scientists with a refined list of potential off-targets to screen in vitro. In silico models to predict ADRs can be built-in house, found in the literature, or licensed from companies that specialize in building tools to predict pharmacology.

Loss- or gain-of-function mutations in potassium voltage-gated channel subfamily KQT member 1 (KCNQ1), caveolin 3 (Cav3), sodium voltage-gated channel alpha

subunit 5 (SCN5A), human ether-à-go-go related gene (hERG), and other genes have been associated with long QT syndromes. Inhibition by hERG, a voltage-gated potassium channel, by pharmaceutical agents, has also demonstrated the ability to prolong QT intervals. Astemizole, grepafloxacin, terfenadine, and cisapride are examples of small molecules with unintentional hERG inhibition that caused cardiac arrhythmias associated with QT interval prolongation resulting in their withdrawal from the market [6]. Efforts to develop predictive models of hERG blockers have been largely successful due to it being a promiscuous protein with binding largely influenced by lipophilicity, aromatic moieties, and basic nitrogens. Due to the wide structural diversity of ligands that result in hERG blockade, the more successful hERG quantitative structure-activity relationship (QSAR) models tend to be classification models using molecular descriptors, rather than 3D docking models [7].

Endocrine disruption is one in vivo toxicology endpoint that groups are trying to forecast using QSAR models of in vitro pharmacological endpoints. Endocrine disruption involves interference in the hormone (or endocrine) system, which may result in developmental or functional effects. Endocrine toxicity may manifest as reproductive, carcinogenic, or immunogenic effects. QSAR models of estrogen receptors (ER), ERα and ERβ, androgen receptors, and 17β-hydroxysteroid dehydrogenase 3, to name a few, have been developed to attempt to predict the likelihood of endocrine disruption in novel chemistry [8–10]. Several groups have used a comprehensive approach by combining the QSAR models of multiple targets associated with endocrine disruption to predict a molecule's risk for hormone perturbations.

To evaluate a specific QSAR model's prediction, it is first important to understand the performance of that QSAR model in measures such as accuracy, predictive squared correlation coefficient, sensitivity, specificity, positive predicative values (PPV), and negative predictive values (NPV). Secondly, knowledge of the applicability domain of the model will allow the user to determine if the novel chemistry can be reasonably predicted by the model. Additional considerations would include confidence measures for the prediction, internal evaluation of that QSAR model's concordance to in vitro outcome against an internal test set, and evaluation of the in silico-in vitro concordance for other compounds from the same scaffold.

Depending on the degree of confidence in the in silico prediction from the QSAR evaluation, you can decide how to use that information. If in silico-in vitro concordance of other compounds from that scaffold is high, then it may make sense to use the QSAR model to prioritize which compounds progress. However, if there is less confidence in a prediction or no establishment of scaffold-specific in silico-in vitro concordance, then further progression of the molecule into in vitro enzyme screening at that ADR may be your best option.

5.3.2 Enzyme Safety Pharmacology

Even with the plethora of advantages of in silico pharmacological screening, there are several drawbacks to only utilizing QSAR approaches over in vitro assays.

Given the vast number of protein-coding genes and the fact that a molecule can modulate a protein via agonist, inverse agonist, antagonist, allosteric, covalent, and protein-protein disruption interactions, there are more potential endpoints to test than there are reliable in silico models. As QSAR models are only as good as the data used to build them and need to have an endpoint that can be modeled, certain pharmacological endpoints have limited numbers of potent molecules with low chemical diversity in available enzyme datasets. Furthermore, in silico models may have poor in silico-in vitro concordance for a given scaffold, thus making an in silico screening strategy ineffectual for that chemical series. The wide range of enzymatic screening that can be developed internally or that are available via contract research organizations allows greater flexibility and confidence in identifying ADR risk in multiple species.

Kinases have shown a wide range of therapeutic potentials with Gleevec (imatinib) and ibrutinib being marketed examples of oncolytic kinase inhibitors. Despite the large potential for patient benefit, identifying druggable kinase targets has been a challenge due to difficulties identifying selective ligands. Active kinase conformations have a large degree of structural overlap in their binding sites often with homology spanning kinase subfamilies and groups [11]. Several kinases are known ADRs for gastrointestinal, cardiovascular, reproductive, and bone marrow toxicity. Companies like DiscoveRx (DiscoveRx Corporation, USA), ActivX (ActivX Biosciences, Inc., USA), and CEREP (Eurofins Discovery Services, France) screen submitted compounds in predefined or customized biochemical or cell-based assay panels. However, in vitro profiling of concentration response in even one compound at the roughly 500 kinases can be an expensive prospect, so alternative approaches have been taken. One approach is to screen compounds in a "sentinel" kinase panel that includes the more promiscuous or central kinase proteins out of networks of kinases with high pharmacological similarity [12]. This allows for estimation of promiscuity rather than specific kinase interactions. Another approach is to generate single point data at each kinase for compounds and then generate concentration-response curve values only at those kinases that showed strong single point responses.

Drug-induced valvulopathy in patients treated with the 5-hydroxytryptamine receptor 2B (5-HT2B) receptor agonists is a serious ADR that can result in myocardial dysfunction, congestive heart failure, and sudden mortality. The drug combination fenfluramine and phentermine, coined fen-phen, resulted in some patients requiring valve replacement due to 5-HT2B stimulation by fenfluramine. Valvulopathy seems to be associated primarily with high-affinity 5-HT2B receptor agonists like ergotamine, pergolide, and fenfluramine rather than 5-HT2B antagonists or low-affinity agonists at therapeutic doses [13]. Due to the serious potential side effects with the activation of this serotonin receptor and the lack of predictive animal models for drug-induced valvulopathy, in vitro screening remains a key risk mitigation strategy.

Hepatotoxicity is a key risk in drug discovery and it is important to avoid liver transferases, bilirubin, and direct hepatic effects in animal and human testing. Hepatic metabolism regulation by nuclear hormone receptors (NHRs) has been one mechanism by which hepatotoxicity is suspected in vivo. Due to their

involvement in expression of transport proteins and metabolizing enzymes, NHRs like pregnane X receptor (PXR), constitutive androstane receptor (CAR), hepatocyte nuclear factor 4-alpha (HNF-4α), farnesoid X receptor (FXR), liver X receptor (LXR), and peroxisome proliferator-activated receptor (PPAR) play roles in drug metabolism, bile acid homeostasis, drug transport, lipid homeostasis, cholesterol regulation, and adipogenesis [14]. Pharmacological counter-screening against NHR modulation may help to avoid untoward effects on the liver.

Investigation of covalent inhibitor ADR risk can be especially difficult due to the differing drug kinetics between traditional and covalent inhibitors. Even relatively weak interactions with an off-target protein may result in covalent modification if the protein contains the conserved nucleophilic residue and the compound's electrophile is in the proper configuration to interact. Due to the differing kinetics, profiling targeted covalent inhibitors in enzymatic pharmacology screens at multiple incubation times and utilizing click-chemistry approaches have been proposed to provide a more accurate "selectivity" perspective than traditional single time point screens afford [15, 16].

Other pharmacologies that have been linked to adverse effects include cyclooxygenase-1 (COX-1) inhibitor-related abdominal pain, histamine H1 antagonist-associated sedation, and muscarinic receptor inhibitor-induced SLUDGE (salivation, lacrimation, urination, diarrhea, GI upset, emesis) to name a few [17, 18]. Dr. Laszlo Urban is a leading expert in the field of safety pharmacology with an extensive list of publications on in silico and in vitro ADR screening strategies. Urban and colleagues have nicely described ADRs and the possible adverse effects associated with modulating these targets. These sources would be valuable for those wanting to explore a more comprehensive list of ADRs and their connection to preclinical and clinical toxicities.

Once activity at an ADR has been identified, then several considerations must be made to determine the impact on a molecule's progression. Although an ADR by definition can result in an adverse toxicity, the degree of tolerability of that toxicity must be considered with regard to the severity of the disease state being modulated, brain penetration for ADRs beyond the blood-brain barrier, patient population, duration and frequency of treatment, and the adverse toxicity itself [18]. Nausea and vomiting side effects may be more acceptable if the medication is dosed weekly for the complete remission of a late-stage terminal cancer, than for daily administration to help minimize seasonal allergy effects. The potency of the drug for the ADR in comparison to the on-target potency and the in vivo pharmacokinetic profile of the molecule can determine whether modulation of the ADR is likely within an in vivo setting. Taken together, in vitro ADR profiling and the considerations discussed above can help reduce the risk of non-tolerable side effects.

5.3.3 Summary

In silico and in vitro safety pharmacology screens are pivotal to identifying ADR risks early in safety assessment. Unintentional modulation of an ADR can put the

patient's safety at risk and delay the delivery of valuable therapeutics to patients. In silico pharmacology screens should be used to inform enzymatic testing to identify ADRs early and avoid preclinical and clinical safety liabilities. An understanding of patient population, disease state, toxicokinetics, tolerability, and structure-activity relationships helps to inform the dosing, progression, and/or chemical modification of molecules with ADR risks.

5.4 In Silico Preclinical Predictive Modeling

Developing novel, timely, and informative approaches for toxicity risk assessment is imperative with the push for reduction of animal usage. This push comes from three main fronts: the advent of the Tox21 vision and strategy for the future of toxicology, the concern for ethical treatment of animals, and the financial impact of increasing costs associated with preclinical in vivo toxicity studies [19, 20]. These fronts have created an increased emphasis on computational approaches to assess the risk of molecules.

Utilizing QSAR models of in vitro and in vivo toxicological findings in early safety assessment can help in prioritization of molecules in early phases of drug discovery. Computational approaches are particularly appealing due to their ability to be high-throughput with minimal resources. Prior to chemical synthesis, virtual compounds can be screened using QSAR models to determine the potential risks associated with the chemistry. This can save chemists valuable time and resource by de-prioritizing synthesis of compounds with high predicted toxicological risk. As with any QSAR model, limitations include, but are not limited to, the:

Quality of the data for the endpoint in which you are modeling

Diversity of the compounds used to make the model

Balance of compounds with positive and negative results for the modeled endpoint training set

Performance of the QSAR model in cross-validation and prospective test set evaluations

Applicability domain of the novel compound to the training set compounds

Here we will discuss the application and considerations when using computational tools and data trends to forecast in vitro and in vivo safety risk. We will emphasize the use of physicochemical properties, QSAR, and structural tools to assess risk. Finally, we will discuss approaches to forecast in vivo adverse toxicities.

5.4.1 Physical and Chemical Properties

Molecules have inherent physicochemical (physchem) properties associated with them. Many biological processes of drugs are driven or contributed to by the physchem properties of the drug. The ionization, lipophilicity, protein binding,

solubility, and polar surface area are some of the physchem properties that affect the ADMET properties of drugs.

Manipulating physchem parameters do not always affect ADMET properties and efficacy equally, so optimization of one parameter may be at the expense of another parameter. Lipophilicity, aka hydrophobicity, is one such parameter where the magnitude or direction of change is not consistent from one parameter to the next. For example, increases in mitochondrial uptake have been shown to correlate with increased lipophilicity [21]. Therefore, improved efficacy via mitochondrial targets may be driven by increasing log *P*, a measure of lipophilicity. Conversely, increased lipophilicity has been reported to be correlated with the risk of decreased cell viability as measured by depletion of cellular ATP [22]. Using an internal cytolethality dataset of rat primary hepatocyte (RPH) LC50 values generated from lactate dehydrogenase (LDH) release calculations, the trend of higher measured log *P* being associated with increased cytolethality risk can be observed (Fig. 5.2). Promiscuity, activity at non-intended targets, has also been demonstrated to have a strong correlation to lipophilicity increasing the potential of pharmacologically mediated toxicities [23]. Thus, multiparameter optimization is crucial to develop safe and efficacious drugs.

The extent of ionization at a basic amine is determined by the pK_a of that basic group and the pH of the system, so the degree of ionization would differ between the stomach and the large intestines. Higher pK_a values indicate more basic functional groups and molecules may be comprised of multiple basic centers. Ionization state is crucial for determining absorption of a molecule as protonated basic molecules are less lipid soluble, thus limiting transport across a biological membrane. Increased basicity of molecules has been associated with increased apparent volume of distribution and tissue partitioning, which would increase a compound's distribution into non-target tissues possibly increasing the chance for off-target toxicity [24]. Accumulation of basic compounds within lysosomes is known as lysosomotropism or lysosome trapping; the acidic nature of lysosomes results in basic molecules becoming protonated and trapped within the lysosome. The relationship between basicity and lysosomal accumulation has been well documented [25], and an example of this relationship is shown in Fig. 5.3.

As physchem properties are drivers of many in vitro and in vivo toxicity endpoints, it is no surprise that physchem descriptors often rise to the top when identifying optimal descriptors for toxicology in silico or rule-based models. Research by Hughes et al. found that calculated log *P* (clogP) and total polar surface area (tPSA) showed strong correlations to adverse outcomes that were suspected to not be related to the primary pharmacology of the molecule [26]. This finding was coined the 3/75 Rule. Since that time, some other companies have reported that the 3/75 Rule did not hold for their internal chemistry, which could be the result of differences in analyses or chemical space. Thus, it is always important to evaluate what works best for your own unique chemistry.

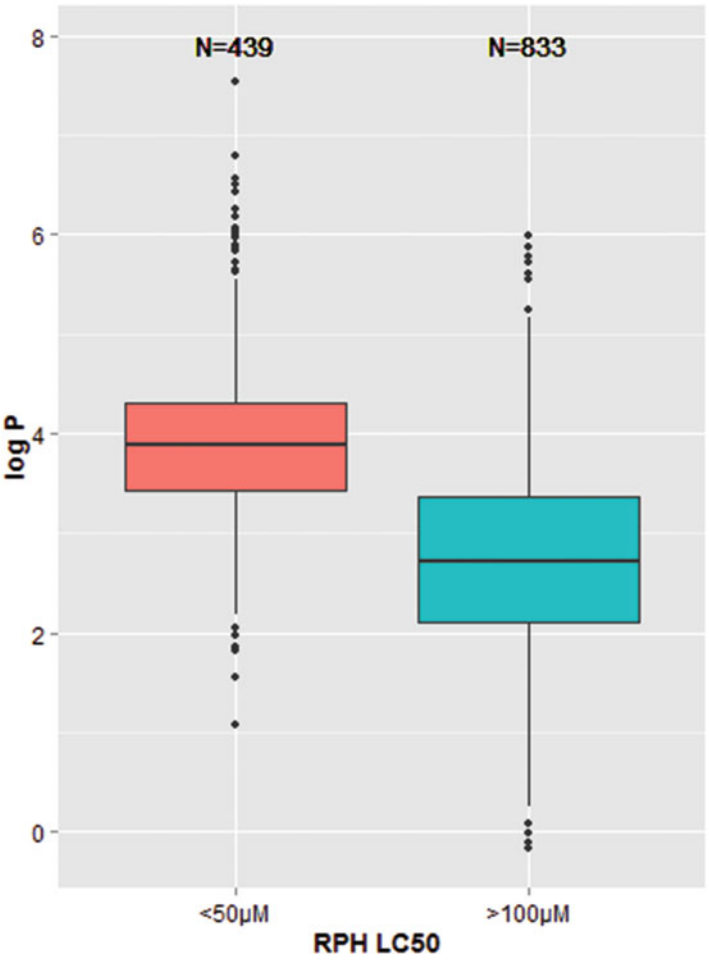

Fig. 5.2 Influence of lipophilicity on cytolethality risk. Individual molecules are separated into a low cytolethality (>100 μM) and high cytolethality (<50 μM) risk bins based on RPH LC50 values. High-cytolethality-risk compounds have an average log *P* of 3.9, while low-cytolethality-risk compounds have an average log *P* of 2.7 for this internal dataset. There is a significant difference in log *P* between the high-risk and low-risk groups of −1.2 (95% CI, −1.3 to −1.1) using the *t* test

5.4.2 Structural Risk Assessment

Structural risk assessment is a broad catchall term to describe the information we can derive around toxicological risk from chemical structure alone. Much of structure risk assessment is focused on using historical knowledge to identify relationships between substructure, structure, or reactive metabolites and a given toxicity endpoint. Matched molecular pair analyses won't be discussed here but do show promise in identifying viable replacements to functional groups or cores associated with toxicity

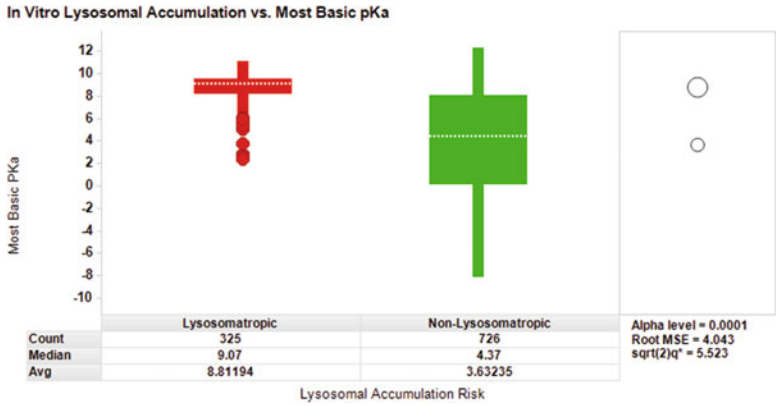

Fig. 5.3 Relationship between lysosomotropism and most basic pK_a. Internal data from HepG2 cells treated with compounds and LysoTracker Green DND-26 (Molecular Probes) to measure maximum fluorescent object count (Rmax). Molecules were classified as lysosomotropic if Rmax > 1000 at a concentration <40 μM, while non-lysosomotropic molecules had Rmax < 200 at concentrations >40 μM. Lysosomotropic molecules had an average most basic pK_a approximately five units higher than non-lysosomotropic molecules

risk. While structural risk assessment can provide valuable, testable hypotheses early in a compound's development, utility may also be derived in later stages when unexpected toxicities are identified via in vivo toxicology studies.

One important piece of structural risk assessment is determination of structural reactivity. The intrinsic reactivity of certain functional groups, like quinones, alkynes, nitrosamines, acyl halides, or epoxides, present in parent or in a reactive metabolite formed from the parent compound, has been associated with drug-induced liver injury (DILI), genotoxicity, and carcinogenicity. Fortunately, many of these overtly reactive groups in parent molecules are known and filtered out by medicinal chemists. Reactive metabolites are less likely to be caught until identified using site-of-metabolism prediction, glutathione trapping, or other liquid chromatography-mass spectrometry (LC-MS) detection tools [27, 28]. However, the presence of a reactive metabolite does not necessarily translate to toxicity, so other factors like dose burden must be considered.

Just as reactive metabolite presence does not imply toxicity, the results of structural risk assessments need to be considered holistically with dose, exposure, and available in vitro or in vivo data. This section will focus on two areas of structural risk assessment, similarity and substructural analyses, to identify potential risks associated with molecules.

5.4.3 Similarity Analyses

Structural similarity is a measure of similarity or distance of one compound versus another compound. These parameters are calculated using algorithms that look at

the characteristics that are similar or dissimilar between the two molecules. Structures may be mapped to molecular fingerprints (i.e., bit strings of 0 and 1 to indicate the absence or presence of each described molecular feature, respectively) or mapped using field-based functions to determine 3D molecular similarity to calculate similarity or distance measures. Tanimoto similarity and Euclidean distance are two of the more common measures to determine similarity with the range of similarity values being between 0 and 1. Despite having the same range, they have an inverse relationship. As Tanimoto similarity approaches "1," the molecules are closer in similarity, while as Euclidean distance approaches "1," the molecules are less similar to each other. It is important to know which measure you are looking at to interpret the result.

Chemical similarity can be measured and interpreted in many different ways; thus the concept of "similarity" may vary person to person. Structural similarity, the similarity between structures, is the more common interpretation of chemical similarity. With structural similarity, the molecule is deconstructed into substructures or fingerprints to enable comparison between molecules. However, within a scaffold of high similarity minor functional group, changes can greatly affect the property similarity between compounds. Addition of a carboxylic acid can drastically alter the binding and electronics surrounding a molecule, thus making the molecules dissimilar from a property standpoint. Biological similarity may also vary with minute chemical modifications, such as addition of an acrylamide, resulting in molecules that may have high structural and property similarity, but that vary greatly in biology. In contrast, two molecules may differ greatly in structural similarity, yet share property and/or biological similarity due to the steric properties of those molecules [29].

Read-across is one evolving approach that considers more than just structural similarity alone to make a prediction on toxicological risk. Those developing read-across methods identified that structural similarity alone was not enough to impact regulatory decisions. More recent read-across analyses consider property, bioavailability, metabolism, and biological similarities alongside structural similarity to guide risk predictions [30]. Given the novelty of chemicals in drug discovery, satisfying the multiple measures of similarity seems unlikely in practice.

There are advantages that both internal and external similarity searches can provide in early safety assessment. Many companies have chemical libraries of molecules that are used to screen "hits" in active assessment screens. One compound may end up being a "hit" for multiple projects; thus a given library could have a wealth of historical pharmacological, ADME, and toxicology data associated with it. Identifying structural similarity to internal chemistry with historical knowledge can provide valuable information on potential risks to enable early mitigation strategies to be formed. External tools for similarity searches can provide valuable information on literature and marketed compounds:

- MetaDrug (Thompson Reuters Corporation, USA)—expert-checked summarization of property, biological, and/or toxicity data on over 700,000 compounds

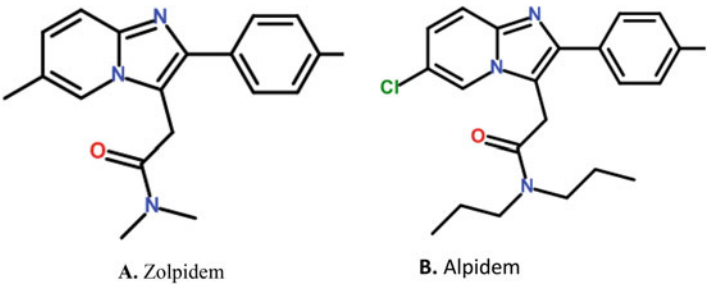

Fig. 5.4 Illustrations of the structures of (**a**) zolpidem and (**b**) alpidem

- ChemSpider (Royal Society of Chemistry, UK)—free, comprehensive database of freely available property and/or toxicity data on 35 million compounds from almost 500 data sources
- PharmaPendium (Elsevier B.V., USA)—preclinical, clinical, and post-marketing toxicity data from approval documents

Using external sources for similarity searches greatly expands the chemical space and information sources to further probe risks for early chemistry.

While structural similarity can provide potential insights into the risk for newer chemistry or to explain in vivo adverse effects, one should be weary of using similarity alone to guide their decisions. Just as minute changes in chemical structure can result in a detrimental loss of potency, minor chemical modifications can greatly alter a compound's toxicity profile. Zolpidem, a $GABA_A$ potentiator primarily used for the treatment of insomnia, thus produces sedative effects as part of its mechanism of action. The structurally similar molecule alpidem (Fig. 5.4), a peripheral benzodiazepine receptor ligand, was prescribed for the treatment of anxiety. Despite the two molecules sharing similar structural features, alpidem avoided the sedative effects of zolpidem at therapeutic doses. Furthermore, alpidem was withdrawn from the market for hepatotoxicity in humans, while Zolpidem is not hepatotoxic and is still a marketed product [31].

5.4.4 Substructural Analysis: Identification of Toxicophores

The term structural alert was first coined by Ashby and Tennant, who defined structural features associated with mutagenicity and genotoxic carcinogenicity [32]. Structural alerts on toxicological endpoints are also known as toxicophores, which are very similar to the concept of pharmacophores (substructures that are associated with activity at a certain pharmacological target within a defined context). Toxicophores are substructures that are associated with an increased likelihood for a certain toxicity within a defined molecular or biochemical context. Just as the presence of a pharmacophore in a structure does not ensure potency, the

presence of a toxicophore does not guarantee toxicity. Toxicophores may be driving toxicity risk via reactive metabolism formation, interaction with an ADR, modulation of physicochemical properties, or other mechanisms.

Substructures may be statistically associated with a particular toxicity (i.e., "suspect" groups), but they may not necessarily be toxicophores. The true liability could actually be related to another substructure that is often contained in molecules that bare the "suspect" group. Another possibility is that the "suspect" group has only been contained in compounds for a single project that had an on- or off-target toxicity that was unrelated to the "suspect" group. These confounding factors may mislead researchers if not identified and interrogated.

For those wishing to identify toxicophores, it is important to set toxicokinetic exposure limits for the test and training sets. Otherwise, large differences in exposure could account for the toxicity differences, thus confounding the analysis. To better separate "suspect" groups from toxicophores, it is important to ensure that substructures have been in a representative number of molecules and in more than one chemical series. This will improve the statically robustness of the analysis and help to mitigate confounding factors mentioned previously.

Determining the context in which a toxicophore has an increased toxicity risk is an important step to pruning out "suspect" groups. The context of risk may be related to chemical features on the toxicophore. Some potential chemical features that may alter the toxicity risk for a toxicophore could include addition of steric bulk, incorporation of nitrogens into rings, shortening alkyl chains, removal of electron-withdrawing groups, or addition of ring substituents. Expert alert systems like Derek Nexus (Lhasa Limited, UK) and CASE Ultra (MultiCASE, USA) incorporate the context in which a structure has increased toxicity risk into their predictions; therefore, a furan may be a hepatotoxicity alert for one molecule, while a different furan-containing compound may not have the alert due to a difference in surrounding chemical features.

Substructural analyses can provide early alerts to identify genotoxicity, hepatotoxicity, and other toxicity risks. These tools may also be employed to determine substructures that may be driving toxicity identified in an in vivo toxicity screen. Collection of in vivo information for diverse chemistry containing the toxicophore can enable identification of the context surrounding the toxicophore and/or the mechanism driving the toxicity. Toxicophore alerts should not halt the progression of a molecule, but rather guide decisions on chemical modification, in vitro ADR or toxicity testing screens, or early Ames mutagenicity profiling.

5.4.5 In Silico Models for In Vitro Tox Endpoints

In vitro screening approaches have enabled earlier evaluation of toxicity risks for a larger number of molecules than plausible with in vivo toxicology studies. Improvements in technology and screening methods enable higher throughput of an expanded number of endpoints in cellular systems. Primary cells (primary

human hepatocytes, rat primary hepatocytes), immortalized cell lines (HepRG, HepG2, H9c2), and induced pluripotent stem cells (iCell cardiomyocytes, iCell neurons) are examples of in vitro models used to characterize cellular perturbations of molecules [14, 33–35]. Functional and cell state endpoints measured in cellular systems include, but are far from limited to, viability, cell morphology changes, mitochondrial function, proliferation rate changes, glutathione loss, and QT prolongation [36–39].

In silico models of in vitro toxicology endpoints allow chemists to prioritize lower-risk molecules for chemical synthesis. There are a number of public in vitro assay datasets that are available to use for toxicology in silico model building. The EPA ToxCast and Tox21 program generated in vitro toxicology data for thousands of chemicals at over 800 assay endpoints generating one of the largest public toxicology datasets. PubChem BioAssay and ChEMBL databases are large public repositories of multiple assay endpoints including toxicology bioassay outcomes [40, 41]. In curating data, great care should be taken to ensure data quality, accuracy, and validity; otherwise any in silico model generated using those data will contain erroneous information. As efforts expand to identify alternatives to animal testing, the number of public toxicology datasets continues to proliferate.

Development of in silico toxicology models often involves filtering available data to those compounds that lack potent cytolethality (for non-cytolethality in vitro endpoints) and that have favorable solubility relative to the concentration ranges used in vitro. If one is modeling in vitro phospholipid accumulation, for example, potent cytolethality may result in artificial phospholipid fluorescent probe signals that are associated with cellular death and not a true phospholipid response. Using the same example of modeling phospholipid accumulation, if a compound has very poor aqueous solubility, it may show no in vitro phospholipid accumulation due to compound not getting into solution. "Negative" in vitro signals for aqueous insoluble compounds could be "positive" in vivo once formulated to better solubilize, thus resulting in false negative in vitro results. Removing cytolethal and poor solubilizing molecules from toxicology in silico model datasets will remove some variability and unknowns from your in silico models.

For novel scaffolds, in silico models can often provide valuable information to enable selection of compounds for in vitro toxicology testing. Rather than going "in blind" to in vitro screening for new chemistry, one can select a range of compounds from each prediction class to assess in silico-in vitro alignment. For example, in Fig. 5.5, assessing in vitro cytolethality of a new scaffold selection of potent molecules with QSAR predictions of both "high risk" and "low risk" enables early understanding of model performance for the novel chemistry. Figure 5.5a shows a project scaffold where high- and low-risk in silico predictions show good concordance to in vitro cytolethality risk, so using the in silico model to identify a lower-risk in vitro chemical space should work well for this scaffold. Figure 5.5b shows a project scaffold where the in silico model predicts high risk, while the in vitro risk is generally low to mid risk; therefore, using the in silico model may overpredict the risk for molecules from this scaffold.

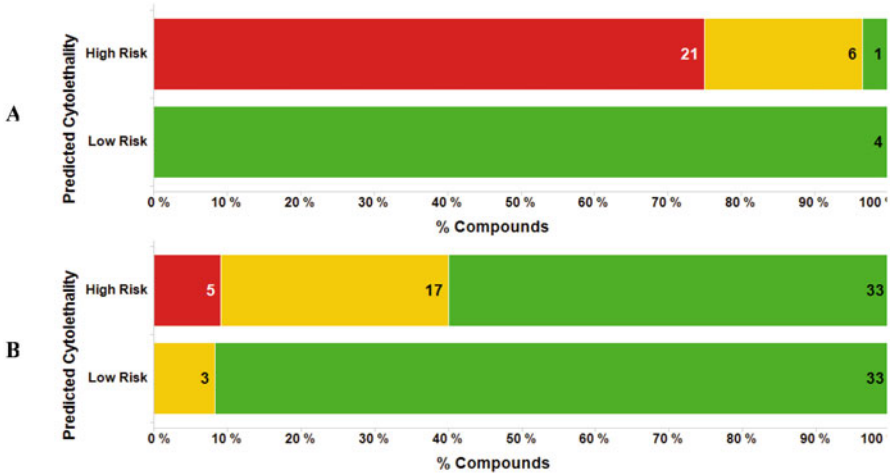

Fig. 5.5 In silico-in vitro cytolethality model concordance for two different scaffolds. Bars represent in silico model predictions and bars are colored on the actual in vitro risk. (**A**) Representation of good in silico-in vitro concordance indicates in silico prioritization may be warranted. (**B**) Representation of poor in silico-in vitro concordance for high-risk predictions and good connectivity for low-risk predictions. In vitro evaluation of high-risk predictions would be optimal due to in silico-in vitro differences

5.4.6 In Vivo Tox Prediction

There are many ongoing efforts to predict in vivo toxicity, many of which are led by the following initiatives or consortiums:

- Registration, Evaluation, Authorization and Restriction of Chemicals (REACH)
- European Partnership for Alternative Approaches to Animal Testing (EPAA)
- Safety Evaluation Ultimately Replacing Animal Testing (SEURAT-1)
- EPA's Toxicity Forecaster (ToxCast)/Tox21
- eTOX

For example, the eTOX collaboration project to identify novel in silico strategies and tools to improve toxicological risk assessment in early drug development has developed the eTOXsys platform. This platform contains a number of in vivo toxicological in silico models including cardiotoxicity and phospholipidosis [42]. eTOX has used the concept of combining the multiple Molecular Initiator Events (MIE) that may contribute to an Adverse Outcome Pathway (AOP) to build a single model [43]. For example, instead of one model for drug-induced liver injury (DILI), there may be multiple MIE models (i.e., total bilirubin increase, hepatocellular necrosis, alanine aminotransferase increase, and bile salt export pump inhibition) used as a consensus model for a DILI AOP prediction.

Prediction of carcinogenicity risk for compounds is an area that has been heavily explored. Regulatory requirements for evaluating chemical carcinogenicity involve 2-year rat screening, a prospect that costs valuable time and money in getting

medicines to patients. Therefore, those in the pharmaceutical industry use in silico, in vitro, and in vivo models of the easier-to-test endpoints of chromosome aberration (CAB) and bacterial mutagenicity to screen compounds to determine which compounds to advance into the 2-year screens. Most in silico models center around prediction of DNA reactivity as assessed by Ames mutagenicity testing as DNA reactivity is considered a hallmark of carcinogenicity [44] although data show only 50% of marketed genotoxic (i.e., mutagenic, clastogenic, aneugenic, epigenetic) compounds result in carcinogenicity [45, 46]. Commercially available Ames mutagenicity in silico prediction tools that have demonstrated favorable prediction accuracy include Derek Nexus (Lhasa Limited, UK) and Leadscope (Leadscope, USA), although it has been demonstrated that the addition of in-house chemistry to a model training set helps improve model performance [47].

There are a variety of programs available that offer prediction of in vivo toxicological endpoints including hepatobiliary injury, carcinogenicity, skin sensitization, and acute toxicity. The datasets behind these models are often a combination of public and proprietary data. Examples of programs to predict toxicological endpoints are highlighted in Table 5.2. Several of these applications include non-toxicological endpoints such as absorption, distribution, metabolism, or elimination (ADME) and physicochemical or pharmacological models. A few of the programs also include or offer the option to license the databases behind these models.

Table 5.2 Applications for prediction of in vivo toxicological endpoints with brief details on their offerings

Application	Source	Details
ACD/Percepta	ACD/Labs	Predictions for over 25 physchem and ADMET endpoints (seven of which are toxicological). Model output includes measures of prediction reliability
ADMET Predictor	Simulations Plus, Inc.	Rapid prediction of over 25 toxicological QSAR models. Model building, visualization, and additional physchem and ADME model tools also available
Derek Nexus	Lhasa Limited	Rule-based expert system using chemical structure alerts to predict over 50 toxicological endpoints
Discovery Studio TOPKAT	Accelrys, Inc.	Statistical-based QSAR models for 14 toxicological endpoints. Additional predictive science applications available
eTOXsys	eTOX	Contains 20 toxicology models and 19 safety pharmacology models where the prediction is the result of the outputs of multiple models. Ability to query the database behind eTOXsys
Leadscope	Leadscope, Inc.	Comprised of nine statistical or expert alert models spanning 86 toxicological endpoints. Toxicity databases are also available
MetaDrug	Thompson Reuters	Over 70 QSAR models to predict ADMET and therapeutic activities. Metabolism prediction tools, toxicity pathway maps, and large comprehensive databases also available
REACH QSAR	Molcode	QSAR prediction for 30 endpoints primarily focused on toxicity risk

Despite many efforts to predict specific in vivo toxicity endpoints in preclinical species and humans, these models in general have poor accuracy. The prevalence of adverse events is <10% for many toxicities, especially when filtering out compounds that cause multi-target organ toxicity driven by poor physicochemical properties and/or poor selectivity. The low prevalence for compounds to cause a given toxicity often results in models with low PPV due to a higher proportion of false positives compared to true positives. An additional complicating factor to prevalence is that a specific toxicity may result from multiple MIEs, so compounds resulting in toxicity via a particular MIE may be poorly represented in a model's training set. Thus, compounds causing toxicity via an MIE that is poorly represented may not be predicted positive by the model driving down the NPV. One additional factor that causes difficulties in building in silico models for in vivo endpoints is creation of the training and test set used to build the model. This is due to compounds differing in dose, exposure, intrinsic clearance, plasma protein binding, CNS penetration, and other crucial ADME properties that make comparison of compounds difficult. For example, "Compound A" may have only been dosed up to a total Cmax of 1 μM with no cardiac necrosis observed, whereas "Compound B" may have been dosed higher, and cardiac necrosis was observed at a dose resulting in a total Cmax of 100 μM. In this example, due to exposure differences, the endpoint of cardiac necrosis cannot be easily compared between "Compound A" and "Compound B" at total Cmax concentrations exceeding 1 μM. Therefore, ADME properties need to be taken into account along with prevalence when building in silico models of in vivo endpoints.

In recent years, alternative approaches to predicting in vivo toxicity have been investigated to try to improve accuracy. Incorporation of in vitro concentration responses as biological descriptors and their maximal responses were shown to improve acute rodent toxicity QSAR model accuracy [48]. Therefore, combining in vitro toxicological data with general molecular descriptors may provide for improved model predictivity. Setting specific exposure cutoffs has been one approach to improve comparison across compounds when building model training and test sets [26]. In modeling the total Cmax at the lowest observed adverse effect level (LOAEL), our group showed that increasing apparent volume of distribution (Vd, area) and increasing cytolethality drastically reduced the average LOAEL, while decreasing Vd and cytolethality greatly increases the average LOAEL [49]. Figure 5.6 shows internal data demonstrating the relationship between Vd, area, RPH cytolethality, and adverse histopathology in oral dosed rat 4-day toxicology studies. This work has shown the value of incorporating in vitro endpoints, exposure, and additional ADME parameters to determine toxicological risk.

5.4.7 Summary

Given the push for reduction of animal use in preclinical testing, utilization of predictors for in vivo toxicological risk is imperative. Physicochemical properties,

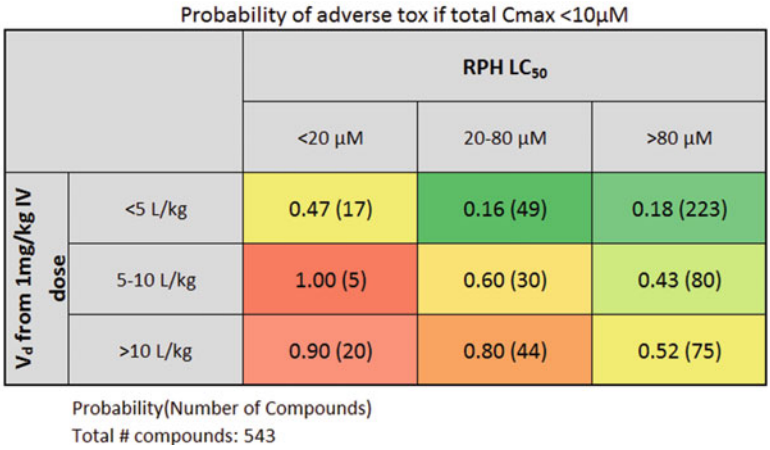

Fig. 5.6 Correlation of Vd, area, and RPH cytolethality to rat oral dosing 4-day adverse histopathology. Internal dataset of 4-day rat orally dosed compounds. Probabilities indicate the proportion of molecules in that "bin" with adverse histopathology findings in any of the eight primary target organ tissues examined where at least 20% of the animals had an adverse effect in the same tissue where the total Cmax at that dose was <10 μM. Those compounds with no adverse effects at a total Cmax of 10 μM or higher were considered "clean." These data show that higher Vd, area (5–10 L/kg), and lower RPH LC50 (<20 μM) internal compounds have historically shown a high probability of adverse histopathology at total Cmax values <10 μM, whereas lower Vd, area (<5 L/kg), and high RPH LC50 (>20 μM) internal compounds have shown a low probability of adverse histopathology at total Cmax values <10 μM

in silico models, and structural risk assessment can be used in conjunction with in vitro models to identify toxicity risks prior to in vivo screening. These tools show great value in scaffold risk assessment to identify lower-risk chemical series and in development of better-informed in vitro, biomarker, or in vivo toxicology screening strategies. While in silico and structural tools are not a replacement for animal testing, they do provide opportunities for finding safer molecules earlier in development to protect animals and patients.

5.5 Cellular Systems: General Screening and Models of Key Target Organs

Cell-based models in toxicology have been applied in numerous ways often with unique strategic intent. The most widely used approach is the evaluation of general cell health. This phenotypic approach interrogates generalized cellular function in a higher-throughput screening paradigm which can be applied in a proactive manner to de-risk chemistry in early discovery. Alternatively, cell-based systems are applied for cause in a directed screening effort or in a hypothesis-driven target organ approach to better understand mechanism of action and facilitate lower-throughput screening efforts.

5.5.1 General Screening

General cell health screening involves the application of endpoints such as cytolethality, phospholipidosis, steatosis, mitochondrial membrane potential, and redox status to name a few. In this paradigm, a cell model is usually chosen that is both a good general reflection of a mammalian cell of toxicological interest (hepatocyte) and has the characteristics of reproducibility, robustness, and ease of culturing (a cell line). The human hepatoma cell line HepG2 is a good example of a cell model that has those characteristics.

General cell health screens have been applied at various points in the drug discovery model. Often, you will see these data being generated and applied just before project teams begin to optimize lead molecules with short-term live-phase studies. Unfortunately, at this point in the drug discovery process, the available chemical space has been narrowed leaving little room for structural diversification. However, if you can integrate your general cell-based safety screening paradigm into an earlier discovery process where biological hits are being profiled, there is ample chemical diversity to optimize safety along with target activity, drug disposition, and biopharmaceutical properties. In this setting, a combination of in silico and informatics tools as described in Sect. 5.4 along with a few key toxicology cell-based screens can be effectively applied to improve the outcome of early live-phase studies.

General cell health screens have been developed using various combinations of cell systems, analytic reagents, and assay platforms. The simplest platforms rely on easily maintained cell lines with simple enzymatic-, fluorescent-, or luminescent-based readouts [50]. These readouts can be single endpoint or multiplexed into "high-content" platforms [51]. A list of cell systems and key endpoints can be found in Table 5.3.

Table 5.3 Collection of cell systems used in early safety assessment screening

Cell system	Endpoint	Biological function	Measurement
Rat primary hepatocytes	Cell death	Measurement of enzyme leakage (lactate dehydrogenase) from a cell which has lost membrane integrity	Enzymatic
Rat primary hepatocytes	ATP	Measurement of mitochondrial function and cell death	Luminescent
HepG2	Steatosis	Measurement of neutral lipid accumulation within the cytoplasm of the cell	Fluorescent
HepG2	Phospholipidosis	Measurement of phospholipid accumulation within the cytoplasm of the cell	Fluorescent
HepG2	Lipodystrophy	Measures expansion of lysosomal compartments with the cytoplasm of the cell	Fluorescent

5.5.2 *Focused Cell Screens*

Beyond general cytotoxicity screening and broadly applicable phenotypic assays (phospholipidosis, neutral lipid accumulation, and lysosomotropy), focused cell models are chosen to reflect the organ and tissue toxicities that are manifested during late drug discovery and early drug development. The cell model choice may be based on previous knowledge of the drug's target organ/tissue distribution and biochemical/signaling pathway impact or on knowledge derived from animal model studies. Using hematopoietic stem cells in and a colony forming assay for screening when the target is a cyclin-dependent kinase is an example of choosing a cell model based on previous knowledge of the target, while establishing a screening assay in skeletal muscle myotubes after noting skeletal muscle injury in an initial rat toxicology study is an example of choosing a model based on study results.

Once the appropriate cell model is identified, the relevant assay endpoint (s) needs to be established. These will vary depending on the nature of the injury (observed or anticipated) and can range from simple cell viability assays, through mechanism and function based screens, and even global gene expression analysis [52]. More and more, cellular injury evaluation involves using multiparameter high-content analysis approaches which incorporate several endpoints and thus provide both more granularity on the nature of the injury and more selection power for ranking molecules [51].

As early chemistry and drug safety groups have become more efficient at identifying inherent compound physical-chemical property-based risk and have developed in silico predictive models, more molecules with liability based primarily on compound structure properties are removed earlier in the preclinical development process. Because of this success, cell injury models and assays today reflect more mechanistic and functional toxicity screening approaches, where the nature of the injury is usually more subtle and often reflects a negative impact on cell function not manifested as overt cytotoxicity. Two examples of these newer function or mechanism-based cellular injury models would include screening for compounds that negatively impact vesicular trafficking in retinal epithelial cells using a high-content imaging-based approach or screening compounds with potential cardiac arrhythmia risk using cultured ventricular cardiac myocytes and multielectrode array field potential duration measurements [53].

Another trend in the development of tissue- and organ-based cell models has been the major move toward the use of human cell models over animal cell models. The rationale is that human cells will more accurately reflect the biology and thus relevant molecule risk in the intended treatment population than animal derived cell models. Until recently, the use of human cell models was limited, in that most of the available human models are transformed cell lines which have inherent drawbacks as to their relevance to the in vivo human status. The inability, except in a few cases such as hepatocytes, to obtain primary cells from humans has until recently limited the widespread use of human cell models in drug safety screening. New potentials

for using human cell models have arisen with the advent of technologies that allow for the production of many differentiated cell types from human-induced pluripotent stem cells (iPSC) generated, relatively noninvasively, from donor's skin or blood cells [54]. A number of these human iPSC-derived cell models are now being evaluated in many drug safety organizations and in a few cases are being routinely used in drug safety assessment screening [55].

In the following section, we will provide examples of how organ-/tissue-specific cell models are being used in early drug safety screening and emphasize the movement toward mechanism and function-based screening approaches as well as the move toward extensive use of human cell models.

5.5.3 Liver Injury Cell Models

Drug-induced liver injury is the leading cause for adverse toxicity in the clinic and in severe cases can lead to the need for liver transplantation and sometimes result in death [56]. Although many compounds causing acute liver failure are identified during preclinical testing, the use of both immortalized cell lines and primary hepatocytes during early drug discovery is essential for screening large compound sets to identify a safe chemical space while maintaining potency at the target of interest. During later stages of drug development, focused experiments can be utilized to understand mechanism of on- vs. off-target toxicity using these in vitro models.

While standard cytolethality screens have been suggested to correlate with nonspecific organ toxicities [49], these are not clear predictors of liver-specific toxicity. Nonetheless, it is essential to understand a compound's in vitro cell death profile prior to interpreting results of other functional assays. To measure cytolethality, either primary hepatocytes or immortalized liver cell lines are treated with a concentration response curve, and cell death is measured by either lactate dehydrogenase release or high-content imaging using nuclear staining. The concentration which results in 50% cell death (LC50) relative to total control is reported, and compounds within a chemical scaffold are banned from most cytolethal (low LC50) to least cytolethal (similar to vehicle control).

Following generation of cytolethality curves, further endpoints can be produced to understand functional changes within the hepatocyte. These endpoints are often multiplexed within the same well using high-content imaging. Examples range from understanding relative amounts of neutral lipid accumulation (i.e., phospholipidosis or steatosis) to perturbations of vesicular trafficking (i.e., lysosomotropism and inhibition of autophagic flux) or mitochondrial function with either fluorescent probes or fluorescently labeled proteins [57] (Fig. 5.7). When implementing new functional assays, it is essential to utilize a test set of molecules known to perturb the cellular system of interest, as well as negative controls. Having a set of positive and negative controls is essential to set parameters for data interpretation, such as fold change and relative fluorescence.

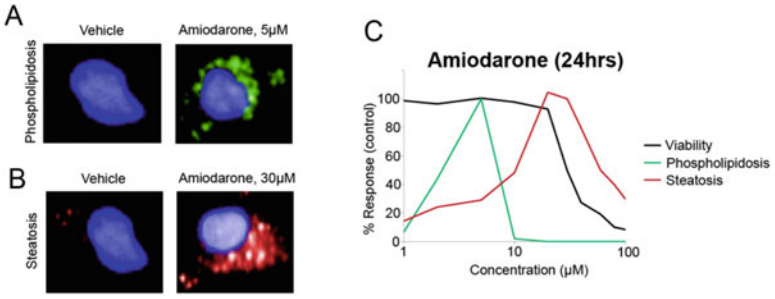

Fig. 5.7 (**A**) HepG2 cells were treated with either vehicle of 5 μM amiodarone and LipidTox Green phospholipidosis detection reagent for 24 h, followed by fixation with preference and 5 μg/mL Hoechst prior to fluorescent imaging on the PerkinElmer Opera. (**B**) HepG2 cells were treated with either vehicle of 30 μM amiodarone for 24 h. Following fixation with 5 μg/mL Hoechst, cells were stained with LipidTox Deep Red for 2 h prior to imaging on the PerkinElmer Opera. (**C**) HepG2 cells were treated with either vehicle or a concentration response curve of amiodarone from 100 to 1 μM and processed as described in (**A**) and (**B**)

5.5.4 Gastrointestinal Injury Cell Models

Intestinal epithelial cells (IECs) have a critical function in the absorption of nutrients and act as a physical barrier between our body and the outside world. Damage and/or death of the epithelial cells lead to the breakdown of this barrier function along with inflammation as a result of access of the immune system to the intestinal flora. Intestinal epithelial damage is frequently associated with various inflammatory disorders as well as drug-mediated toxicity. The lumen of the gut, although at first glance may seem to be a simple mucosal epithelia with primarily absorptive properties, is in reality a much more complex and nuanced system of multiple cell types, which also interfaces with a complex microbial biome [58]. Most cell lines used for GI toxicity screening purposes are intestinal epithelial in nature such as the rat IEC-6 and human Caco-2 cell lines and thus reflect primarily properties of an enterocyte cell [59, 60]. Under proper culturing conditions, the IEC-6 cell model, having been derived from the crypt region of a juvenile rat small intestine, can display a mixed cell morphology which reflects multiple cell types when differentiated over time in culture (Table 5.4). In our laboratory, IEC6 cells have been able to discriminate GI injury risk with a positive predictivity of 68% that separates GI toxicants from other more general cytotoxicants.

Intestinal organoids, "mini guts," are now being generated in culture that better replicate the GI with cryptal regions giving rise to villous structures [61]. Intestinal organoids are showing promise as a physiologically relevant surrogate system for large- and mid-scale in vitro testing of intestinal epithelium-damaging drugs and toxins and for the investigation of cell death pathways [62].

Table 5.4 Relative gene expression levels of selected intestinal epithelia genes from the rat IEC-6 cell line at 1 day and 7 days in culture under differentiating conditions

Expression of intestinal mucosal epithelial genes in IEC-6 cells (PCR analysis)				
Gene name	Gene symbol	Predominant cell type	IEC-6 1d	IEC-6 7d
Intestinal alkaline phosphatase	Alpi	Enterocyte	–	++
Villin	Vil1	Enterocyte	++	++
Sucrase isomaltase	Sim	Enterocyte	+	+
Kruppel-like factor 4	Klf4	Enterocyte, goblet cell	++	+
Kruppel-like factor 5	Klf5	Stem, enteroendocrine, paneth cells	+	+++
Cholecystokinin	Cck	Enteroendocrine	+	++
Mucin1	Muc1	Goblet cell	+	+
Mucin2	Muc2	Goblet cell	–	–
Intestinal trefoil factor	Tff3	Enterocyte, goblet cell	+	+
Ephrin type B2 receptor	EphB2	Villus	+	++
Ephrin B2 ligand	Efnb2	Villus	+++	+++
Hairy enhancer of split-1	Hes1	Stem, enteroendocrine, paneth cells	+	++
Musashi-1	Msi2h	Stem cell	+	++
Notch 1	Notch1	Stem cell	+	++

5.5.5 Heart Injury Cell Models

Heart injury caused by compound treatment may be due to direct action at the cardiac myocyte but may also be due to indirect effects on cardiac function, such as hemodynamic changes, that eventually may lead to cardiac myocyte death. Mirroring those indirect effects on cardiac myocytes in a single cell in vitro model is very challenging if not impossible. Until the advent of iPSC-derived human cardiac myocytes, researchers have been limited to cell models that recapitulate only partially the attributes of an adult cardiac myocyte. Primary cardiac myocytes are difficult to isolate and maintain in culture; neonatal rat ventricular myocytes (NRVM) are very fetal in nature and often do not display a uniform synchronous beating pattern, and the rat H9c2 cardiomyoblast cell line, though expressing a number of cardiac myocyte-specific genes, lacks the ability to spontaneously beat in culture [63]. Now with the availability of human iPSC-derived cardiac myocytes, many of the shortcomings associated with earlier cardiomyocyte cell models have been addressed (Table 5.5) [64].

Table 5.5 Comparison of rat H9c2 cardiomyoblast cell line to human iPSC-derived cardiac myocytes for a number of indicators of cardiotoxicity. With the exception of contractile activity, little difference was seen between the two cell models

	Cytolethality-LC50			Mitochondrial tox-IC50		Cardiac stress gene panel: fold induction	Peak frequency/BPM increase EC50
	Doxorubicin	Staurosporine	Imatinib	Rotenone	Imatinib	Imatinib	Isoproterenol
Rat H9c2	98 μM	20 μM	39 μM	0.1 μM	54 μM	22.3	Does not beat
Human iPSC-CM	10 μM	38 μM	0.1 μM	59 μM	21.7	22 nM	10 μM

5.5.6 Skeletal Muscle Injury Cell Models

Skeletal muscles represent a considerable mass in the organism (36–42% of body mass in human adults) [65] and have a key role in regulating overall organismal bioenergetics and thus are a major target for xenobiotic-mediated injury. Although abundant and grossly similar in morphology, skeletal muscles are not a uniform tissue as location and function of skeletal muscle fibers vary and thus their susceptibilities to toxicants also vary accordingly. "Fast twitch" glycolytic fibers respond differently than "slow twitch" oxidative fibers, and different muscle groups can be one form or the other or a mix of both types of fibers. Skeletal muscle, like cardiac muscle, is a bioenergetically active tissue susceptible to injury either directly or indirectly. Skeletal muscle injury by drugs such as statins and PPAR agonists can be mirrored by in vitro models consisting of both cell lines such as the mouse C2C12, and rat L6 and H9c2 cell lines, and primary myoblasts isolated from animal and human muscle tissue. The availability of human iPSC-derived skeletal myoblasts should allow for a consistent source of cells for screening for skeletal muscle injury risk [66].

5.5.7 Injection Site Irritation

Injection site reactions (ISRs) are a common occurrence with parenteral drugs, and few in vitro assays exist which accurately predict the occurrence of ISRs in vivo. Small molecules are often developed as parenteral compounds to increase bioavailability or to avoid intestinal toxicity, whereas all biologics are developed as parenteral products. Because ISRs are an acute local toxicity at the injection site, normal in vitro cytolethality assays, which are designed to predict chronic systemic toxicities, are not predictive for ISRs. To screen for ISR potential prior to running in vivo studies with parenteral compounds, L6 rat myoblasts are differentiated into myotubes and treated with compounds formulated in 5% mannitol and adjusted to pH 4.0–10.0 to maintain maximal solubility [67–70]. Cell membrane perturbation is measured by conversion of nonfluorescent calcein-AM to fluorescent calcein, where lower fluorescence is indicative of higher ISR potential. We have shown that acute membrane perturbation, as measured by decreased fluorescence, correlates to a high extent with clinical ISRs for small molecules (Table 5.6). One important observation is that many of the small molecules inducing clinical ISRs are dosed at a higher dose concentration than the measured L6 IC50. For example, doxorubicin is dosed at 2 mg/mL, which causes nearly 100% cell membrane perturbation in the L6 assay at this concentration in the absence of cell death, but the L6 IC50 is tenfold lower at 0.2 mg/mL. For this reason, the L6 assay has been used not only to prioritize compounds for lowest ISR risk but also for optimizing dose concentrations for in vivo experiments as well as selecting alternative formulations to minimize risk of ISRs. It is important to note that a percentage of ISRs are caused

Table 5.6 L6 correlates with clinical ISRs

Drug	Clinical ISR (Y/N)	Clinical dose concentration (mg/mL)	L6% cell membrane perturbation at clinical dose concentration	L6% cell death at clinical dose concentration	L6 IC50 (mg/mL)
Doxorubicin	Y	2	97	0	0.2
Mitoxantrone	Y	0.5	88	0	0.1
Vinorelbine	Y	2	84	2	0.8
Metoprolol	N	1	0	0	>10
Atenolol	N	0.5	0	0	>10

by large molecules, which have been suggested to have an immune component associated with the manifestation of inflammation and necrosis at the injection site [71]. However, while the L6 assay might have utility to understand structure and charge-related irritation associated with large molecules, ISRs due to immunogenicity might not be picked up with this assay and new tools will need to be explored.

5.5.8 Hematopoietic System and Hematopoiesis

Hematopoiesis is the process by which bone marrow stem cell progenitors give rise to the mature cell populations of circulating peripheral blood in animals. Injury to the bone marrow compartment causes hematological toxicity, or hematotoxicity, and is frequently observed in administration of drugs eliciting antiproliferative effects. Hematotoxicity, leading to myelosuppression and neutropenia, is the most common clinical dose-limiting toxicity (DLT) encountered during development of oncolytic therapies. The antiproliferative effects are often desired pharmacology of oncolytics but manifest as undesired or off-target pharmacology in other therapeutic classes. In vitro cell-based models such as the bone marrow progenitor or colony formation unit (CFU) assays are utilized to measure off-target risk for a given set of candidate drugs, but, perhaps more importantly, to measure and help predict the clinical risk profile of neutropenia [72].

The in vitro CFU assays are highly specialized clonogenic assays and are utilized to measure the differentiation and proliferative capacity of specific hematopoietic progenitor cells. The assays are guided by incubation with specific cytokine cocktails that promote differentiation and growth of primitive hematopoietic cells. Qualitative endpoints of hematotoxicity include the following: measurement of multiple lineages, scheduled treatment (continuous vs. pulsed exposure), combination treatment, multispecies sensitivity, and rank order of a chemical series [73].

Although there are many CFU assay endpoints used to measure direct effects on bone marrow lineage precursors, the key assay endpoints used in early safety assessment are CFU-GM (myeloid, granulocyte-macrophage progenitor) and BFU-E (erythroid progenitor). These progenitors give rise to the myeloid and erythroid cells that differentiate and proliferate to form components of the peripheral blood. Drug-induced reduction of hematopoietic progenitors are measured in each of these assays and used to predict multiple elements of hematotoxicity. The CFU-GM assay has served as a "gold standard" for predicting the clinical maximum tolerated dose (MTD) and plasma concentrations where neutropenia is likely to occur [72].

Under treatment conditions lasting for the duration of the assay, typically 1–10 days, inhibition dose response curves can help the in vivo plasma concentrations predictive of a clinical grade III neutropenia [73]. Treatment time periods in the assay are typically continuous that last throughout the 7–10-day assay duration. However, noncontinuous or "pulsed" followed by washout treatment periods allows flexible exposure time of the progenitor cells to the test molecule. Pulsed exposure times, e.g., $\leq$24 h, have been applied to investigate effects of targeted (cell cycle kinase inhibitor) therapies [74]. Differential effects in the CFU assays are observed with pulsed vs. continuous exposures, and these effects can be applied to predictive PK/PD models that predict a safer and more effective clinical starting dose [73]. Additional noncontinuous treatment periods, compounds can be added in combination and in a pulsed or continuous treatment to evaluate combination therapy effect on bone marrow progenitors.

Current formats of the assay and utilization of appropriate cytokine cocktails allow for multispecies comparison. Species-specific effect can be evaluated in order to help reduce animal toxicity studies and identify the most sensitive preclinical species that may translate to a clinical risk.

CFU assays are lower throughput and reflect accurate effects on mechanism due to the longer 7–10-day incubation periods in the culture system. However, when higher-throughput efficiency is needed to screen potential drug candidates, alternative models are useful. Suspension cell cultures using a variety of cell types (e.g., mononuclear bone marrow cells, CD34+ cells, myeloblastic cell lines, etc.) are incubated with compound and assessed for phenotypic changes. Higher content technologies are used to assess cell viability, cell density, and proliferative index [75]. Subsequently, these higher content and throughput assays serve as an effective prefilter to the CFU assay such that only safest profile candidates are evaluated for predictive neutropenia risk.

Preclinical hematotoxicity may be driven by multiple biological, chemical, and physicochemical properties. An effective strategy includes the use of multiple tools to minimize or mitigate hematopoietic toxicity leading to neutropenia or other cytopenias**.** Intrinsic chemical properties, like lipophilicity and basicity, overt toxicity to nonproliferating cells, and higher-throughput viability/proliferation assays, should be incorporated in a screening strategy prior to subsequent use of more definitive bone marrow CFU assays. This strategy minimizes expense and maximizes probability of true positive results by minimizing the number of false positives due to chemical-based or off-target toxicity (Fig. 5.8).

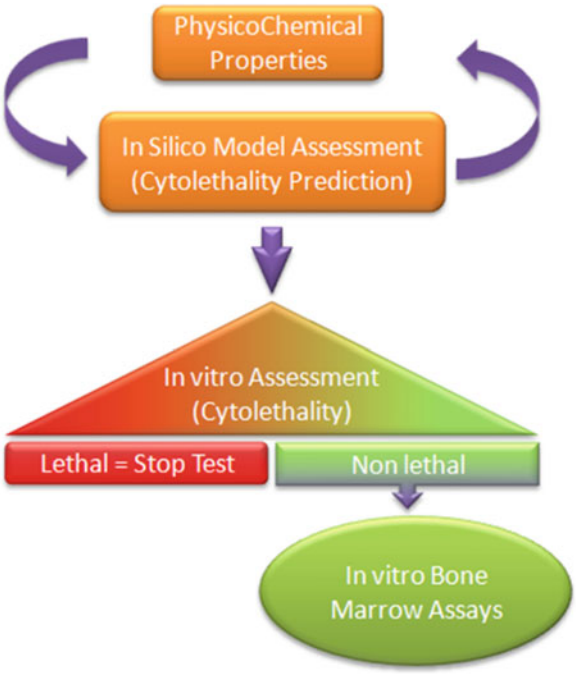

Fig. 5.8 Cell-based strategy designed to limit overt chemical-based toxicity in the highly specialized bone marrow progenitor colony formation assays

5.5.9 iPSC-Derived Cell Models

The advent of human stem cell technologies, especially the ability to produce induced pluripotent stem cells (iPSC), has provided an opportunity to address long-standing limitations on the use of in vitro cell models for risk screening, including the lack of species relevance, lack of phenotype that reflects the in vivo environment, tumor-derived cells, and high variability/poor reproducibility using primary cells [76].

The iPSC technology allows for a uniform and continuous source of cells with the same genetic background which can be used to generate any differentiated cell population present in the organism, as long as the appropriate factors driving the differentiation are understood [77]. As the iPSC reprogramming approach can be done starting with any somatic cell, moral and ethical issues concerning the use of human embryo-derived cells are avoided. Another significant advantage that the iPSC approach provides is the ability to generate patient-specific cells, which will contain the genetic background associated with that patient's particular condition or disease, allowing for characterization of toxicity in the context of the disease state (Fig. 5.9) [78–80].

Several human iPSC-derived cell types of toxicological interest are available in industrial amounts from commercial sources. The most widely used cell types are cardiac myocytes, hepatocytes, and neurons. Additional iPSC-derived cell types, including endothelial cells, skeletal myoblasts, astrocytes, and macrophages, are

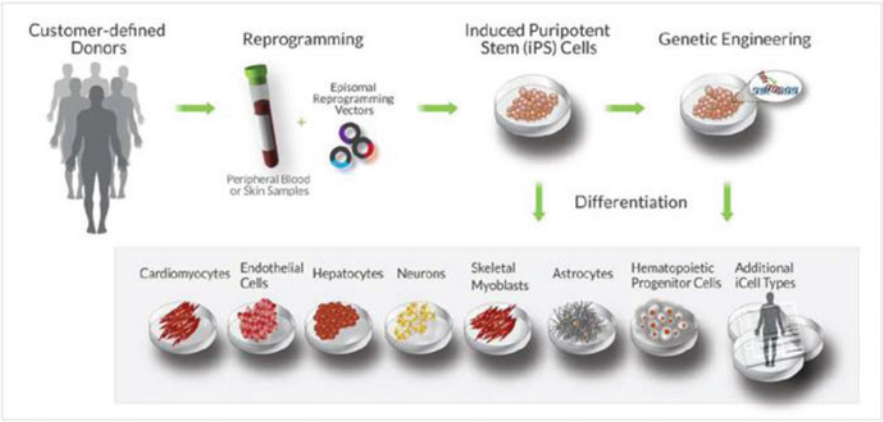

Fig. 5.9 Using iPSC technology, organ toxicity in humans can be assessed in the relevant cell type from both normal and diseased patient populations (Figure courtesy of Cellular Dynamics International, a FUJIFILM company)

available or are being developed but have a more limited utility for toxicity profiling. The ability to more fully characterize functional endpoints such as cardiac contractility and neuronal synaptic plasticity in an accessible and reproducible experimental format provides a significant advantage over previous cell models.

Although iPSC-derived human cells have proven to be a valuable tool for in vitro cell-based safety assessment, there are several negatives associated with the cells, which may in certain cases impact the overall utility of the cells. Differentiated cells derived from iPSCs tend to be somewhat immature in nature in their gene/protein expression patterns and resulting functional attributes. Maintenance of the cells in culture for longer durations ameliorates the fetal nature of the cells to some degree but not completely. Possibly related to the somewhat immature nature of iPSC-derived cells is the observation that their epigenetic marks may differ from a differentiated cell derived from a pluripotent stem cell of normal embryonic origin and may actually contain epigenetic marks of the somatic cell used to generate the iPSC. This may not be a concern depending on the test articles and endpoints being evaluated but should be taken into account if epigenetic impacts are anticipated. Finally, from a purely logistical standpoint, the expense and somewhat more complex culturing conditions of the cells make them a more challenging choice for extensive screening applications. The iPSC-derived human cells are likely best used in a tier-2 setting where prefiltered molecules can be evaluated for specific mechanistically relevant functional endpoints.

The iPSC-derived cell model that has received the most attention and which is most widely used today in safety assessment is the human cardiac myocyte model. The ability of the iPSC-derived cardiac cells to beat with spontaneous rhythm when grown densely enough to form a syncytium provides a useful model for assessing impacts on contractile function of cardiac myocytes in an in vitro setting. Using

various analytical approaches, such as measuring cytoplasmic calcium flux, probe-free cell shape monitoring, and membrane voltage potential change, many groups have shown that these cells have the ability to correctly identify and categorize known cardioactive compounds [81–83]. In addition to the iPSC-derived cardiac myocytes, the development of iPSC-derived human neurons has provided toxicologists with the ability to grow a homogeneous population of synaptically active human cortical or peripheral neurons for assessing aspects of neurotoxicity, including neurodegeneration, impaired synaptic activity, and seizure induction [84, 85]. Development of hepatocytes derived from human iPSCs would be a particularly attractive cell type as drug-induced liver injury (DILI) is a major drug development concern, and nonhuman in vitro models are poorly predictive of effects in the human patient population [86].

5.5.10 Microphysiological Culture Systems

Microphysiological organotypic culture systems are rapidly advancing to more readily create in vitro tissues/organ models by co-culturing in appropriate ratios, and often in a three-dimensional architecture, defining cell types that comprise an organ or complex tissue [87]. These platforms incorporate complex factors found in vivo, including extracellular scaffolding, three-dimensional structure, cellular interactions, perfusion, biomechanical stresses, electrical stimulation of excitable tissue, and hormone responses to list a few. These features are present in preclinical animal models, but some aspects of animal physiology do not accurately represent those of humans. National Institutes of Health (NIH), Food and Drug Administration (FDA), and the Defense Advanced Research Projects Agency (DARPA) collaborated to launch the Microphysiological Systems (MPS) Program in 2012 (http://www.ncats.nih.gov/research/reengineering/tissue-chip/tissue-chip.html). Ten major organ systems were identified for funding as part of this program (intestine, liver, central and peripheral nervous system, blood-brain barrier, vascular system, skeletal muscle/innervated motor unit, heart, lung, kidney, and female reproductive system) along with key contributions from bioengineering, stem cell biology, cellular and molecular biology, physiology, toxicology, and pharmacology. In the end, these unique culture platforms are being funded to offer viable options for surrogate human tissue testing.

5.6 In Vivo Biomarker Screens

Biological markers (biomarkers) are objective indications of disease, injury, or pharmacology that can be measured accurately and reproducibly in an organism [88, 89]. When applied during in vivo drug development studies, biomarkers can give a sensitive and quantitative measure of test article-related tissue injury or

changes in homeostasis. As part of early safety assessment, the use of in vivo biomarkers is a step in the progressive dedication of resources as a project advances. With the goal of assessing a compound's safety as early as possible, the inclusion of biomarker measurements makes sense after in silico and in vitro endpoints have been utilized to prioritize compounds more likely to be tolerated in animal studies and as soon as in vivo studies are conducted. The use of biomarkers has been encouraged by the US Food and Drug Administration to reduce the time and cost of drug development [90].

The "decision gate" analogy is a useful paradigm in drug development. It holds that drug development can be divided into several go/no-go decisions, including whether the drug works in humans, and whether it can be marketed [91]. Lead optimization is an important decision gate, during which a molecule's suitability for human dosing is determined by establishing the maximum tolerated dose and dose-limiting toxicity in nonclinical studies. Early safety assessment supports this process by assessing a molecule's safety as early during development as possible, helping to narrow the possible candidates to safer choices. Elimination of molecules with strong structural similarities to known toxicants or undesirable effects on cultured tissues, such as cytotoxicity, and those that cause changes (often increases) in safety biomarkers during in vivo studies improves the chances of success in finding a molecule that will be successful during lead optimization and tolerated in human studies.

Each tool available during early safety assessment supports application of the next. As we have discussed, a thorough understanding of the risks associated with target modulation, based on available literature and previous experience, informs the entire project, predicting lesser or greater investment of resources based on lower or higher risk of dose-limiting toxicity and possibly pointing toward specific target organs. In silico modeling and in vitro screening help initially narrow the number of molecules to choose from based on previous experience with similar chemistries and direct effects on cells in culture. After these tools have helped prioritize which molecules are more likely to be tolerated in human studies, those with the ability to bind to the target and some measure of bioavailability are chosen for the first in vivo studies, often in a mouse model of a disease state consistent with the intended indication for the drug program. As development progresses and molecules show efficacy and tolerability in mouse models, rat studies may be conducted at higher doses to begin to identify target organ toxicity and establish maximum tolerated doses in order to set dose levels for longer duration rodent studies (e.g., 14 days to 3 months) and studies in larger animals during nonclinical safety assessment. The application of blood- and urine-based biomarkers during these initial studies in mice and rats will be the focus of this section.

The use of biomarkers in early safety assessment is dependent on the availability of assays for safety-related endpoints or the investigator's ability to develop an assay for the desired endpoint. Platforms commonly used to measure biomarkers in blood samples include (but are not limited to) enzyme-linked immunosorbent assays (ELISA), electrochemiluminescent immunosorbent assays (ECLIA), polymerase chain reaction (PCR), mass spectrometry, and enzyme activity assays.

Common blood chemistries used in human patients can also be included in rodent assays, including (but not limited to) albumin, total globulins, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, direct and total bilirubin, blood urea nitrogen (BUN), calcium, chloride, cholesterol, creatinine, gamma-glutamyl transferase, glucose, iron, phosphate, potassium, total protein, sodium, triglycerides, and complete and differential blood counts. These endpoints offer insight into liver function, kidney function, muscle injury, metabolic state, acid/base balance, immune status, hydration status, and hematopoiesis. They come with the advantage of decades of investigation into their biological significance with well-established reference intervals allowing the flagging of concentrations above or below typical values in healthy animals, including rodents. Results are best interpreted with the aid of a trained pathologist certified by an organization such as the American College of Veterinary Pathology.

Safety biomarkers can also include more recently developed "novel" markers, such as cardiac troponins I and T [92]. In a short (2-day) mouse screen, cardiac troponin I (cTnI) was used to rapidly explore the structure-activity relationship (SAR) of a large number of molecules in mice administered two oral doses, allowing higher throughput than would be possible with histopathology focused studies [93]. Public-private consortia continue to advance the science of safety assessment using biomarkers targeted for both nonclinical and clinical studies [94]. In focus groups dedicated to a specific target organ injury and in collaboration with the US Food and Drug Administration (FDA), these consortia qualify clinically relevant biomarkers for use in preclinical and clinical drug development studies [95–98].

Used in conjunction with histopathology, in vivo biomarkers can increase the sensitivity and quantitative value of early safety assessment studies; however, by combining multiple biomarkers into panels and forgoing direct examination of tissues, the number of molecules that can be assessed can be increased. In rat studies, the relatively greater amount of serum or plasma available (compared to mice) allows for the combination of multiple novel biomarkers and traditional clinical chemistries into a more comprehensive biomarker screen. For instance, the use of kidney injury markers measured in urine, such as osteopontin (OPN) or kidney injury molecule-1 (KIM1); blood-based markers, such as cardiac and skeletal troponins I, natriuretic peptides, and microRNA-122 (miR122); and markers of inflammation, such as lipocalin-2 (LCN2) and tissue inhibitor of metalloproteinase-2, allows for the detection of major target organ toxicities, such as kidney, heart, skeletal muscle, and liver, and general or systemic inflammation [99–102]. Expectation of other target organ toxicities through literature-based target evaluation, in silico modeling, or previous target experience may require the addition of other biomarkers, or histopathology if no suitable blood-based markers are available. For example, the use of pancreas-specific microRNAs may be warranted for targets in the pancreas; however, the expectation of lesions in the brain may warrant histopathology [103].

Changes in biomarker concentrations outside established reference intervals, or outside the range established by a control group, should be considered evidence of

possible organ injury. When used in a panel, cumulative changes in multiple biomarkers can be regarded as strong evidence of compound-related toxicity. Similarly, greater magnitude of change from control may indicate more severe injury. Taken together, molecules that cause changes in multiple biomarkers, or changes of greater magnitude, can be deprioritized compared to molecules that cause changes in fewer markers, or changes of lesser magnitude. Preferably, molecules that cause no changes in biomarkers or clinical chemistries in short duration screens (e.g., 2–4 days) can be advanced into longer duration safety assessment studies.

5.7 Technologies

5.7.1 Multiplex and High-Content Approaches

The ability to measure multiple endpoints (high content) or multiple targets (multiplex) in the same sample has become a well-accepted and widely utilized approach in cellular and molecular biology [40]. Multiple endpoint measurement is best exemplified by the high-content imaging of cells, where using a set of probes, each with unique tagged properties and each measuring different cellular endpoints or components, can provide valuable information about what is occurring in individual cells or populations of cells. As an example, using the high-content imaging approach, one can measure viability (nuclei staining), cytoskeleton complexity (actin staining), ROS content, and mitochondrial integrity all in the same cell. This high-content imaging capability has become possible with the simultaneous development of new sensitive and specific fluorescent probes and tagging approaches coupled with state-of-the-art multi-camera imaging systems [104, 105]. Multiplexing of analytes (of the same type) has also become commonplace. Examples that come to mind are monitoring the transcriptome by gene arrays (Affymetrix) and interrogating panels of cytokines by xMAP bead-based technologies (Luminex). Both of these multiplex approaches have been made possible by the development of highly specific probes that can reliably detect specific analytes (mRNAs or proteins) coupled with sensitive detection systems.

Live content imaging of cells, which can be defined as the acquisition, analysis, and quantification of images from living cells that remain unperturbed by the detection method allowing for repeated measurements over long periods of time (days to weeks), is the latest advance in sophisticated high-content cell imaging approaches [106]. The value of obtaining these kinetic readouts versus endpoint readouts, particularly for early safety assessment, has not been clearly elucidated. One case where there may be value for using this approach is in assessing the impacts of compound treatment over time on neurite outgrowth in neuronal cultures [107]. The application of both high-content and multiplex approaches in ESA allows safety screening for more mechanistically based or multifactorial toxicities where cell viability measurements alone are insufficient for characterizing compound treatment impacts on cells.

5.7.1.1 Gene Expression Approaches (Toxicogenomics)

A fundamental response of a cell to xenobiotic perturbation is often an alteration of gene expression. Therefore, monitoring changes in the cell mRNA population is a useful tool for assessing cellular injury in response to exposure to a xenobiotic agent. Approaches for the analysis of these gene transcript changes can range from global analysis of the entire mRNA population via microarrays or RNA sequencing, DNA sequencing or interrogating individual transcripts using QPCR, or branched-chain DNA assays.

Global transcript analysis using statistical-based analysis of gene expression data has allowed for the development of gene signatures that can be diagnostic for various toxicological endpoints [108]. In addition to using panels of genes as biomarkers for direct screening, gene expression analysis, because of its high informational content, can provide insight into mechanisms of compound mediated toxicity, which can lead to the development of appropriate mechanism-based screening assays [109]. Various gene enrichment statistical methods, coupled with extensive gene ontology and knowledge-based systems, allow for the identification of causal signaling or metabolic pathways and regulatory networks that may underlie the observed toxicity [110]. Coupled with the development of sophisticated gene expression analysis tools has been the generation of very large comprehensive toxicogenomic databases that link gene expression data with extensive phenotypic and pathology data for a large number of compound treatments in rats. While these large toxicogenomic databases have enhanced the power of gene expression analysis for predicting compound treatment-induced injury, coupling these tools with techniques such as cellular knockouts (i.e., CRISPR/Cas9) and ChIP-seq will allow for deeper understanding of the underlying mechanisms of cellular injury [111].

5.7.1.2 In Vitro Measurement of Cellular Electrical Activity

Improvements in relevant cell models and in electrical activity/excitability measurement technologies has provided drug safety scientists with the ability to more easily evaluate xenobiotic treatment effects on populations of electrically active/excitable cells (neurons, cardiac and skeletal myocytes). This enhanced ability has had a significant impact on assessing compound-mediated neurotoxicity and cardiotoxicity earlier in drug development [112]. The advent of iPSC-derived human cardiac myocytes and neurons has promoted higher-throughput approaches for in vitro evaluation of compound treatment impact on electrical activity in neurons and cardiac myocytes. The advantage to using iPSC-derived human cells is their consistent phenotype and low variability. Using these cells, coupled with multi-well multielectrode array technologies (MEA), which sensitively measure changes in cellular membrane potential, gives researchers the ability to screen large numbers of compounds for undesired impacts on cellular electrical activity [113].

5.7.1.3 Gene Editing

Throughout drug development, there is often a need to understand mechanism of toxicity, such as determining if a given finding is on- or off-target. Recent advances in molecular biology have allowed for the manipulation of genomic DNA, mRNA, and even proteins to allow for the interrogation of these concepts. One of the most basic experiments to understand mechanism of toxicity, in an in vitro system, is to prevent protein translation of a suspected on- or off-target mRNA by small interfering RNA (siRNA) or short hairpin RNA (shRNA). The resulting protein knockdown allows for further experimentation, including many of the assays described in previous sections, to understand if functional changes at the cellular level are due to the protein of the interest [114]. While siRNA allows for rapid turnaround, shRNA allows for the generation of stable knockdown cell lines, allowing for the ability to propagate cells for multiple functional assays.

It is important to note that while both siRNA and shRNA provide results in a very short time period, they are only knockdowns. Recent advances in molecular biology have enabled scientists to generate in vitro knockout cell lines and even perform homologous recombination to generate nonfunctional protein using CRISPR/CAS9 [115]. CRISPR/CAS9 is composed of two main elements: 1) a guide RNA (gRNA) which targets a specific sequence and 2) the Cas9 protein which creates a double-stranded break on the DNA. The Cas9 protein requires a conserved DNA sequence called the protospacer adjacent motif (PAM) just upstream of the gRNA binding region. Many newer techniques utilize lentivirus delivery systems to introduce both the gRNA and Cas9 with selectable markers prior to clonal selection. If the goal is homologous recombination, several publications have shown it to be helpful to introduce an inducible version of Cas9 followed by the gRNA with a separate selectable marker in combination with a nonhomologous end joining inhibitor [116, 117].

Following characterization and functional analysis of either knockdown or knockout cells, it is often useful to develop a high-content assay using either a promoter or 5′ untranslated region of mRNA tagged to luciferase or GFP for a gene of interest. This will allow for rapid compound screening after the known mechanism of toxicity has been established. Additionally, proteins within a biological pathway themselves can be tagged with fluorescent proteins. This is a useful technique, especially in combination with CLICK chemistry, which enables the scientist to image co-localization of the molecule itself to a specific organelle.

5.8 Organizational Framework for Early Safety Assessment Activities

Small molecule drug discovery in general follows a process that is well established across large pharma. Using this framework, our toxicology organization has built tools and established cross-functional partnerships to embed early safety

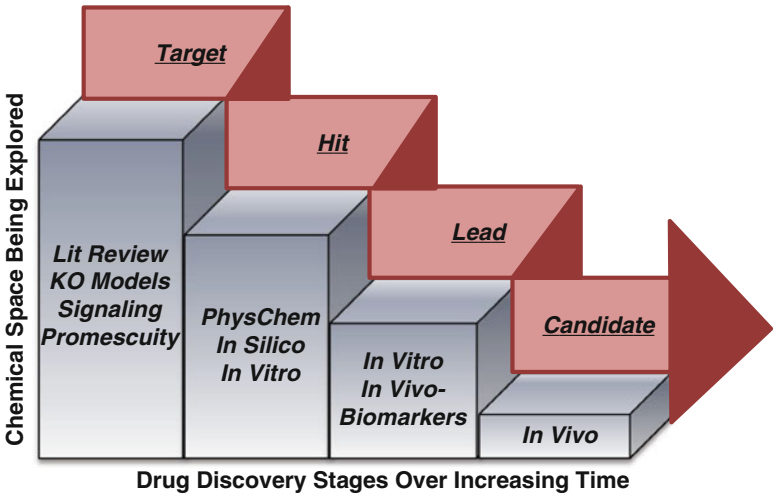

Fig. 5.10 Organizational framework based on the general pharmaceutical development pipeline

assessment into the general drug discovery process. As depicted in Fig. 5.10, the elements discussed within this chapter establish an organizational framework through which early safety assessment can be effectively implemented through each step of the drug discovery process.

While implementation of screening tools and data generation are key elements of the organizational framework, one often overlooked element is the application of these data in a decision-making process. One approach that seems to be most effective is weight of evidence. In this approach, the data for a given structural series or scaffolds are combined to make a judgment-based decision. While this process is more ambiguous, alternative considerations can be included such as therapeutic indication. An alternative approach is a rule-based approach where clear cutoffs are defined for individual assays with compounds being classified as positive or negative. In this rule-based approach, compounds are clearly classified leaving little room for ambiguity. This approach often suffers from high false-positive rates to ensure false-negatives don't slip through the screening process. The application of biostatistics coupled with decision science principles can be used to define the best approach given the data streams available to an organization.

5.9 Summary

Safety assessment in early drug discovery has made significant advancements over the last 20 years through the application of in silico and in vitro models and the development of numerous screening modalities such as high-content imaging and genomic profiling along with the development and application of key target organ-based

biomarker panels. More recently advances in next-generation sequencing have enabled the application of RNA-Seq and ChIP-seq technologies along with the advancement of gene editing tools that can be used to evaluate the impact of gene silencing. With these advancements, molecules entering live-phase animal testing have improved properties leading to a higher probability of technical success, thus reducing animal consumption and overall dwell time before pivotal first in man safety and efficacy testing.

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Part III
Optimizing Lead Molecules into Drug Candidates

Chapter 6
Integrated Lead Optimization: Translational Models as We Advance Toward the Clinic

Bianca M. Liederer, Xingrong Liu, Simon Wong, and Daniel R. Mudra

Abstract Drug discovery requires the convergence of molecular attributes including magnitude and duration of exposure, tissue distribution, target engagement, and pharmacological action. To this end, during lead optimization, discovery scientists must leverage integrated data sets and translatable models to offer projections of clinical performance and thereby make informed decisions on the merits of individual molecules. This chapter presents methodologies to predict human clearance, drug-drug interaction (DDI) risk, and penetration of the blood-brain barrier (BBB) and exposure to the central nervous system during various stages of discovery with emphasis on immediate preclinical stages. By focusing on current state and best practices of the contemporary lead optimization scientist, we discuss the use of human-derived model systems and multiparameter optimization to drive the discovery of clinical candidates with favorable human ADME/PK properties in mind. We present strategies to predict and mitigate DDIs at different stages of drug discovery and development by evaluating CYP involvement in metabolism as well as achieving an assessment of a DDI's clinical significance. We introduce concepts related to brain penetration from the perspective of small molecule drug discovery and discuss how to effectively address BBB issues in lead optimization. Emphasis is given to creation and application of preclinical data and methodologies that provide a mechanistic understanding of drug disposition leading to translatable models to predict clinical outcomes, assess developability risk, and help address simple to complex "what-if" scenarios. Predictive models of clearance, CNS penetration, and DDIs will be presented and discussed including comprehensive case studies to highlight integrated approaches used to discover drug candidates suitable for the safe exploration of clinical hypotheses.

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Keywords Drug-drug interaction • Human clearance • Brain penetration • Translational models • Preclinical lead optimization

6.1 Introduction

The discovery of molecules suitable for testing pharmacological hypotheses in the treatment of human disease requires a convergence of attributes including, but not limited to, safety, magnitude, and duration of exposure, tissue distribution, and target accessibility. Prior to 1988, unexpectedly poor human pharmacokinetics (PK) was the leading cause of failure in clinical trials, accounting for nearly half of all development program terminations [1]. A subsequent analysis revealed that between 2000 and 2010, out of 157 oral compounds entering phase 1 clinical trials, only 25 terminations (16%) were due to unexpected human PK [2] suggesting that technical advances in predicting PK have manifested positive results in clinical development. However, another report attributed up to 30% of clinical attrition from 2005 to 2010 to an inability to achieve sufficient exposure at the therapeutic target and, with that, an inability to achieve clinical efficacy [3] suggesting that there are still improvements to be made in translating preclinical to clinical data. Meanwhile, the cost of pharmaceutical research and development (R&D) continues to increase even as fewer innovative drugs achieve FDA approval [4]. A series of analyses reviewing the last 25 years of R&D (adjusted for inflation and presented in 2015 US dollars) spending and successful drug approvals demonstrated that the average cost of bringing a single drug to market increased from $490 million in 1991 to $1.1 billion in 2001 to $3.6 billion in 2013 [5–7]. Together, the evolving clinical attrition data and the economic statistics illustrate that the research engine responsible for designing, discovering, and developing novel therapies for human diseases and disorders is becoming increasingly strained over time and those responsible for its caretaking must consider if the continued cost of failure will, at some time, become unsustainable. Therefore, opportunities to improve the predictive accuracy of clinical performance stand to produce benefits for both the sustainability of research programs and the patients they serve.

In recent decades, laboratories from around the globe made important advances in biopharmaceutics and oral absorption [8–10], drug metabolism enzymology [11], tissue distribution [12–14], and the understanding of drug-disease, drug-food, and drug-drug interactions [15–17], changing the way discovery scientists evaluate molecules for potential clinical testing. The subsequent emergence of broadly accessible, high-quality, specialized tools, such as human-derived systems for the study of absorption or metabolism, and improved methodologies to better connect preclinical data with clinical outcomes, for example, in vitro-in vivo extrapolation (IVIVE) and physiologically based pharmacokinetic (PBPK) models, has begun to significantly decrease the likelihood that poor human PK or insufficient unbound drug exposure at the pharmacological target will cause the termination of clinical development.

An advanced appreciation has developed within the industry for the importance of focusing on drug-like properties throughout the course of drug discovery. The process of molecular design and drug discovery is most often initiated by a subregion of "drug space" defined by one or more potent target "hits." And while the therapeutic target might often bias the structure-activity relationship (SAR) to a particular region [18], it is the objective of a "hit expansion" phase to test the regional boundaries of potency and in doing so identify, where possible, regions that trend toward enhanced drug-like properties. In the past, it was not uncommon for discovery teams to find themselves discovering molecules in a chemical space rich with exquisitely potent molecules (e.g., IC_{50} values <1 nM) but which produced few, if any, with drug-like properties often as a result of the hydrophobic character favored by target ligand-binding sites. Such focus on target binding tends to produce "high-affinity ligands" that bear structural attributes (e.g., high log*P*) that impart extensive tissue binding and/or enzymatic lability, resulting in an insufficient exposure of circulating free drug [19]. The risk of an SAR producing local minima rich with high-affinity ligands that present with few if any drug-like properties, also known as a "high-affinity trap" [20], can be lessened by installation of ADME resources early in the discovery process, in particular at the time of hit and pre-lead as described by Joshi et al. in Chap. 3 and elsewhere [19, 21, 22]. By the time a lead is declared, identification of key ADME issues endemic to an otherwise promising chemical scaffold is critical to establishing vectors in a chemical space that can result in the convergence of potency and drug-like or human ADME properties. During optimization of the lead, it is imperative to turn attention toward integrated data sets and translatable models that offer reliable projections of clinical performance and thereby permit informed decisions on the merits and value of individual discovery molecules. Based on these models and the best available data, if the putative clinical candidate can be expected to perform in a manner that allows for testing of clinical hypotheses (i.e., safety and efficacy) and has an acceptable probability to be developed as a drug product, then a decision to advance to clinical development is reasonable. Alternatively, if the data and models predict untoward risk in the clinic (be it uncertainty in the projection of human PK or the likelihood of drug-drug interactions), then a data-driven decision can be made to return to unexplored chemical options until such time as a suitable molecule can be found.

This chapter presents the current state and best practices of the contemporary lead optimization scientist using integrated preclinical data sets, human-derived model systems, and multiparameter optimization to drive the discovery of clinical candidates with human ADME/PK properties in mind. Emphasis is given to creation and application of preclinical data and methodologies that provide a mechanistic understanding of drug disposition leading to translatable models to predict clinical outcomes, assess developability risk, and help address simple to complex "what-if" scenarios. Predictive models of clearance, central nervous system (CNS) penetration, and drug-drug interactions will be presented and discussed including comprehensive case studies to highlight integrated approaches used to discover clinical drug candidates suitable for the safe exploration of clinical hypotheses.

Note:

*This chapter focuses on the principles and application of a variety of prediction methodologies including advantages, disadvantages, and applications in the contemporary industrial drug discovery setting. It will not, as a general rule, address technical aspects of assay conduct or variability in underlying laboratory measurements (*e.g.*,* in vitro *or* in vivo *models and/or associated bioanalytical methods). It is acknowledged that technical differences and variability in model systems can contribute significantly to apparent statistical outliers and/or differences between laboratories. This is not intended to be an exhaustive delineation of techniques nor a comprehensive comparison of all available methodologies. For such information the reader is guided to the numerous references included herein. Rather, we present a collection of methods demonstrated to present applicability with integrated* in vitro *and* in vivo *data sets with high translatability and prediction accuracy*.

6.2 Integrated Approaches to Assess and Predict Human Clearance

Clearance (CL) is the volume of plasma or blood from which drug is completely and irreversibly removed per unit time. Expressed in units of volume/time, it is a simple relationship between the amount of drug in the body (Xo) and exposure as shown in 6.1

$$\mathrm{CL} = \frac{Xo}{AUC_{0-\infty}} \tag{6.1}$$

where $AUC_{0\text{-}\infty}$ is the area under the concentration-time curve from the time of dosing to infinity. This expression illustrates that clearance of drug from plasma or blood is the sole determinant of the dose required to elicit a particular exposure (AUC = Do/CL). Therefore, if AUC correlates with an efficacious outcome, drug clearance (assuming intravenous administration; more on oral administration will be presented later in this section) determines the dose at which efficacy can be achieved. For this reason, understanding the molecular properties associated with clearance pathways and the prediction of clearance in humans is often of central importance in a lead optimization (LO) drug discovery program. Clearance predictions are typically conducted by one of two distinct approaches. Allometric scaling is an empirically derived, regressive relationship with which clearance for a "standard" human (e.g., 70 kg body weight) can be extrapolated or interpolated from in vivo pharmacokinetics observed in preclinical species [23–28]. Alternatively, physiologically based or mechanistic scaling methods are reductionist approaches where drug properties (e.g., permeability, intrinsic clearance in microsomes or hepatocytes, and plasma protein binding) are applied to mathematical

models of physiological systems (e.g., systemic and/or organ blood flow models) to predict pharmacokinetic outcomes [24, 29–36]

6.2.1 Allometric Scaling

Following seminal work by Thompson and later by Snell, both of whom reported on relationships between body size and anatomical structure, Sir Julian Huxley demonstrated in 1932 that a logarithmic relationship exists between organ weight (Y) and total body weight (X) across species. Termed the "allometric equation," this formula assumes that a given parameter (Y) scales predictably with body weight (X) across an unlimited range of species (i.e., simple allometry).

$$\log Y = b\log X + \log a \tag{6.2a}$$

$$Y = aX^b \tag{6.2b}$$

As shown in Eqs. 6.2a and 6.2b, when organ weights from different species are plotted on 2D–log-log axes, a is the y-intercept (at $x = 1$) and b is the slope of the line which ranges from 0.70 to 0.99 for a variety of organs including the principle clearance organs, the kidney, lung, and liver. In addition to organ weights, allometric relationships have been shown to exist for a variety of physiological parameters including tidal volume ($b = 1.0$), creatinine and urea clearance ($b = 0.69$ and 0.72, respectively), basal oxygen consumption ($b = 0.73$), and liver blood flow ($b = 0.89$) [24, 28, 37–40]. Simple allometry provides reasonably accurate predictions, within a twofold error, of human clearance for a number of drugs including felbamate, ketamine, meloxicam, midazolam, nicardipine, propranolol, sildenafil, sumatriptan, and troglitazone. However, numerous cases exist in which human clearance cannot be explained by the allometric equation, and simple allometry produces only 50% of predictions within twofold error (56% within threefold error) [27]. Notwithstanding the statistical uncertainties caused by extrapolation beyond the regression curve, the term "vertical allometry" has been used to describe cases in which predicted clearance markedly exceeds the observed data (i.e., human clearance is overpredicted), typically with a prediction error of tenfold or greater [25, 28, 41]. Examples of drugs that exhibit vertical allometry include antipyrine, diazepam, reboxetine, susalimod, tamsulosin, valproate, and warfarin with predicted human clearances for these drugs reported between 10 and 53 times higher than observed [27, 42]. In order to anticipate cases of overprediction, several structural property thresholds can be used to alert investigators to compounds that are likely subject to vertical allometry. Such property alerts include (1) log$P > 2$ and a ratio of rat/human plasma protein binding >5, (2) extensive binding to plasma α-1-acid-glycoprotein (AAG), and (3) large differences between unbound and total clearance across species [26, 28, 41]. And while such guiding principles can be predictive of

vertical allometry risk in some compound data sets, to date there are no universally trusted rules.

Cases of vertical allometry can be seemingly incongruent with the knowledge that mammalian liver weight, liver blood flow, and total energy consumption (kcal/day) scale to body weight with the simple allometric exponents (b) of 0.85, 0.89, and 0.75, respectively [23]. Boxenbaum [23] tested a hypothesis that corrections for binding and liver blood flow would improve prediction accuracy by allometrically scaling calculated hepatic intrinsic clearance (CL_{int}) [43] instead of plasma clearance according to Eq. 6.3

$$CL_{int} = \frac{(Q_h \times CL_h)}{f_b(Q_h - CL_h)} \tag{6.3}$$

where f_b is the blood binding, Q_h is liver blood flow, and CL_h is hepatic clearance. CL_{int} was calculated for a series of five drugs (antipyrine, bromazepam, clonazepam, chlordiazepoxide, and phenytoin) cleared by the liver and exhibiting varying degrees of allometric verticality, from 11 different species spanning more than 4-log units of body weight with measured f_b for each and Q_h equated across species at 1.5 L/min/kg liver weight [23, 37]. Despite this correction for mechanism, allometrically scaled human CL_{int} values were still 4.2–11.4 times higher than the observed human CL_{int} [23, 44], leading some to hypothesize that humans possess a lesser intrinsic metabolic capacity compared with other species "lower" in the evolutionary continuum. Put simply, such hypotheses state that smaller mammalian species metabolize more rapidly per unit body mass compared with larger species [23, 44].

6.2.1.1 Neoteny, Dedrick Plots, and the Rule of Exponents (ROE)

The hypothesis that species-specific physiological growth rates contribute to vertical allometry was tested by analysis of plasma concentration-time profiles across species. Elimination rates for two renally cleared drugs, ceftizoxime and methotrexate, the total clearances for both of which are well predicted by simple allometry [28], reveal an equating principle across otherwise disparate physiologies. Ceftizoxime, an iminomethoxy aminothiazolyl cephalosporin, exhibits markedly different plasma elimination half-lives ($t_{1/2}$) across species, ranging from 15 min in rodents to 50 min in dogs and monkeys to nearly 120 min in humans. Mordenti [24] demonstrated that rather than expressing ceftizoxime $t_{1/2}$ in minutes but instead by the number of heartbeats, all species studied eliminated the drug at approximately the same rate: 50% of the dose in 7253 heartbeats. Similarly, when dose-normalized methotrexate plasma (or serum) concentrations in mice, rats, dogs, monkeys, and humans are plotted together on a semilogarithmic plot, concentration-time profiles are scattered across multiple log units and seemingly not predictive of one another. As observed with ceftizoxime, elimination was fastest in rodents, moderate in dogs and monkeys, and slowest in human subjects. In both cases, an empirically

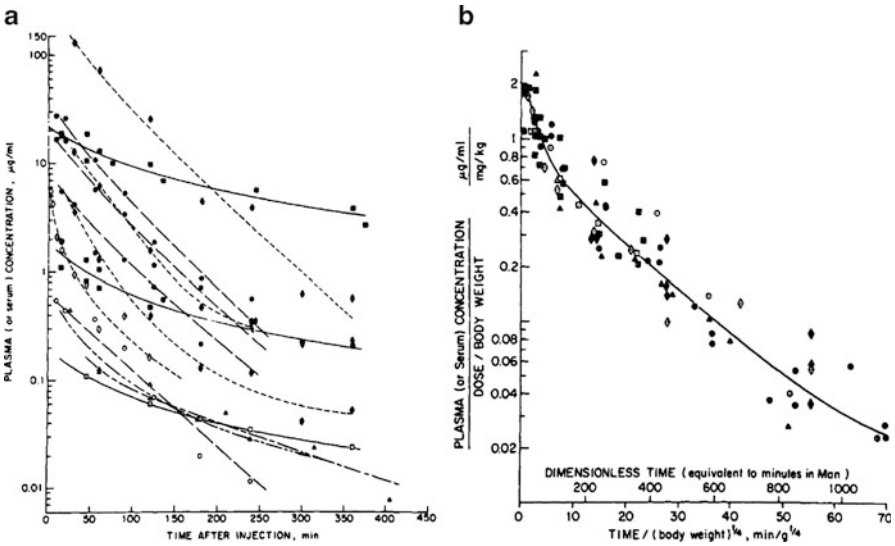


Fig. 6.1 Semilogarithmic plots of methotrexate plasma and/or serum (**a**) concentration versus time in mouse (---), rat (——), monkey (—·—), dog (—··—), and human (——) and (**b**) concentration versus time after normalization of the *x*-axis as time/$BW^{0.25}$ and the *y*-axis as dose/BW (Reproduced from [24])

determined biological time constant (i.e., a time frame specific to each species and distinct from chronological time) scales with body weight (BW) with an exponent (*b*) of 0.25, and when the time axis is expressed as time/$BW^{0.25}$, known as a Dedrick plot, the concentration-time curves from different species are near perfectly superimposable [24, 45, 46].

The superimposable nature of methotrexate concentration-time curves through Dedrick plot analyses (Fig. 6.1) is consistent with the hypothesis that smaller species possess a quicker physiological "tempo" when compared with larger species. According to this hypothesis, in order to relate a chronologically measured pharmacokinetic event across species, different biological time scales must be equated by extending the time scales for smaller species and compressing those for larger species. The varying biological time scales are thought to be a manifestation of neoteny, differences in the relative rates at which physiological events occur between species, differences that do not scale with body weight. For example, it has long been understood that humans undergo sexual maturation at a slower rate than nonhuman species, including laboratory animals. Similarly, humans exhibit relatively prolonged brain and cranial growth well beyond the developmental time at which other mammalian species cease to add brain weight (BrW). Simple allometric coefficients derived from a diverse set of mammals can accurately predict human total body surface area, liver weight, liver blood flow, and cardiac cycle. However, simple allometry also predicts a human brain mass of 275 g (compared to a typical observation of 1200–1400 g) and a human maximum life span potential (MLP) of only 27 years [45, 47–49].

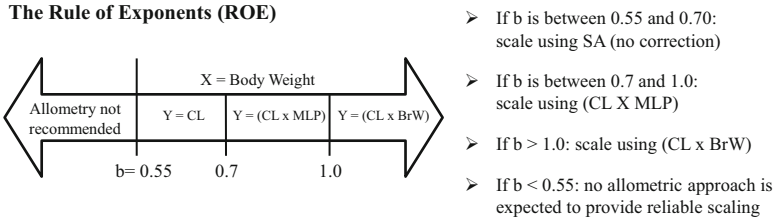


Fig. 6.2 The rule of exponents (ROE) is the systematic application of an MLP or BrW correction based on the empirically derived allometric exponent (*b*)

Neoteny is theorized to be one cause of vertical allometric relationships observed with human drug clearance [45]. In fact, when the antipyrine and benzodiazepine data sets were plotted as unbound CL_{int} versus MLP, human CL_{int} is well predicted by a log-linear regression, meaning that unbound intrinsic metabolic drug clearance decreases as body weight *and* life span increase [45]. Boxenbaum went on to suggest that all species have a similar metabolically active mass (the "ergosome") and expend that metabolic activity (per kg of body weight) at rates inversely proportional to their MLP. In short, small animals metabolize at relatively high rates over short lives, whereas larger animals have slower metabolic rates over longer life spans [23, 24, 44, 45] As such, it stands to reason that parameters influenced by neoteny, such as BrW or MLP, could be used to adjust simple allometric regression and thereby account for cases of vertical allometry.

The rule of exponents (ROE) is a rule-based methodology to determine when BrW or MLP correction should be applied to a simple allometric regression or when to apply no correction at all [50]. The ROE states that upon inspection of simple allometric regression, the value of the exponent (*b*) determines the correction to be applied according to the boundary conditions depicted in Fig. 6.2.

In a test set of 37 compounds with simple allometric exponents (*b*) greater than 0.7, for which 49% were poorly predicted (> twofold error), application of the ROE improved prediction error (to within twofold) for 72% of the poorly predicted compounds (13 of the 18) including diazepam, cefpiramide, quinidine, norfloxacin, propafenone, thiopentone, and warfarin. Notably, a more consistent improvement in error was observed for compounds with $b > 1.0$ for which a BrW correction was applied. However, ROE worsened the accuracy, changing prediction from within a twofold error to more than twofold error, for 42% of the compounds (8 of 19) including midazolam, troglitazone, and thiopentone. In theory, an MLP correction (when $b > 0.7$ and <1.0) will produce a predicted human clearance (hCL) of 1/3–2/3 the value derived from simple allometry, whereas a BrW correction (when $b > 1.0$) will result in a predicted hCL of 1/5–1/2 that which would otherwise be obtained from simple allometry [50]. In practice, the prediction change brought about by MLP or BrW correction is a function of both the number and types of species selected, which may be an underlying cause for the reported errors and varying degrees of success realized with ROE-based clearance predictions [26, 51, 52].

Regardless of the correction method applied, allometric-based scaling of drug PK parameters tends to elicit criticism based on its inherent retrospective nature and the knowledge that the enzymatic and physiological systems underlying the empirical data are multifactorial, and therefore, by nature, predictions based on such data necessitate a higher dimension of mathematical complexity. It has been challenged that any physiologically relevant allometric approach ought to encompass the following features [49]:

1. Empirically derived quantities that permit superimposability of pharmacokinetic data across species (e.g., Dedrick plots).
2. Defined thermodynamic constants and mechanical terms (i.e., well-understood biology for all relevant species).
3. A stable set of parameterized equations that accurately model the data across the relevant geometry describing either intrinsically linear or nonlinear events (i.e., a group of models that covers all relevant species, kinetics, and chemical matter).

Coincidental with the emergence of in vitro tools of drug metabolism, arguments such as this supported many efforts in drug discovery programs to integrate mechanistic knowledge of drug or compound clearance and disposition into allometric scaling methods.

6.2.1.2 Semi-Mechanistic Allometry

Inclusion of mechanistic-based corrections to allometric regression based on known or measurable species differences has become an increasingly common approach in attempting to improve prediction accuracy of simple allometry. With a set of marketed drugs for which both preclinical and clinical clearance values were available, human clearance was more accurately predicted (21 drugs with a predicted/observed ratio of 0.34–2.2) when scaling unbound clearance ($CL_u = CL/fu_{plasma}$ where fu_{plasma} is the fraction unbound in plasma) as opposed to total clearance. A greater improvement in prediction accuracy was reported for drugs with known species differences in protein binding (e.g., tamsulosin, remoxipride, cefotetan, and RO25-6833), whereas little improvement was observed for drugs with lesser differences in binding between species. Of the 27 drugs investigated, five (propranolol, antipyrine, diazepam, valproate, and midazolam) exhibited vertical allometry with predicted/actual ratios $\geq$9.0. However, without exception, these poorly predicted drugs exhibited allometric exponents (b) > 0.85, and when a BrW correction was applied, CL_u predictions for these were in line with the other 22 drugs [42]. Predictions were not improved for highly plasma-bound drugs (fu < 0.1) with this method; however, applying the ratio of rat-to-human plasma protein binding (Rfu) demonstrated statistically significant benefit. The result was introduction of the fraction unbound correction intercept method (FCIM) in which a is the y-intercept from simple allometry and the exponent (b) was fixed at 0.77 as seen in Eq. 6.4.

$$\mathrm{CL} = 33.35 \times \left(\frac{a}{Rf_u}\right)^{0.77} \tag{6.4}$$

With FCIM, total clearance scaled across 61 drugs produced significantly improved accuracy (78% absolute percentage error, APE) compared with both ROE and simple allometry (185% and 323% APE, respectively) [26]. A systematic comparison of 18 allometric methods to predict human clearance for 19 development molecules showed FCIM to be the most accurate with more than twofold prediction error in 72% of cases, opposed to simple allometry which tends to produce predictions within twofold error only 50% of the time [25, 26]. It is noteworthy that the FCIM modification is based on the ratio of plasma protein binding in rat and human, irrespective of *which* or *how many* species are included in the regression. And as accepted as FCIM has become in some drug discovery programs, this fundamental ambivalence to species selection underscores, despite appearance, the ignorance of mechanism associated with this methodology.

Testing the hypotheses that allometric prediction accuracy could be improved by including species-specific metabolism, scientists at Hoffmann-La Roche demonstrated that inclusion of in vitro hepatocyte clearance improved the prediction accuracy by regression of observed plasma clearance values corrected by in vitro data according to Eq. 6.5.

$$\mathrm{CL}_{\mathrm{animal}} \times \frac{\mathrm{CL}_{\mathrm{human(hepatocytes)}}}{\mathrm{CL}_{\mathrm{animal(hepatocytes)}}} = aBW^b \tag{6.5}$$

This correction resulted in a marked improvement in prediction accuracy (only 20–40% deviation from observed) along with a decrease in overprediction bias for ten extensively metabolized compounds (antipyrine, bosentan, caffeine, mibefradil, midazolam, mofarotene, RO24-6173, propranolol, theophylline, and tolcapone) when integrating at least three preclinical species [53]. While principally of benefit to compounds undergoing hepatic metabolism as the primary route of clearance, this method demonstrates a means to integrate mechanistic in vitro data into the allometric prediction and offers a rational correction in light of the aforementioned hypothesis that humans exhibit less metabolic capacity (per kg bodyweight) than other species included in the allometric regression [45, 53].

The long-standing and widely accepted method of allometry allows investigators to generate predictions of human plasma clearance based on preclinical in vivo data. Regardless of the correction method employed, allometric-based predictions fundamentally rely on the assumption that drug clearance scales in a predicable manner with body weight, regardless of the species used in preclinical studies. Correction methods that attempt to standardize for neoteny such as Dedrick plots or ROE adjust biological time scales across species and may be useful across a variety of clearance mechanisms (e.g., metabolism, renal excretion, etc.). Other biochemical- and physiological-based corrections to allometric inputs, including the use of drug- and species-dependent terms such as plasma protein binding or hepatocyte

CL_{int}, also offer serviceable correction methods. And while to date there is no reported methodology known to reduce allometric prediction error universally across all compounds, such approaches provide discovery scientists with a number of integrated methods to combine the in vivo relevance of allometry with mechanism-based knowledge of species differences in drug clearance.

6.2.2 Mechanistic Scaling

It is well accepted that all methods to predict human clearance bear uncertainty and the utility of any given method is harnessed best when the uncertainty can be minimized and properly managed to provide meaningful guidance, be it with decisions to advance or terminate discovery compounds or in crafting designs for future clinical development trials. Incidences of vertical allometry along with contemporary knowledge of species differences in metabolism, enzyme and transporter expression, and substrate affinities present challenges to the reliability of a simple log-linear correlation between body weight and total clearance [11, 24, 49, 54–58]. Furthermore, scaling according to body weight can provide only an estimated value of predicted human clearance with no intrinsic knowledge of the underlying pathway(s) of drug elimination. A more highly valued prediction would offer a projection of the operative drug clearance pathways in healthy human subjects or patients, thereby enabling the forecast of potential drug-drug interactions and expected PK in special populations (e.g., healthy subjects compared with renally impaired patients compared with hepatically impaired patients). To this end, a mechanistic approach to a clearance projection will independently scale, from preclinical data, each elimination pathway for a summation of total clearance (CL_{total}) according to Eq. 6.6.

$$CL_{total} = CL_h + CL_r + CL_{other} \tag{6.6}$$

When a circulating compound is in rapid and free equilibrium with the liver, meaning the unbound concentration in plasma is equal to unbound concentration in the liver intracellular compartment (Kpuu $\cong$ 1), the hepatic clearance (CL_h) can be simply thought of as metabolic clearance plus the direct excretion of unchanged parent drug in bile ($CL_{metab} + CL_{bile}$). However, when free drug concentration in the liver is limited by either passive permeability or active uptake into the liver, this requires a more integrated approach to determining CL_h.

6.2.2.1 Clearance Classification Systems

The use of physical-chemical properties to guide the prediction of operative human clearance pathways is a commonly accepted approach in many discovery programs. Discovery scientists at Pfizer report the routine application of compound property

categorization to understand and predict human clearance pathways. Based on the Biopharmaceutical Drug Disposition Classification System [59], this approach hypothesizes that for highly permeable compounds, unbound drug in plasma crosses the hepatocyte sinusoidal membrane establishing a free drug equilibrium such that the unbound plasma concentration will be equal to the unbound liver concentration ($Kpuu_{liver} \cong 1$). The result is that clearance from circulating plasma is determined principally by the intrinsic rate of metabolism ($CL_{int,met}$). By contrast, the unbound liver concentration for low permeability compounds is limited by hepatic uptake, and therefore, while these compounds may undergo metabolism, clearance from plasma for these compounds is determined by the intrinsic uptake (influx) into the liver (PS_{inf}, a summation of active and passive transport) [60]. Additional granularity to this method was offered by introduction of the Extended Clearance Classification System (ECCS), a categorical approach shown to be 92% accurate (in a 307 compound test set) at predicting a single predominant clearance pathway accounting for ≥70% of total clearance. Using structural properties including ionization state, molecular weight (MW), and passive membrane permeability, compounds were categorized according to the grid shown in Fig. 6.3a.

According to the ECCS, the plasma clearance of highly permeable ($Papp_{MDCK} > 5 \times 10^{-6}$ cm/s), basic, and neutral molecules (Class 2) is principally metabolic, whereas the plasma clearance of low permeability basic and neutral molecules (Class 4) is principally determined by renal elimination. In the case of acids and zwitterions (Classes 1 and 3), plasma clearance for low-MW compounds (<400 g/mol) with high permeability (Class 1A) is metabolic, whereas for low-MW compounds with low permeability (Class 3A), the free drug does not reach the intracellular compartment to any substantive degree, and, in turn, clearance from plasma is dictated largely by renal elimination. The clearance of high-MW (>400 g/mol) acids and zwitterions is dictated by hepatic uptake or a mixture of hepatic uptake and renal elimination, Classes 1B and 3B, respectively [61]. While principally designed to be qualitative in nature, such classification of a discovery molecule can guide the collection of laboratory data necessary to drive a quantitative prediction of human clearance.

In a similar but further quantitative approach, Novartis scientists presented the Extended Clearance Concept Classification System (ECCCS, Fig. 6.3b) that produced >90% accuracy in the prediction of human clearance (within threefold error) by first determining hepatic clearance (CL_h) by the extended clearance model (ECM) and subsequently incorporating the predicted CL_h into the well-stirred liver model as shown in Eq. 6.7a

$$CL_{int} = \frac{PS_{inf} \times (CL_{int,\,sec} + CL_{int,met})}{(PS_{eff} + CL_{int,\,sec} + CL_{int,met})} \tag{6.7a}$$

(Gillette, 1971; Rowland et al. 1973; [34, 62]) to produce a predicted total human clearance according to Eq. 6.7b

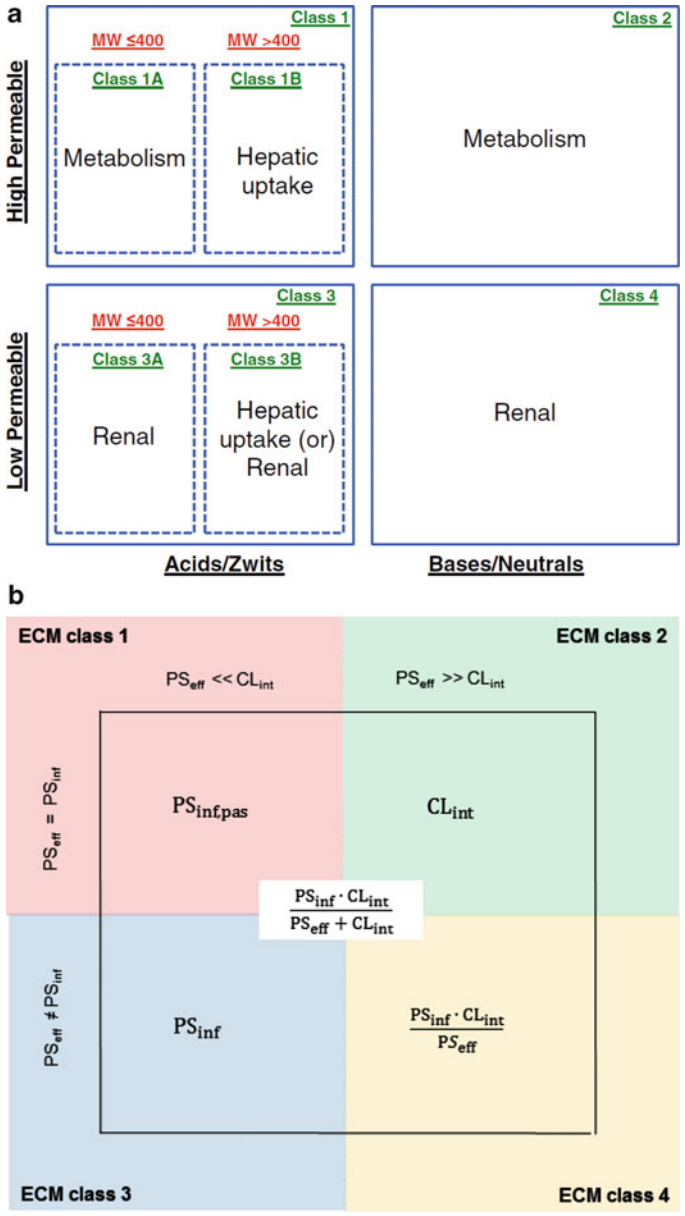


Fig. 6.3 A comparison of two quadrant-based classification systems. (**a**) The Extended Clearance Classification System (ECCS) framework illustrates the predominant mechanism that determines systemic drug clearance (Reproduced from [61]). (**b**) The Extended Clearance Concept Classification System (ECCCS) illustrates the rate-determining hepatic clearance mechanisms as they relate to the extended clearance model (ECM) based on expressed property conditions (Reproduced from [54])

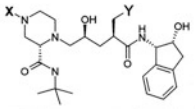
$$CL_h = \frac{Q_h \times fu_b \times CL_{int}}{Q_h + fu_b \times CL_{int}} \quad (6.7b)$$

where Q_h represents hepatic blood flow; fu_b is the free fraction of drug in the blood (fu_{plasma}/blood/plasma); $Cl_{int,sec}$ and $CL_{int,met}$ are the in vitro secretory (biliary) and metabolic intrinsic clearances, respectively; and PS_{inf} and PS_{eff} are the hepatocyte influx and efflux permeabilities, respectively, determined from suspended and sandwich-cultured hepatocyte systems [29, 35, 54]. Consistent with the qualitative classification systems, the ECCS demonstrates that for highly permeable molecules with no active uptake in the liver ($Kpuu_{liver} \leq 1$), plasma clearance is determined by $CL_{int,met}$ (ECCCS Classes 1 and 2). However, plasma clearance of compounds exhibiting active hepatic uptake (ECCCS Classes 3 and 4) is determined by a mix of hepatobiliary and renal elimination best described by the ECM [29, 33, 35, 54]. As a discovery team approaches a decision of whether or not to advance a particular molecule into clinical development, scaling methods that predict the operative clearance pathways enable risk assessment for a variety of clinical development scenarios. To afford such opportunity, a thorough understanding of the likely operative clearance pathways along with a preclinical (in vitro and in vivo) data package to describe the clearance as quantitatively as possible should be amassed to produce a mechanistic understanding of total clearance.

6.2.2.2 Structural/Chemical Rationale

Underlying such classification and calculable prediction methodologies is the physical-chemical and structural attributes of a molecule that determine the in vivo disposition. Lead optimization design cycles, in the focused pursuit of potency and selectivity, often drive a lead chemical series toward hydrophobicity (increased high log*P*/*D*) and high molecular weights [20, 63, 64], which in turn bias compounds toward extensive hepatic metabolism, high nonspecific binding, low solubility, and other nondrug-like properties as described previously [19, 65–67]. In many cases, seemingly minor and even one-atom changes in structure can elicit significant changes in hepatic metabolism. Indinavir, a potent HIV protease inhibitor, also potently inhibits cytochrome P450 (CYP) due to a type II binding interaction between the unhindered pyridyl-N and the CYP heme, which in turn limits the systemic clearance of indinavir through saturation of metabolism. Merck scientists demonstrated that structural modification of the pyridyl ring by addition of gem-dimethyl substituents, direct methyl pyridyl substitution, or even isomerization of the pyridyl nitrogen resulted in an up to 33-fold increase in the CYP-IC_{50} (i.e., less inhibition) and a 12-fold increase in metabolic clearance. Results were concordant with changes in the P450-binding spectra indicating that the structural modifications directly altered the manner in which the compounds coordinate with

a



No.	X	Y	P450 Binding Spectra	Metabolic Clearance (ml/min/kg)	IC_{50} (μM)
Indinavir			II	75.0	0.451
I			II	71.0	0.873
II			I	763	15.1
III			I	854	8.70
IV			I	636	19.3
V		H	II	53.7	1.32
VI			II	70.7	0.990
VII			II	61.2	0.625

b

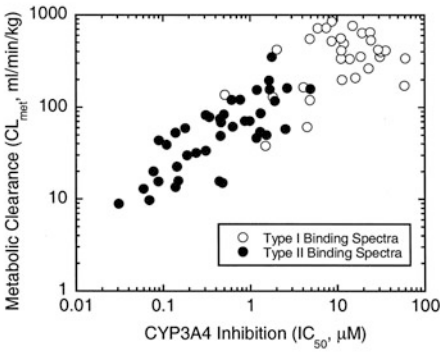


Fig. 6.4 (**a**) The structure-property relationship between indinavir and its structural analogues and their P450 binding spectra, metabolic clearance, and HIV protease potency (IC_{50}). (**b**) The relationship between metabolic clearance and CYP3A4 inhibition (IC_{50}) upon co-incubation of indinavir analogues and human liver microsomes (Reproduced from [68])

the P450 heme, changing both drug-drug interaction (DDI) potential and clearance of the parent drug (Fig. 6.4) [68].

Similarly, scientists at Bristol-Myers-Squibb reported the discovery of a pyrazole amidine series as potent inhibitors of coagulation Factor Xa due in part to the compound's strongly basic benzamidine moiety (p*K*a 10.7). The lead series demonstrated 1–10 nM potency against the target but limited potential for clinical development due to low oral bioavailability (<5%) and short $t_{1/2}$ in preclinical species. In the lead optimization phase, it was recognized that, despite the exquisite potency that could be achieved, the benzamidine moiety was a significant determinant of low volumes of distributions contributing to short elimination plasma $t_{1/2}$ [69]. Consequently, medicinal chemistry focused on replacing the benzamidine with less basic substituents. This strategy increased passive permeability, reduced hepatic intrinsic clearance (80% decrease), and modulated the distribution, while achieving adequate potency to drive an in vivo pharmacological effect through increased oral exposure (>260-fold), relative to the benzamidine. The resulting benzylamine-containing clinical development compound was orally bioavailable in humans, with an absence of food effects, a plasma $t_{1/2}$ of 27–35 h, and measurable increases in prothrombin time [19, 70, 71].

These examples of directed and apparently small structural modifications resulted in marked changes in in vivo clearance and illustrate the power of understanding mechanism in particular in the LO setting as programs traverse chemical diversity in search of clinically developable molecules.

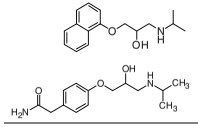
Hepatic CL_{int} and total plasma clearance can be significantly modulated by small structural diversity within a series, but so too can the contributing elimination

pathways be substantially altered by changes in structural properties. Most drugs and discovery compounds with a log$P > 4$ exhibit high liver microsomal CL_{int} that decreases with lower log$P$ as described previously by Desai et al. in Chap. 4 and elsewhere [65]. Therefore, during the course of LO, medicinal chemistry design cycles' intent on increasing microsomal stability, often by lowering log$P$/D, tends to incorporate polar or ionizable groups thereby increasing polar surface area (PSA) or charge potential (lowering acidic pKa or increasing basic pKa). These types of structural properties increase the tendency of unbound hepatic CL_{int} to underpredict in vivo clearance due to increased contribution of non-CYP metabolic pathways and excretion pathways [65, 72, 73] as illustrated by comparison of atenolol and propranolol. Used in the treatment of cardiac arrhythmias, angina, and hypertension, these β-adrenergic receptor antagonists exhibit similar human plasma $t_{1/2}$ but vastly different clearance pathways (Table 6.1; [19]).

Propranolol exhibits no measurable biliary or renal excretion and rather is nearly completely metabolized to such an extent that its observed clearance from plasma provides a reliable in vivo estimate of total liver blood flow. Conversely atenolol, which bears a terminal amide as opposed to propranolol's naphthalene, undergoes no measurable metabolism and is eliminated almost entirely by the kidneys, necessitating clinical dose reduction in patients with renal insufficiency [62, 74, 75]. These differences in clearance pathways may be somewhat surprising based on the apparent structural similarities between atenolol and propranolol and the lack of any significant differences in MW or pKa. However, inspection of data from a larger set of structurally related β-antagonists reveals that within this series the balance of metabolism and renal excretion of unchanged drug is a function of differences in log$D_{7.4}$, carbon SP_2 hybridization, and PSA with hydrophobicity driving compounds toward metabolism and increased polarity biasing compounds toward renal excretion (Fig. 6.5) [19, 60, 67].

Similar inspection of a set of 3-hydroxy-methylglututaryl coenzyme A (HMG-CoA) reductase inhibitors illustrates how structure and physical-chemical properties within a chemical series can influence pathways of clearance. Atorvastatin (Lipitor™), fluvastatin (Lescol™), pitavastatin (Livalo™), and rosuvastatin (Crestor™) belong to the family of synthetic statins, each with a C_7-aliphatic carboxylic acid linked to an unhindered F-phenyl by a 5- or 6-membered N-containing mono- or bicyclic aromatic ring system. Common to the clearance of all four is the role of OATP1B1 and other active hepatic uptake transport mechanisms, accounting for between 75 and 99% of hepatic uptake. Where these drugs differentiate is in their fates of clearance beyond that point (Fig. 6.6). Atorvastatin and fluvastatin (PS_{inf} of 198 and 544 mL/min/kg, respectively) are almost exclusively eliminated through metabolism by hepatic CYP enzymes albeit by differential predominant isoforms, CYP3A4 (atorvastatin) and CYP2C9 (fluvastatin). However, atorvastatin AUC is significantly increased by inhibition of OATP1B1 (six- to eightfold increase when coadministered with rifampicin or cyclosporine), whereas fluvastatin AUC is far less sensitive to OATP inhibitors indicating atorvastatin's clearance is dictated by hepatic uptake (PS_{inf}) and fluvastatin's clearance is determined by $CL_{int,met}$. Conversely, pitavastatin

Table 6.1 Comparison of physical-chemical and ADME/PK properties of propranolol and atenolol

		MW (g/mol)	p*K*a	cLogD 7.4	PSA (Å^2)	F,SP3	$t_{1/2}$ (hr)	CL, int, u (mL/min/kg)	CL, renal (mL/min/kg)	Vd (L/kg)
	Propranolol	259	9.7	0.92	44	0.375	3–4	470	ND	50
	Atenolol	266	9.7	−1.24	94	0.5	3–5	ND	2	1

Modified from [19]

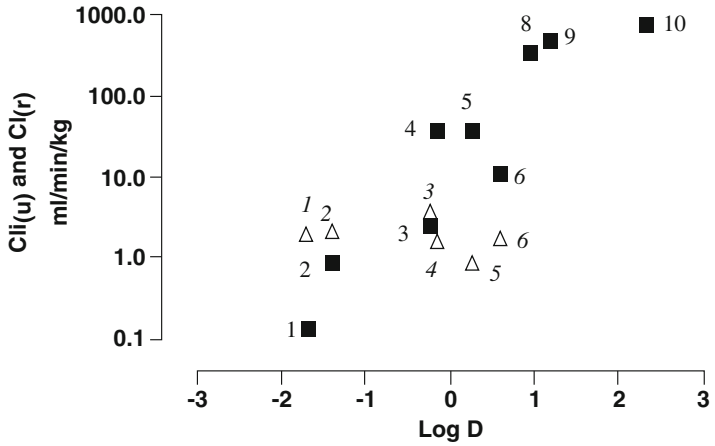


Fig. 6.5 The correlation of lipophilicity (log $D_{7.4}$) and unbound hepatic intrinsic clearance ($Cli_{(u)}$, *filled squares*) and unbound renal clearance ($CL_{(r)}$; *open triangles*). Compound numbering refers to β-receptor analogues included in reference from which figure is reproduced [67]

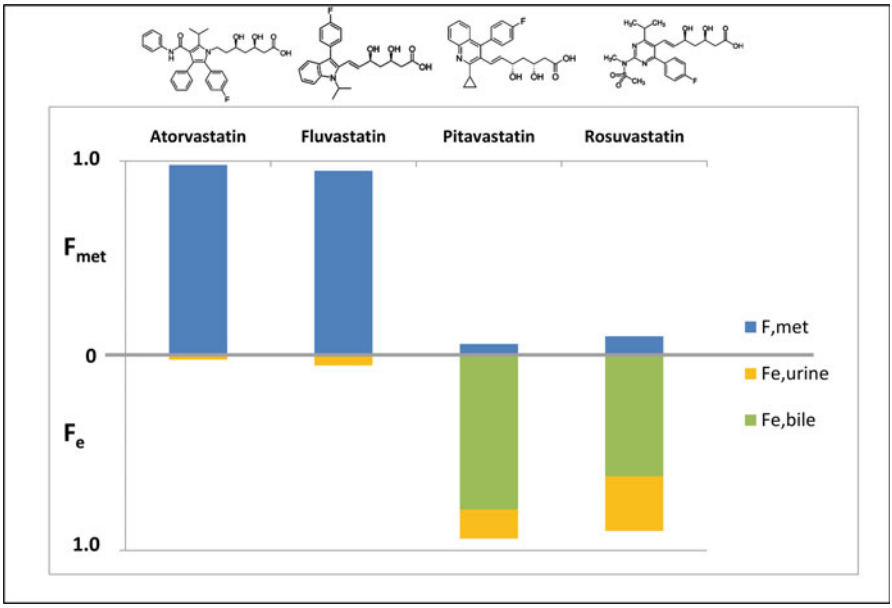


Fig. 6.6 The fractional clearance of four statins including fraction metabolized (Fmet) and fraction excreted (Fe), further divided into fraction excreted in urine and fraction excreted in bile (fe,urine and fe,bile, respectively). Data were compiled from [76–79]

(PS_{inf} = 623 mL/min/kg), which differs from fluvastatin only by the introduction of a cyclic-propyl-pyridine ring, undergoes minor metabolism and instead is excreted principally unchanged (78%) in the bile, mediated by MRP2 secretion, and exhibits three- to fivefold increases in plasma AUC when co-dosed with OATP inhibitors. Only about 15% of pitavastatin dose is excreted unchanged in the urine, and notably due to its high passive permeability, pitavastatin is thought to undergo enterohepatic circulation (biliary excretion followed by reabsorption) giving it the highest oral bioavailability (51%) of the statins described here. Finally, rosuvastatin (PS_{inf} = 52 mL/min/kg), the most hydrophilic of the four, undergoes a minor degree of metabolism (principally by CYP2C9) and is 90% excreted unchanged in feces (62%) and urine (28%) [76–79].

The chemical diversity illustrated by the series of molecules above is consistent with the range of structural and property diversity commonly explored in a medicinal chemistry LO initiative [65]. By extension, the diversity of clearance pathways observed within these series, from nearly complete metabolism to nearly complete excretion of unchanged drug, is representative of the diversity in clearance pathways that could be encountered in an active discovery lead series. Even within a space of highly metabolized compounds (e.g., atorvastatin and fluvastatin), it is not uncommon to observe significant shifts in the relative contribution, or fraction metabolized (fm), from different drug-metabolizing enzymes (e.g., CYP2C9 vs. CYP3A4) as well as the processes that determine clearance from plasma (PS_{inf} vs. $CL_{int,met}$). As polarity and hydrophilicity are introduced in a chemical series, metabolism is likely to reduce in contribution to total clearance, and rather biliary and urinary pathways begin to take over with varied contribution from secretory transporters (e.g., MRP2 and OAT3). It is those compounds cleared by hepatic metabolism (in particular CYP metabolism) for which most industrial drug discovery programs are well positioned to optimize clearance through microsomal and hepatocyte screening assays, and as such many DMPK programs routinely invoke scaled in vitro $CL_{int,met}$ in projections of human clearance [11, 31, 80, 81]. The extent to which discovery programs can also include both qualitative and quantitative knowledge of uptake and excretion mechanisms can lead to increased prediction accuracy, informed chemistry design cycles, and an overall improvement in the understanding of compounds advanced into clinical development.

6.2.3 Mechanistic Prediction of Human Clearance

Categorical approaches such as the ECCS and ECCCS illustrate means by which a mechanistic projection method can capture contribution from a variety of operative clearance mechanisms and elimination pathways and, in doing so, predict each molecule based on its unique properties. A strategy to determine the utility of such an approach with any given discovery molecule is to quantitatively assess the accuracy of a mechanistic prediction in preclinical species where with greater the

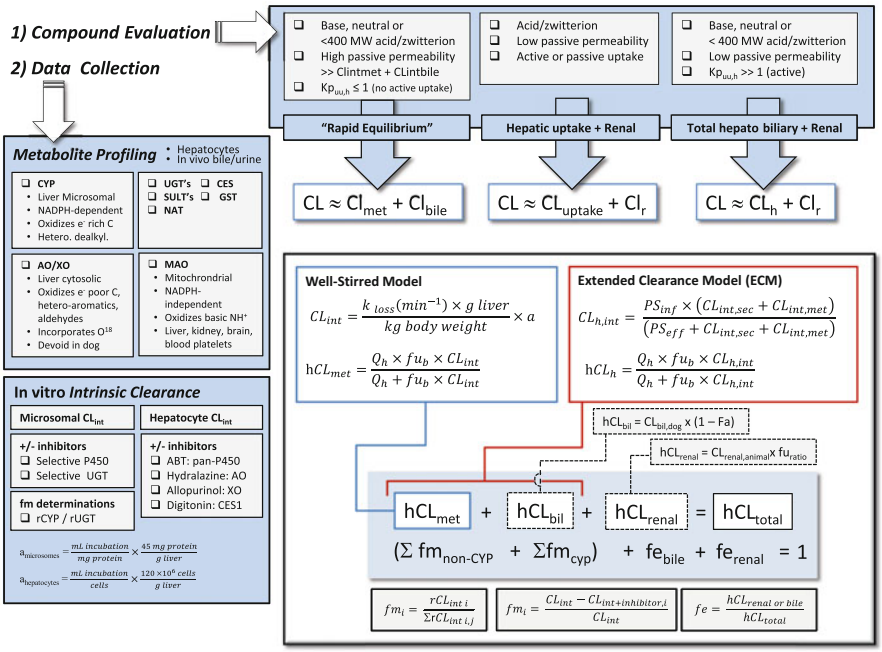


Fig. 6.7 A schematic diagram depicting compound evaluation according to integrated classification systems (ECCS/ECCCS) and data collection methods to guide and support the mechanistic scaled clearance pathways leading to the summation of predicted total human clearance

prediction accuracy, the greater the confidence in subsequent human predictions for that molecule. Provided the summation of scaled pathways (e.g., CL,met + CL, bile + CL,renal) amounts to 70% or more of total clearance observed in preclinical species, then the mechanistic approach is considered validated for that compound, and in turn the summation of scaled pathways can be used with increased confidence for the prediction of human clearance. Compound evaluation for projecting human clearance, both qualitatively and quantitatively, can be approached in stages as illustrated in Fig. 6.7.

6.2.3.1 Compound Evaluation: Physical-Chemical and Property Space

A systematic evaluation of physical-chemical and ADME properties can guide LO teams in both qualitative and quantitative prediction of human clearance and operative clearance pathways. Independently, the ECCS and ECCCS present methodologies that provide such benefits, and while they do not precisely superimpose in property endpoints or in categorical cutoffs, the two systems are more alike than they are distinct, and a reasonable amalgamation by integrating the approaches can be derived [35, 59–61]. Congruent with both systems (Fig. 6.3a, b), basic and

neutral compounds as well as low-MW (<400 g/mol) acids and zwitterions that exhibit high passive permeability and no evidence of hepatic uptake (e.g., in vivo [liver concentration$_{unbound}$/plasma concentration$_{unbound}$], liver Kpuu$_{hepatic} \leq 1$) will have a plasma clearance principally determined by metabolic clearance (i.e., $CL \approx CL_{met}$). The clearance of acidic and zwitterionic compounds that exhibit low permeability (passive and active) will be largely determined by the summation of hepatic uptake clearance mechanisms (PS_{inf} from the ECM) and renal elimination where low-MW compounds will be biased toward renal elimination. Finally, for bases, neutrals, and small MW acids and zwitterions that exhibit low passive permeability with a large portion of uptake from active processes (e.g., Kpuu,liver $> > 1$), clearance will be dictated by the totality of hepatobiliary clearance (best determined by the ECM). In all cases, an understanding of the metabolic pathways of a given molecule is essential to the construction of the mechanistic prediction.

6.2.3.2 Data Collection

Experimental objectives toward qualitative assessment of metabolic pathways are of two varieties: (1) metabolite profiling and (2) reaction phenotyping. In metabolite profiling it is important to identify the operative metabolic processes both in vivo across preclinical species and in vitro (hepatocytes) in all species including human. While not intended to be comprehensive, Fig. 6.7 captures some of the commonly observed metabolic routes including oxidative, conjugative, and hydrolytic. Once the operative pathways are identified (in vitro and in vivo, including profiling metabolites from excreta; bile and urine), it is important to determine the enzymes or enzyme systems involved in the observed metabolic pathways. Drug oxidation pathways are commonly mediated by CYP enzymes located in microsomal fractions; they are most abundant in the liver and intestine and catalyze reactions in an NADPH-dependent manner oxidizing drug substrates by incorporating elemental oxygen from O_2. Commonly observed reactions catalyzed by CYPs include the hydroxylation of electron-rich carbon (aliphatic or aromatic C), double-bond epoxidation, and heteroatom dealkylation [11]. The cytosolic enzymes aldehyde oxidase (AO) and xanthine oxidase (XO) highly expressed in the liver, lung, and kidney are known to contribute to the oxidation of electron-poor carbons, such as aldehydes and aromatic carbons within heteroatom cyclic- and bicyclic ring systems [58]. In order to identify a relevant in vitro system for the study of a given molecule, it can be very useful to delineate between CYP- and AO/XO-mediated oxidation by exploring microsomal versus cytosolic function, the ability of the reaction to incorporate O^{18} from heavy water (a hallmark of AO/XO-catalyzed oxidation) or the strict absence of the metabolic pathway in dogs [58, 82]. Non-oxidative pathways are most commonly identified by profiling metabolites in hepatocyte incubations. Pathways catalyzed by UDP-glucuronosyltransferase (UGTs), sulfotranferases (SULTs), N-acetyl transferases (NATs), or hydrolytic reactions by carboxylesterases (CESs) and hydrolases can be assessed for their possible contribution to a compound biotransformation by recombinant-expressed

systems or, in the case of hydrolases, blood or plasma stability. Measured hepatocyte $CL_{int,met}$ can be deconvoluted through co-incubation of selective inhibitors against several of the enzymes mentioned. In such experiments, inhibition of a particular pathway by a selective enzyme will indicate the fraction of total CL_{int} attributed to that pathway (fm) according to the equation: $fm = (CL_{int} - CL_{int+inhibitor})/CL_{int}$. Caution must be exercised however with respect to inhibitor selectivity and the concentrations used. For detailed descriptions of experimental conditions, selection of hepatocyte and subcellular fractions, selectivity of inhibitors and substrates, and information on additional enzymes and enzyme system readers are directed to the many detailed references on the matter [11, 15, 81–90].

For cases in which CL_{met} is the primary determinant of plasma clearance ($CL \approx CL_{met}$), human clearance can be determined by scaled intrinsic clearance as described in Fig. 6.7. In the other cases, either $CL \approx CL_{uptake} + CL_r$ or $CL \approx CL_h + CL_r$, the projection of human clearance from in vitro data can be made from the ECM [35, 54, 60, 61]. Establishing the intrinsic metabolic lability of the molecule is fundamental regardless of the scaling method employed (e.g., well-stirred or extended clearance model; see Fig. 6.7). Compounds for which the in vitro human metabolite profile and inhibitor/recombinant studies are consistent with only oxidative, CYP-mediated metabolism, CL_{int} is determined from the in vitro rate (k) of substrate loss as shown in Eq. 6.8a [81, 83].

$$CL_{int}\left(\text{ml min}^{-1}\text{ kg}^{-1}\right) = k_{loss}\left(\text{min}^{-1}\right) \times \frac{\text{g liver}}{\text{kg body weight}} \times \frac{\text{mL incubation}}{\text{mg protein}} \times \frac{45\text{ mg protein}}{\text{g liver}} \tag{6.8a}$$

This equation holds true provided the reaction is conducted under linear conditions, most likely met when substrate concentrations ([*S*]) are well below the Km such that $(0.5 \times [S])/K_m << 0.693$. Alternatively, in cases where one or more non-CYP metabolism pathways contribute a substantial degree (>25%) to total metabolism, metabolism should be measured in human hepatocytes and CL_{int} determined according to Eq. 6.8b.

$$CL_{int}\left(\text{ml min}^{-1}\text{ kg}^{-1}\right) = k_{loss}\left(\text{min}^{-1}\right) \times \frac{\text{g liver}}{\text{kg body weight}} \times \frac{\text{mL incubation}}{\text{cells}} \times \frac{120 \times 10^6\text{ cells}}{\text{g liver}} \tag{6.8b}$$

For details on recommended assay conditions, readers are directed to detailed descriptions on the topic [32, 80, 84, 85, 89]. In application of the ECM, the $CL_{int,met}$ is determined from the in vitro model best suited to capture the relevant metabolic pathways (e.g., microsomes, hepatocytes); PS_{inf} and PS_{eff} (active and passive uptake terms) are measured in hepatocyte suspensions, whereas $CL_{int,sec}$ data are generated from sandwich-cultured hepatocytes as described elsewhere [29, 35, 54].

In the absence of the data to determine CL_h by the ECM, microsomal or hepatocyte CL_{int} scaled to CL_{met} by the well-stirred model can be added to predicted human biliary clearance (hCL_b) to achieve an estimate of total hepatobiliary elimination. However, compounds with high permeability are likely to be extensively reabsorbed across the intestinal epithelia, thereby reducing the overall contribution of CL_b to the total clearance. Therefore, predicted CL_b should be estimated as follows where $CL_{bile\text{-}dog}$ is the biliary clearance of parent drug measured in a bile duct-cannulated dog and fa is the fraction (of an oral dose) absorbed across the intestinal epithelia.

$$hCL_b = CL_{bile,dog} \times (1 - fa) \tag{6.9}$$

The $(1 - fa)$ term accounts for the non-reabsorbed fraction of biliary secreted drug, thereby attenuating the predicted CL_b to capture only the portion irreversibly eliminated by this pathway. In all cases (whether determining CL_h by ECM or by summation of $CL_{int,met} + CL_b$), the predicted renal elimination of unchanged drug should be accounted for by the prediction of human renal clearance (hCL_r) for which a recommended approach is to scale from dog or monkey renal elimination including a correction for species protein binding.

$$hCL_r = CL_{renal,dog\ or\ monkey} \times \left(\frac{fu_{human}}{fu_{dog\ or\ monkey}}\right) \tag{6.10}$$

Using in vivo observed renal clearance in intact animals with correction differences in species plasma protein binding, human renal clearance was predicted within twofold from dog or monkey renal clearance, for a set of 36 chemically diverse drugs ($r^2 = 0.84$) exhibiting either active secretion or net reabsorption. This scaling approach produced less error and underprediction bias compared with both simple allometry and Mahmood's corrected allometric scaling [91].

Considerations for Oral Administration

Most commonly, although not exclusively, contemporary industrial discovery programs designing and synthesizing small molecule entities are seeking orally bioavailable therapeutic agents. Therefore, the bioavailability (F) of the dose across the intestinal absorption barrier and the first pass of the liver is of important consideration for both the oral dose to be administered (oral clearance = CL/F) and also the enzymatic extraction in both the liver and the gut as it pertains to victim DDIs. Oral bioavailability (F,oral) is defined in Eq. 6.11

$$F, oral = F_a \times F_g \times F_h \tag{6.11}$$

where F_a is the fraction of dose absorbed from the intestinal lumen, F_g is the fraction of dose escaping the intestine into the mesentery unmetabolized by gut

enzymes, and F_h is the fraction of dose available in the portal vein that reaches the systemic circulation unmetabolized by liver enzymes [32, 36]. The fraction absorbed is most commonly limited by solubility, dissolution, and/or permeability properties of the molecule and dose formulation. Compound absorption should be calculated as described in the previous chapter and the resulting F_a determination referenced for a mechanistic calculation of F as discussed here. Alternatively, the fraction of dose absorbed can be estimated from rodent or dog F,oral according to Eq. 6.1.

$$F_a = F/F_h \times F_g \tag{6.12}$$

However, this approach assumes that the discovery lot solubility and dissolution parameters will estimate those observed in the clinic as well as assuming that the F_g in rodents or dogs will be approximately 1.0, a commonly invalid assumption with monkeys in which CYP3A is highly expressed in the intestinal epithelia [92]. Extraction by intestinal metabolism requires careful consideration and has been studied extensively with reliable methods currently employed in many discovery programs. A study of 14 drugs in the perfused rat intestine alongside 48 drugs with rodent and human oral bioavailability demonstrated a high correlation ($r^2 = 0.8$) for intestinal absorption (F_a) but no correlation ($r^2 = 0.29$) observed for oral bioavailability, suggesting species differences in metabolic extraction ($F_g \times F_h$) were largely responsible for lack of predictability from rodent to human. In fact, intestinal CYP3A and UGT alone were reported to be present at 12- to 193-fold differences between species [93]. Human F_g can be projected based on experimental data according to Eq. 6.13

$$F_g = \frac{Q_{gut}}{Q_{gut} + fu_{gut} \times CL_{int,g}} \tag{6.13}$$

where Q_{gut} is a determination of gut permeability taking into account the blood flow surrounding the enterocytes, fu_{gut} is the fraction unbound in the intestinal lumen (often assumed to equal 1.0), and $CL_{int,g}$ is the unbound intrinsic clearance in the gut normalized for human CYP3A4 expression [94]. This calculation is based on human in vitro model systems, whereas to harness preclinical in vivo data, the following alternate method of calculating F_g based on in vivo monkey data has been proposed:

$$F_{g,human} = \frac{F_{g,monkey}}{F_{g,monkey} + \left(1 - F_{g,monkey}\right) \times \frac{CL_{int,HIM}}{CL_{int,MIM}}} \tag{6.14}$$

In Eq. 6.14, $CL_{int,HIM}$ and $CL_{int,MIM}$ are the in vitro intrinsic metabolic clearance measured in human intestinal or monkey intestinal microsomes, respectively [92]. Given the 2.3-fold higher expression of CYP3A in monkey and human intestines (in contrast to rodent and dog which bear little to no intestinal CYP3A), this method utilizes preclinical in vivo data while accounting for the ratio of metabolic differences (utilizing intestinal microsomal preparations)

between the model species (monkey) and the prediction species (human). Finally, following a projection of CL_h based on experimentation and calculations described in Fig. 6.7 (either CL,met + CL,bile or CLh calculated according to the ECM), F_h is calculated according to Eq. 6.15.

$$F_h = 1 - (CL_h/Q_h) \tag{6.15}$$

The resulting prediction of F,oral should be factored into oral clearance calculations and oral dose projections accordingly in addition to being considered in static and dynamic models for projection of potential DDI risk in the clinic (see Sect. 6.3).

6.2.3.3 Constructing a Mechanistic Profile

Upon conclusion of a mechanistic prediction of human clearance, there is opportunity to assess the fractional clearance pathways as they relate to clinical disposition. The following is a hypothetical but representative example of the knowledge that should be expected for a compound interrogated as described for such a projected human clearance. In this example, in vivo data for compound A obtained from dog renal and biliary excretion studies and scaled as described above indicate that 35% of the total projected human clearance is expected to be through the excretion of unchanged parent drug ($f_{exc} = 0.35$), whereas 65% is expected to be through metabolism ($f_{met} = 0.65$). Of the fraction excreted, the majority (71%) is expected in the urine with less in the bile. The majority (85%) of total metabolism in hepatocytes was inhibited by co-incubation with the irreversible and nonselective CYP inhibitor 1-aminobenzotriazole (ABT) and is, therefore, attributed to CYP metabolism with 15% attributed to non-CYP pathway(s). Follow-up recombinant work demonstrated that 90% of the CYP activity is due to CYP3A4 with a small portion (10%) due to CYP2C9. Therefore, the fm,CYP3A4 is equal to 0.55 (0.9×0.65) and the fm,CYP2C9 is 0.06 (0.1×0.65).

Given this profile (Fig. 6.8), both the discovery team and the clinical development scientists want to understand (as quantitatively as possible) the implications of renal or hepatic impairment on the clearance of compound A. It would be valuable to forecast the effect of a concomitantly administered CYP3A4 inhibitor or inducer on compound A disposition including clearance and half-life. Given such understanding of a discovery molecule, a project team may determine that the forecasted risk is appropriate given the indication (e.g., in the case of an unmet medical need) and may choose to progress the molecule into clinical development, perhaps with a modified clinical plan as the risk profile dictates, for example, exclusion of subjects with moderate or severe hepatic impairment given that 75% of the clearance of compound A is expected to rely upon the liver ($f_{met} = 0.65 + fe_{bile} = 0.10$). Alternatively, a program team may determine the forecasted risk is more than can be reasonably tolerated (e.g., in the case of a projected high victim DDI risk for a compound with a narrow therapeutic index) and may elect to revisit the

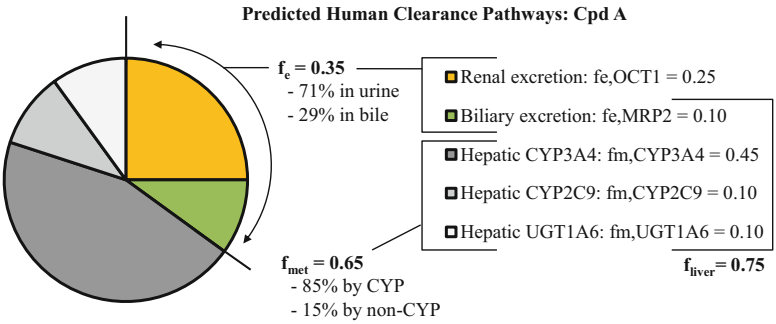


Fig. 6.8 An illustration of a fractional clearance profile on example discovery molecule A derived from a mechanistic prediction of human clearance

structure-activity relationship in an attempt to discover compounds bearing less risk in this particular area. Regardless of the ultimate decision, the value of this prediction method is manifested by availing the team of the forecasted clinical risk and permitting appropriate clinical progression both for product development and patient safety-benefit profile.

6.2.4 Summary

In the LO discovery setting, the prediction of human clearance for a molecule or a lead series of molecules is perhaps the single most important druggability property available to the discovery scientist. Projected human clearance is essential to anticipating drug performance in the clinic including the compound's exposure (AUC), its rate of elimination ($t_{1/2}$), and by extension the dose required to elicit a pharmacological response (efficacy). Without a promising or reliable projection of clinical performance, discovery teams are left only to continue their design-synthesis cycles until an optimally projected compound can be identified. As for methods of clearance projection, simple allometry is a historically relied-upon methodology for many reasons, and in some cases useful prediction accuracy can be demonstrated over large sets of molecules making its frequency of use not without justification. Mathematical corrections and modifications based on experimental preclinical data can improve allometric uncertainty and prediction error in some instances, but there are no universal rules for a correction method that works for all compounds. Data suggest that for compounds known to be cleared primarily by hepatic metabolism, correction of allometry by the ratio of animal-to-human in vitro intrinsic clearance may provide the most promising of the rational, semi-mechanistic approaches. Given no information on such mechanism, the FCIM approach provides perhaps the best available correction over all compounds in terms of reducing uncertainty and risk of overprediction bias (i.e., vertical allometry). On the other hand, mechanistic approaches of clearance prediction provide

researchers with methodologies that can (1) in some cases provide increased accuracy in clearance predictions, (2) be validated against preclinical in vivo data, (3) be informed by data obtained from human in vitro model systems, and (4) provide a rational prediction of the operative elimination pathways, the rate-determining mechanisms, and the relative contribution to the total clearance of the drug. With time, the underlying methods, including in vitro techniques and means of calculating parameters, will undoubtedly continue to evolve and improve, but with the steps outlined above researchers can achieve an increase in both prediction accuracy and utility leading to improved decisions in discovery compound selection as well as clinical trial design for patient safety and efficacy.

6.3 Integrated Approaches to Assess Drug-Drug Interactions

Pharmacokinetic drug-drug interactions (DDIs) are one of the most commonly encountered adverse drug reactions in the clinic and typically occur when one drug (the perpetrator) alters the metabolism of a coadministered drug (the victim). DDIs can manifest as an increase in the victim drug's exposure (and a decrease in clearance) due to reversible or irreversible enzyme inhibition, or a decrease in the victim drug's exposure (and an increase in clearance) due to induction of the enzymes responsible for its elimination. DDIs can also occur when a compound alters its own metabolism (autoinhibition/autoinduction), causing changes in pharmacokinetics following repeat administration. The consequences of these changes in drug exposure are dictated by the therapeutic window, and serious adverse reactions can occur when exposure is pushed beyond the efficacious range. Conversely, reduction in exposure due to enzyme induction can lead to a reduction or complete loss in efficacy. Considering the potentially fatal consequences, it is unsurprising that several high-profile drugs have been withdrawn from the market due to adverse DDIs.

Since cytochrome P450 (CYP) enzymes are highly susceptible to inhibition and are responsible for most known oxidative reactions, evaluating the propensity of a compound to inhibit or induce CYP enzymes is essential in drug discovery and is ultimately a regulatory requirement. This section focuses on appropriate strategies to predict and mitigate DDIs at different stages of drug discovery and development by evaluating CYP involvement in metabolism as well as achieving an assessment of a DDI's clinical significance.

6.3.1 Induction

Metabolic enzyme induction is a process in which increased protein synthesis yields elevated enzyme activity and a subsequent increase in metabolic activity.

Consequently, the perpetrator will increase the clearance and decrease the exposure of the victim drug, which can lead to a complete loss in pharmacological effect. Alternatively, a compound can induce its own metabolism, resulting in lower than expected exposure after repeat administration. Since the exposure is decreased in both of these situations, induction generally has a lower potential for adverse reactions compared to inhibition, and the primary concern in this case is a loss in efficacy of the victim drug. It is important to note, however, that an unexpected loss in pharmacological activity can lead to serious adverse effects depending on the desired therapeutic outcome (or lack thereof). For example, the potent CYP3A4 inducer rifampin can lead to serious adverse effects such as opioid withdrawal symptoms when administered with methadone [95] or organ rejection when administered with cyclosporine [96]. One case in which CYP induction could potentially lead to toxicity is when the formation of reactive metabolites increases, as with the alcohol-mediated induction of CYP2E1, which has been associated with elevated hepatotoxicity due to acetaminophen overdose [97]. These examples underscore the importance of identifying the induction potential of a new chemical entity prior to clinical nomination, and, consequently, pharmaceutical companies have incorporated the evaluation of induction potential into standard discovery screening and lead optimization programs [98–100].

Briefly, the traditional approach for assessment of CYP induction potential determines the changes in enzyme activity and mRNA expression of the key CYP isoforms (CYP1A2, CYP2B6, and CYP3A4/5) after treatment of primary cultured hepatocytes for 48 or 72 h [101]. Several in vitro systems are available for evaluation of CYP induction [99], and primary cultured human hepatocytes remain the preferred "gold standard" for the risk assessment of in vivo DDIs. Recently, high-throughput 96-well techniques have been developed that quantitate CYP activity, mRNA expression, protein levels, and cytotoxicity from a single well, all of which is well suited to lead optimization [102].

The two key endpoints determined in a traditional in vivo induction assay are EC_{50} (an indicator of potency, which is the concentration yielding half-maximal induction) and E_{max} (the maximum fold increase in enzyme activity or mRNA level). A high degree of variability exists in reported E_{max} and EC_{50} values for known inducers (such as rifampin) and has been attributed to differences in hepatocyte donors, cell culture conditions, and other experimental variables such as buffer type and the use of an overlay. Consequently, several investigators have recommended calibrating the induction data for new chemical entities using a positive control, which has yielded improved DDI prediction [103, 104].

Considerations on the risk assessment for induction using steady-state and dynamic modeling are included in Sect. 4. The remainder of this section will focus on the mitigation of and risk assessment for adverse DDIs arising from inhibition of CYP isozymes.

6.3.2 Reversible (Direct) Inhibition

The vast majority of clinical drugs are metabolized by the CYP enzymes, with CYP3A4, CYP2C9, CYP2C19, and CYP2D6 responsible for approximately 80% of known oxidative reactions [105, 106]. Due in part to their broad substrate specificity, CYP enzymes are highly susceptible to inhibition, which can be classified into two general categories: reversible (direct) and irreversible (time dependent). Direct, reversible inhibition is characterized by rapid association and dissociation of inhibitor and enzyme, thus preventing the binding of a substrate to the active site. Classical competitive inhibition, in which the inhibitor interferes with the binding substrate (and thus increases the apparent K_m without affecting V_{max}), is one of the most common types of reversible inhibition. In contrast, irreversible inhibition is characterized by either covalent bonding of the inhibitor to the enzyme or the formation of a quasi-irreversible metabolite-intermediate complex (MIC). This type of inhibition will be discussed in Sect. 1.3.

Since metabolism of the inhibitor is not a prerequisite for direct inhibition, reactive species formation is also not required for this type of inhibition. Consequently, in contrast to mechanism-based inactivators, there are no clear "structural alerts" for direct inhibitors. In addition, since this type of inhibition is reversible, the in vivo effects of direct inhibition persist only while the inhibitor is present. Overall, there have been fewer clinically relevant DDIs due to reversible inhibition than due to irreversible (time-dependent) inhibition; however, potent reversible inhibition is still an important liability, and current methodologies to evaluate direct inhibition and predict the likelihood of an in vivo DDI are summarized in Sects. 2.3 and 2.4.

6.3.3 Time-Dependent Inhibition

Time-dependent inhibition (TDI) refers to an apparent decrease in enzyme activity with time, caused by either the formation of inhibitory metabolites or the mechanism-based inactivation (MBI) of CYP enzymes. Experimentally, MBI is characterized by both time- and cofactor (NADPH)-dependent decreases in enzyme activity and can be broadly divided into two categories: (1) quasi-irreversible inhibition leading to metabolite-intermediate complex (MIC) formation and (2) irreversible inhibition due to covalent modification of a CYP heme or apoprotein. Common to both forms of MIB is the formation of a reactive intermediate that either coordinates tightly to the heme (in the case of MIC formation) or covalently binds to the enzyme (in the case of irreversible inhibition). Trends on the reactivity (and inhibitory activity) of specific functional groups known to cause MBI have emerged, and the chemical moieties associated with MBI have been the subject of several excellent and comprehensive reviews [107–109]. A brief summary is included below.

6.3.3.1 Quasi-Irreversible Inactivation and MIC Formation

The term quasi-irreversible inactivation originated from the observation that in vitro dialysis (typically overnight) can restore microsomal CYP activity following CYP inhibition due to MIC formation [110]. In addition to dialysis, quasi-irreversible inhibition can be reversed in vitro by potassium ferricyanide [111, 112]. Physiologically, however, this process is functionally irreversible in vivo and, therefore, indistinguishable from other forms of MBI (i.e., covalent modification of CYP) with respect to clinical DDIs [113]. In vitro, MIC formation can be readily identified by observing a shift in the characteristic absorption spectrum to 455 nm from 427 nm, which is due to alterations of the UV absorbance of the prosthetic heme [114].

Structure alerts for MIC formation include primary amines (and secondary/tertiary amines susceptible to N-alkylation to a primary amine) and methylenedioxyphenyl derivatives. Primary amines are converted to a highly reactive nitroso intermediate (via a hydroxylamine metabolite) capable of coordination with the prosthetic heme [115], whereas methylenedioxphenyl compounds are converted to reactive carbene intermediates [116].

Compounds susceptible to MIC formation comprise a wide range of chemical matter and subsequently represent the largest number of clinically relevant DDIs compared to the other mechanisms leading to MBI. This class of compounds includes macrolide antibiotics (e.g., troleandomycin [113] and erythromycin [117]) and calcium channel blockers (e.g., diltiazem [111] and verapamil [110]).

6.3.3.2 Covalent Modification of the Heme Prosthetic Group

Irreversible CYP activation, which cannot be reversed following in vitro dialysis, can occur via two distinct mechanisms that are differentiated by the site of covalent modification: the prosthetic heme group or the CYP apoprotein. Although the nitrogen atoms of the heme prosthetic group are relatively weakly nucleophilic, generation of the reactive species in the CYP active site in close proximity to the pyrrole ring can facilitate direct N-alkylation and subsequent CYP inactivation [109]. Common functional groups associated with heme alkylation include alkenes, alkynes, hydrazines, cyclopropylamines, and terminal olefins. Clinically relevant DDIs arising from heme alkylation are relatively rare; however, several examples exist of covalent heme modification leading to N-alkylprotoporphyrin IX formation, which can cause experimental porphyria in animals (for a review, see Marks [118]). Elucidation of the orientation of the heme within the CYP active site was advanced through the use of this class of compounds and their ability to selectively N-alkylate pyrrole nitrogen atoms [119]. In addition, 1-aminobenzotriazole (ABT) elicits covalent heme modification [120], and even though this modification has not been associated with clinical DDIs, it is an important tool that has been extensively used in early ADME discovery programs to understand the contribution of

CYP-mediated oxidative metabolism to total clearance [121]. The nonselective CYP inactivation mediated by ABT is postulated to proceed through the formation of a highly reactive benzyne intermediate capable of binding across two of the pyrrole nitrogen atoms of the prosthetic heme.

6.3.3.3 Covalent Modification of the CYP Apoprotein

In addition to the heme, the CYP apoprotein is an attractive target for irreversible, covalent modification by highly reactive species generated within the CYP active site. This process renders the enzyme functionally inactive or in some cases acts as a signal for proteolysis. Identification of the specific amino acid residues susceptible to adduct formation is now possible due to advances in liquid chromatography/mass spectrometry [122]. For example, the novel CXCR2 antagonist AMG487 was shown to form a highly reactive species (M4) that was responsible for MBI and covalently bound to Cys239 of CYP3A4 [123]. Due to the inherent reactivity of intermediate species, characterization of the putative reactive intermediate (s) involved in MBI is often achieved in vitro through conjugation with nucleophiles (e.g., reduced glutathione (GSH), semicarbazide, or cyanide) and more detailed structural assignment via NMR.

Common functional groups associated with covalent CYP apoprotein modification include furans, phenols, dihaloalkanes, and thiophenes [108]. As observed with compounds that elicit MIC formation, irreversible inhibitors that show apoprotein binding span a wide range of therapeutic areas, including the non-tricyclic antidepressant nefazodone [124], kinase inhibitors (e.g., imatinib) [125], and tetrahydrothienopyridines (e.g., clopidogrel) [126].

6.3.4 Strategies for Mitigating DDI-Related Liabilities

6.3.4.1 In Silico Methods

Since common functional groups are known to be associated with various forms of MBI, attempts have been made to use in silico techniques to predict the likelihood of CYP inactivation. The interactions at the CYP active site are complex, however, and the fate of an inactivating compound is determined by the balance between reactive species formation and metabolism not amenable to MBI. Structure-based computational models have been developed that successfully predicted MBI, but these are largely retrospective in nature and rely on an established mechanistic data set [127].

6.3.4.2 Reducing Lipophilicity and Introducing Polarity

A general association has been observed between increased lipophilicity and decreased CYP inhibition [128], and several investigators have demonstrated success with a strategy of altering lipophilicity to mitigate MBI. Westaway et al. [129] increased polarity in a series of related compounds to reduce CYP inhibition liabilities, leading to identification of the first small molecule agonist of the motlin receptor. Zhao et al. [130] also demonstrated that CYP MBI was attenuated for a series of GLYT-1 inhibitors through reduction of *c*log*P* by 0.6 log units. Similarly, a net reduction of cLogP from 5.5 to 3.5 decreased the CYP3A4 inhibition liability for a series of antigen 4 receptor antagonists [131]. While these examples demonstrate a general trend of improved CYP inhibition profiles with decreased lipophilicity, it should be noted that these structural modifications may also decrease desirable properties, such as target potency or other ADME properties. Consequently, additional, more targeted strategies have been employed to attenuate CYP inhibition.

6.3.4.3 Examples of Successful Medicinal Chemistry Strategies to Address TDI

Attenuation of TDI can be accomplished through replacement of (or blocking access to) the structural motif responsible for reactive intermediate formation with a metabolically unreactive functional group. To support this strategy, the putative reactive intermediate is first typically identified as a GSH conjugate via NMR, leading to synthesis of structural analogs that have various degrees of TDI. An example of this approach was highlighted by Johnson et al. [132], who reported successful modification of the indole core of chemoattractant receptor inhibitors to remove TDI and DDI liability. The mechanistic studies that enabled this successful medicinal chemistry effort were originally reported by Wong et al. [133], who showed that the lead candidate (AMG009, a 2-methylindole containing compound) elicited TDI through covalent modification. A GSH conjugate was unambiguously determined via NMR to be GSH adducted to the C-3 position of the 2-methylindole moiety, and replacement of this motif with an oxindole group prevented reactive intermediate formation and abolished TDI.

6.3.4.4 Analysis of Concomitant Medications (Conmeds)

In addition to mitigating the potential of clinical DDIs by structural design, clinical co-medication analysis for target patient populations can help put a presumed DDI risk into clinical context and diminish it. For example, a drug that inhibits or induces a certain CYP enzyme is unlikely to cause any clinically significant DDIs (severe adverse effects) if co-medications for the target patient population are

primarily cleared by different CYP enzymes. The significance of a clinical DDI also depends on the magnitude of the interaction. For instance, for a CYP3A4 inhibitor, the DDI risk with extensively CYP3A4-metabolized co-medications is likely much higher compared to other, less extensively CYP3A4-metabolized co-medications unless the co-medications have a wide therapeutic index and/or the associated clinical effects are manageable or not severe. Potential DDI risks might also be avoidable if administration of an alternative co-medication is an option, allowing for a bypass of metabolic pathways dominated by CYPs. For accurate assessment of the clinical liability and informed decision-making about compound progression, early cross-functional collaboration between biologists, DMPK scientists, and clinical scientists is valuable.

Bloomer et al. published an excellent review of how an understanding of co-medications in target patient populations can help prioritize or deprioritize DDI assessments and potentially discharge a DDI risk [134]. Common marketed co-medications are shown along with their varying prescription rates for various therapeutic targets, demonstrating that the prescription rate of a co-medication might be zero for a certain target but significant for others. Additionally, drug interactions of the most clinically relevant enzymes (and transporters) and the mechanistically corresponding number of co-medications (>300 evaluated) are shown. The number of co-medications is categorized into strong, moderate, or weak clinical perpetrators and into severe, moderate, or limited clinical risk for victims, all based on the "worst-case" scenario. As expected, CYP3A4 metabolizes the majority of the evaluated co-medications, many of which are strong perpetrators or victims with a moderate to severe clinical risk and, therefore, have clinically relevant interactions. The authors emphasize that the determination of clinical significance of DDI risks also requires a consideration of whether a victim co-medication has a narrow therapeutic index, in other words if a small change in exposure can lead to severe clinical outcomes, as well as the frequency of co-medication use. If the co-medication does have a narrow therapeutic index, further DDI evaluation is needed, and the question becomes one of overall risk-benefit. Is the DDI manageable by dose adjustment, drug monitoring, or toxicity monitoring, is it avoidable by alternative treatment options, or does co-administration need to be excluded? On the other hand, if the victim does not have a narrow therapeutic index and the frequency of co-medication use is low (and no severe toxicities are expected), co-administration is possible and the DDI risk would be very low.

6.3.5 In Vitro Assessment of DDI Potential

Routine evaluation of the potential for CYP inhibition (both reversible and time dependent) has become firmly entrenched in ADME screening paradigms commonly used in the pharmaceutical industry. As discussed previously, a key requirement for a successful medicinal chemistry campaign to reduce DDI liability is a

rapid, high-throughput assay that supports an iterative approach to attenuating CYP inhibition. Experimentally complex studies designed to determine kinetic parameters (K_i, K_I, or k_{inact}) are, therefore, not suitable in a screening paradigm, since longer incubations involving multiple time points are required to capture rates of inactivation at multiple concentrations, all of which is not amenable to a higher-throughput format. To support the assessment of CYP inhibition in a drug discovery setting, several abbreviated assays have emerged and are summarized below.

6.3.5.1 Higher-Throughput Evaluation of CYP Inhibition

Single-Point IC50

Early identification of the potential for direct inhibition has become an integral component of drug discovery screening paradigms. Gao et al. [135] reported an excellent correlation ($r = 0.99$) between the percent inhibition at a single test article concentration (3 μM) and in a traditional ten concentration IC_{50} curve. Abbreviating an IC_{50} experiment that requires multiple test compound concentrations to a single evaluation at 3 μM yields significant time and resource gains in a screening paradigm. While insufficient for detailed risk assessment, screening data from a single-point assay allows for rapid compound binning or rank ordering, which facilitates identification of structure-activity relationships (SARs) and efficient de-prioritization of potent inhibitors.

IC50 Shift

One of the most commonly implemented methodologies for early assessment of time-dependent CYP inhibition is the IC_{50} shift assay [136, 137] in which a left "shift" in the IC_{50} (i.e., an increase in potency) is presumed to be caused by TDI. In this experiment, two IC_{50} values are compared: (1) a standard IC_{50} value representing "direct" inhibition, with a 30 min preincubation in the absence of NADPH, and (2) a left "shifted" IC_{50} (in the case of TDI), with a 30 min preincubation in the presence of NADPH. The fold-shift is calculated as the ratio between the direct and shifted IC_{50} values, and an arbitrary threshold of a fold-shift greater than 1.5 has been proposed to flag compounds as positive for TDI. In general, the IC_{50} shift assay correlates well with TDI potential expressed as k_{inact}/K_I [136, 138, 139]. It is important to note that although the k_{inact}/K_I parameter is frequently used as an indicator of TDI potential as it combines both the potency and inactivation rate, it has no physiological relevance as a predictor of the magnitude of an in vivo DDI.

A key experimental consideration for the IC_{50} shift experiment is the use of a dilution, which has been shown to increase the assay sensitivity [140]. Parkinson et al. [141] summarized the pros and cons of the dilution approach, in addition to

highlighting the key experimental factors to consider when designing an IC_{50} shift experiment.

Several variations of the IC_{50} shift assay have been proposed. The area under the curve (AUC) shift approach [142] addresses the issues associated with weak inhibitors when an IC_{50} value (shifted and/or direct) cannot be calculated due to incomplete inhibition at higher concentrations. The AUC shift approach eliminates the need for a measurable IC_{50} value by comparing the AUC values from the percent activity remaining curve and the concentration curve (with and without NADPH in the preincubation). A threshold value of a percent shift greater than 15% was proposed to classify a compound as positive for TDI.

Another variation of the IC_{50} shift assay was proposed by Li et al. [143] in which, in addition to the two IC_{50} curves (with and without NADPH) generated using the traditional method, two additional IC_{50} values are determined in fresh microsomal incubations containing extracts from the first two incubations (with any metabolite formed during the initial reaction). The key readout from this novel format is that a left shift in IC_{50} from the incubations containing extracts is due to inhibition from metabolites and provides additional insight on the mechanism of inactivation.

Single Concentration Activity Loss Assays (with Dilution)

In contrast to the multiple concentrations required for the IC_{50} shift assay, an abbreviated approach that evaluates TDI at single concentration has been implemented in several drug discovery programs [139, 140, 144]. In one version of this methodology (referred to as "single k_{obs}"), a single concentration (typically set to 10 μM) is preincubated with microsomes and cofactor (NADPH) and then diluted (typically 1/20) into a selective marker substrate assay to measure residual activity. A plot of percent activity remaining versus time facilitates calculation of the inactivation rate (k_{obs}), and this value correlates with k_{inact}/K_I ($R^2 = 0.74$) [139]. Further evaluation of the single k_{obs} approach revealed that, using a database of 400 reference compounds, a k_{obs} value of 0.02 min^{-1} (or 45% inhibition after 30 min) is a good indicator of TDI potential. Wong et al. [145] demonstrated that the single k_{obs} assay can be abbreviated to evaluate percent inhibition at a single concentration at a single time point (30 min), eliminating the need for multiple time points required for determining the inactivation rate. Using this method, good correlations were observed between the percent inhibition at 10 or 25 μM and k_{inact}/K_I. Single concentration TDI assays have also been developed using a cocktail approach [146], in which the potential inhibition of multiple CYP isoforms is determined using multiple substrates in a single pooled incubation. A variation on the single concentration assay approach is the "2 + 2" method originally proposed by Zientek et al. [147], in which two concentrations of an inactivator are evaluated at two time points (0 and 30 min). Regardless of the format, abbreviated methods to identify TDI potential have become important tools in drug discovery and lead

optimization, and careful weighing of the resource savings compared to other methods (such as the IC_{50} shift assay) is required.

Choice of Appropriate Assay to Support TDI Mitigation

Comparison of the various abbreviated assay formats to assess TDI in a discovery setting has been the subject of several reviews [140, 148]. Practical considerations related to ease of automation have favored implementation of the IC_{50} shift approach; since many pharmaceutical companies evaluate direct (reversible) inhibition routinely, it is relatively simple to also determine an IC_{50} value with or without preincubation containing NADPH. Another attractive property of the shift assay includes its high sensitivity (especially if a dilution is used), which can minimize the propensity of false negatives, a primary goal for initial screening assays.

Both the IC_{50} shift and k_{obs} assays are good indicators of TDI potential, but a recent analysis by Wong et al. [145] suggested that the strong correlation between shifted IC_{50} and k_{inact}/K_I was positively biased by the inclusion of potent and efficient inactivators ($k_{inact}/K_I > 30$). When these strong inhibitory compounds were excluded, the correlation (R^2) between shifted IC_{50} and k_{inact}/K_I decreased from 0.8 to 0.6. In contrast, the correlation between single k_{obs} and k_{inact}/K_I remained good ($R^2 = 0.8$) even when potent and efficient inactivators were excluded from the analysis. Therefore, a single concentration assay may be a more appropriate format to support an iterative medicinal chemistry strategy to reduce the TDI/DDI liability for a series of structurally related compounds of similar, but moderate, TDI potential. Several successful implementations of a single concentration assay approach have been reported [132, 149].

6.3.5.2 Kinetic Determination of K_i, K_I, and k_{inact}

Direct (Reversible Inhibition)

A useful parameter for describing reversible inhibition is the dissociation constant K_i, which, unlike IC_{50} (the concentration that yields half-maximal inhibition), is independent of the substrate concentration. The methodology for determining K_i has been well characterized [150, 151] and involves determining the effect of a range of inhibitor concentrations on substrate turnover at various multiples of the K_m (typically 0.5, 1, 2, 4, and 5× K_m). Reciprocal plots (Lineweaver-Burk, Dixon, or Eadie-Hofstee) can aid in the diagnosis of the mode of irreversible inhibition (i.e., competitive, noncompetitive, or uncompetitive) and determination of K_i. The advent of modern software packages has enabled nonlinear regression analysis to fit the various forms of direct inhibition to determine the K_i.

K_i determination is not amenable to a high-throughput evaluation (since multiple substrate concentrations are required), and IC_{50} values are routinely used as the key

initial readout for reversible inhibition. A convenient relationship exists between K_i and IC_{50} for competitive and noncompetitive inhibitors: when the substrate is at its K_m, K_i is equal to one half of its IC_{50}. Haupt et al. [152] evaluated the accuracy of this estimation for 343 compounds, and 92% of the estimated K_i values (calculated as ½ IC_{50}) were within twofold of the actual K_i, supporting the use of IC_{50} as the key parameter for risk assessment for reversible inhibition.

The magnitude of change in victim drug exposure (i.e., the ratio between initial and final AUC values (AUCR)) can be predicted using a steady-state approach that takes into account the inhibitor concentration [*I*] and the K_i. The FDA draft guidance (2012) recommends a threshold value of an AUCR greater than 1.25 (where AUCR = 1 + [*I*]/K_i) to determine the need for a clinical DDI study. Contributions from direct inhibition are incorporated in the mechanistic static model (or net effect model) and are discussed in Sect. 4.1.

Irreversible (Time-Dependent) Inhibition

Predicting the change in exposure of a victim drug following TDI requires knowledge of two properties intrinsic to the inhibitor: (1) a measure of the potency, K_I, which is the concentration yielding half-maximal rate of inactivation and (2) the maximum rate of inactivation, $k_{inact.}$ The experimental procedure for determining these kinetic parameters was originally proposed by Silverman [153], and the fundamental components remain in modern methodologies. Assessment of TDI is accomplished in a two-step assay in which the CYP, cofactor, and inhibitor are preincubated in the first stage, followed by a second activity assay in which the residual enzyme activity from the preincubation is determined. The marker substrate is incubated at saturating conditions to minimize potential direct (reversible) inhibition, and a dilution (of at least 1/20) is used to reduce the inhibitor concentration in the activity assay. Incubation time for the activity assay is kept as short as possible (i.e., enough for sufficient marker substrate activity) to reduce the potential for continued TDI. These strategies (dilution, saturating substrate concentration, and reduced incubation time) all strive to isolate the enzyme inactivation to the preincubation stage and minimize any contaminating effect of the inhibitor during the activity assay.

The key output from the two-step procedure is a series of inactivation rates (k_{obs}, determined by a plot of the natural logarithm of percent remaining activity versus preincubation time) at different concentrations. A plot of k_{obs} versus inhibitor concentration typically yields a classic hyperbolic Michaelis-Menten curve in which K_I is analogous to K_m and k_{inact} is analogous to V_{max}.

Due to their kinetic nature, determination of the parameters K_I and k_{inact} is highly sensitive to experimental conditions such as incubation time, protein concentration, and dilution. Unsurprisingly, initial literature values for otherwise well-characterized inhibitors often had unacceptably high variability (for a review, see [154]). Yang et al. [155] highlighted two factors contributing to uncertainty and variability: inhibitor depletion and additional (contaminating) inhibition during the

activity assay. Thus, short incubation times and at least a 1/20 dilution are recommended, and these considerations have largely been incorporated into the majority of TDI kinetic studies reported in recent years [156].

Experimentally, one key consideration is the high concentrations that, depending on the potency, would be required to accurately characterize the kinetic parameters. For example, assuming that achieving maximal inactivation requires a concentration of about five times the K_I, a compound with a K_I of 25 μM would need to be in solution at a concentration of greater than 125 μM. Typically, the final solvent (DMSO) concentration is recommended to be less than 0.1% to avoid inhibition of CYP isoforms, translating to a stock solution of 100 mM for this example. Creating solutions at this level of concentration can pose a problem for compounds with poor to moderate solubility, and consequently, a common issue associated with adequate risk assessment for such compounds is the uncertainty associated with an incomplete K_I/k_{inact} curve. These limitations should be weighed carefully during the risk assessment phase.

A key criticism of the standard "two-step" approach to determining kinetic parameters is that the second stage activity assay is contaminated by continued inactivation due the incomplete removal of the inactivator. Dilution is the most common strategy for reducing the inhibitor concentration in the activity assay, but this approach requires an increase in protein concentration in the initial preincubation to facilitate sufficient substrate turnover for reliable activity measurement. Increased protein concentration can, in turn, increase the amount of nonspecific binding and decrease the amount of compound available to interact with the enzyme. Another method called progressive curve analysis, which accounts for the change in substrate, inhibitor, and metabolite concentrations in the context of a TDI assay, provides an alternative approach to determining kinetic parameters. This "all-in" approach does not require separation of enzyme inactivation from assessment of remaining enzyme activity, thus eliminating the need for a dilution. Several investigators report improved accuracy in K_I and k_{inact} determination using a progress analysis approach [157, 158]. In addition, recent reports by Nagar et al. [159] and Korzekwa et al. [160] suggest a numerical method that directly determines TDI parameters using kinetic schemes which lead to better estimates of K_I and k_{inact}. While initial reports are promising, further studies are required to determine if K_I and k_{inact} values determined using these modeling approaches yield improved accuracy in the prediction of in vivo DDI.

6.3.6 Assessing Clinical DDI Risk

6.3.6.1 Static Mechanistic Models

Although advances in high-throughput screening and early evaluation of DDI potential have enabled successful mitigation strategies and the mechanism that drives reactive species formation and TDI can be fully elucidated preclinically,

the necessary structural changes to eliminate a CYP liability are often incompatible with attributes required for pharmacological activity. In this common scenario, risk assessment for the likelihood of a clinically relevant DDI is required in order for a molecule with in vitro DDI liabilities to progress to the clinic.

Initial attempts to predict the in vivo consequences of MBI were first proposed by Hall and associates [161, 162], who applied fundamental concepts of suicide-type mechanism-based enzyme activators to CYP enzymes. The primary assumption for this approach is that the inhibitor concentration is constant at steady state, and, over the past decade, this model has evolved to incorporate contributions from gut metabolism and competing metabolic pathways. More recently, these efforts culminated in a "net effect" model that incorporated contributions from direct (reversible) and time-dependent inhibition and induction [163]. This mechanistic static model is now recommended by the FDA in its draft guidance (2012) and consists of three components: (1) reversible inhibition, (2) TDI, and (3) induction. Interactions at the level of the gut and liver are also incorporated into the model.

Although several investigators have reported on the relative predicative accuracy of various input inhibitor concentrations ([I]) in the static model, the FDA recommends the most conservative approach, which translates to a high estimate of the input inhibitor concentration (i.e., free hepatic portal C_{max}). This conservativeness reduces the propensity for false-negative results (i.e., failing to predict a clinically relevant DDI), but increases the potential of false positives, leading to more in vivo studies yielding nonclinically significant results.

The predictive accuracy of static models has been evaluated by several investigators. Fahmi et al. [98] reported that, for a series of 30 clinical DDIs, the combined net effect model yielded an 88% success rate with a mean-fold error of 1.74. However, the success rate was low for compounds that were anticipated to be both inhibitors and inducers, with four out of five such compounds showing predicted exposure changes of greater than twofold higher or lower than the observed values. Guest et al. [164] also observed that a static approach yielded good overall predictions for in vivo DDIs (77% success rate). However, a review [165] of the multitude of retrospective analyses reveals that conclusions on the predictive accuracy of static models are highly dependent on the input parameters (k_{deg}, K_I/k_{inact}, $[I]$, gut contribution) as well as the perpetrator-victim pairs considered for each study. In Wong's analysis, low estimates of certain parameters (such as k_{deg}) were shown to affect conclusions on the apparent improved prediction accuracy when selecting other parameters (such as the input inhibitor concentration). Thus, the perceived success of the static model is highly dependent on the selection of input parameters, and conclusions based on the predictive accuracy using retrospective studies should be caveated by the specific assumptions of the input parameters selected.

The tendency for static models to overpredict the magnitude of an in vivo interaction has been observed by several investigators. Vieira et al. [166] observed that, in general, mechanistic static models tended to overpredict the likelihood of an in vivo DDI arising from TDI. Kenny et al. [167] reported that, for a series of 12 kinase inhibitors, a traditional steady-state approach generally led to an

overestimation of DDI magnitude and several false positives. Taken together, these studies support the FDA's general opinion that the conservative static approach minimizes the propensity for false negatives.

A potential drive for the overestimation of in vivo DDI using a static approach is the inaccuracy of kinetic parameters derived from microsomal experiments as a result of uncertainties around the actual unbound concentration in the liver with access to the CYP active site. Mao et al. [168] reported improved predication accuracy when using K_I and k_{inact} values determined in cryopreserved human hepatocytes suspended in human plasma, suggesting that accounting for plasma protein binding and the requirement for delivery into hepatocytes may improve the effectiveness of a steady-state approach to predict DDI.

Overall, static models tend to minimize the incidence of false negatives, which is desirable from a safety perspective, but tend to overpredict the likelihood of an in vivo DDI. In an analysis reported by Prueksaritanont et al. [17], overpredictions of up to tenfold were observed when using the free hepatic portal concentration as the input inhibitor concentration recommended by the FDA. These authors comment that, while the guidelines are likely successful in avoiding false negatives, essentially no clinical DDI study can exclude compounds that demonstrate any nonnegative inhibitory signal, regardless of the in vitro study outcome.

6.3.6.2 Dynamic Models

In a recent review by Sager et al. [14] of the use of physiologically based pharmacokinetic (PBPK) modeling, the highest percentage of literature articles were on DDIs (27%) followed by clinical pharmacology (23%) and absorption (12%). These statistics are unsurprising considering that a PBPK approach overcomes the need to simplify an interaction to static conditions by accounting for changes in three critical parameters over time: (1) inhibitor, (2) victim, and (3) enzyme concentration. The basic framework for a PBPK model consists of a series of compartments representing individual organs or tissues, with the relationship between these compartments defined by physiological flow (e.g., blood, bile, or pulmonary ventilation). A system of differential equations describes the rate of change of drug concentration or active CYP enzyme with respect to time; thus PBPK models take into consideration the kinetic nature of an interaction involving a DDI (for reviews, see [169]).

Recognizing the mathematical complexity of a PBPK approach, the advent of sophisticated software solutions has enabled broader adoption and application of this technique. Proprietary software systems that are designed for PBPK modeling include SimCYP (Certara), GastroPLUS (Simulations Plus), PK-SIM (Bayer Technology Services), and Cloe Predict (Cyprotex Ltd). Of these packages, SimCYP is one of the more commonly used in the pharmaceutical industry, and this population-based ADME simulator incorporates PBPK modeling to predict plasma concentration-time profiles based on input physiochemical and in vitro derived

parameters [170]. This modeling system has received widespread implementation in the pharmaceutical industry as well as recognition by the FDA as a useful tool for assessing DDIs. The application of PBPK modeling in the pharmaceutical industry has been recently reviewed by Jones et al. [171].

The utility of SimCYP to predict DDIs (arising from CYP inhibition) has been retrospectively investigated by Wang [172], and he found that the majority of studies (87%) were predicted within twofold error of observed values. In a similar retrospective analysis, Einolf [173] demonstrated good (within twofold error) predictive accuracy of the PBPK approach. Overall, both of these retrospective studies suggest a modest improvement in predictive accuracy when using a PBPK approach facilitated by SimCYP compared to the steady-state model, which tended to overpredict.

With respect to induction, Almond et al. [103] reported that the prediction accuracy of a PBPK-based approach improved when in vitro EC_{50} and E_{max} data were calibrated using in vivo data (i.e., the maximum fold in vivo induction, Ind_{max}). These authors emphasized the need for individual laboratories to evaluate how prototypical inducers respond in their own in vitro system and understand the relationship between their in vitro system and in vivo induction in order to improve the risk assessment of induction-mediated DDIs.

In addition to assisting in risk assessment, a key application of dynamic modeling is the ability to simulate clinical exposures and assist in DDI trial design. One example in which PBPK modeling was used to help guide clinical DDI study design was reported by Jones et al. [171]. Compound Y was a late-stage clinical candidate that had marked nonlinear pharmacokinetics, with a sixfold increase in dose-normalized AUC between the 5 and 400 mg doses. It is important to note that compound Y was primarily metabolized by CYP1A2 and the key DDI concern was its susceptibility as a victim to CYP1A2 inhibitors, such as fluvoxamine. When in vitro data was used in a "bottom-up" modeling approach, SimCYP underpredicted exposures at the higher doses. By leveraging SimCYP's Bayesian parameter estimation, a "middle-out" approach determined that threefold higher estimates of K_m and V_{max} were required in order to accurately simulate the supraportional PK observed in the clinic. The default clinical trial design to evaluate the potential for a CYP1A2-mediated DDI would have determined the effect of a 100 mg daily dose of fluvoxamine (a potent CYP1A2 inhibitor) on a low dose of compound Y. However, the PBPK model, refined using the "middle-out" approach to accurately capture the nonlinear PK, predicted that unacceptable CYP1A2-mediated DDIs would occur only at low, subtherapeutic doses of compound Y. With this insight, the clinical DDI trial was designed to include both high and low doses of compound Y and confirmed a minimal risk for CYP1A2-mediated DDI at the higher (and more therapeutically relevant) dose. In the absence of PBPK modeling, only the low dose of compound Y would have been evaluated, and the positive DDI potential would have terminated the program.

6.3.6.3 Microdosing

The potential for DDIs can be relatively easily predicted by any of the previously discussed in silico, in vitro, and modeling and simulation methods. However, the magnitude of the DDI remains far more challenging to predict. This is particularly true at the drug discovery stage when preclinical data is prospectively translated into clinical interactions compared to the early clinical development stage when clinical DDI data is already available and predictions are made retrospectively. Depending on the stage at which a drug is in the pipeline and the target of the drug discovery program, knowing the magnitude of DDIs might not be critical, and the previously discussed methods would provide sufficient guidance. As drug candidates enter the lead optimization stage and a lead compound needs to be selected among several others, the ability to confidently predict the potential and the magnitude of an interaction becomes more and more essential for informed "go/no-go" decision-making and ultimately for the patients' safety and therapeutic benefit. This is particularly true in the case of drug candidates with a narrow therapeutic index, such as anticancer drugs, for which a relatively small change in exposure can lead to detrimental clinical outcomes.

Exploratory microdosing in human, a subset of phase 0 studies, is one promising and emerging tool that can be utilized to predict and quantify the DDI risk based on CYP inhibition for victim drugs. Microdosing has been successfully evaluated and used for more than a decade; however, up till now this technique has been predominantly used for the evaluation of human pharmacokinetics [174]. More recently, the application of microdosing has spread into the evaluation of DDIs [175]. Microdosing refers to a dose for a small molecule that is no greater than 100 μg or 1/100th of the no observed adverse effect level (NOAEL), whichever is lower. Neither therapeutic nor toxic effects are expected to be seen at such a low dose, enabling drugs to be safely evaluated in humans as part of the exploratory lead optimization stage rather than the clinical stage. Compared to traditional phase 1 studies, timelines and regulatory requirements, such those needed for preclinical data, compound amounts, and specifications, are limited for microdosing studies, and thus these studies can be conducted in a shorter time frame at a lower cost. The decreased time and cost of these studies is attractive and makes them feasible at the drug discovery stage as a complimentary tool in the candidate selection process.

Existing published examples for DDIs in human volunteer microdosing studies are still very sparse, but they support the validity and usefulness of this technique and encourage their continued application. Croft et al. showed in a proof of concept study that human microdosing can be used to investigate the DDI risk for victim compounds [176]. Midazolam (a CYP3A4 substrate), tolbutamide (a CYP2C9 substrate), caffeine (a CYP1A2 substrate), and fexofenadine (a P-glycoprotein (P-gp) substrate) were given simultaneously at a microdose of 25 μg each ([176]). PK parameters of the substrates were determined before and after combined, pharmacologically active, repeat doses of ketoconazole (a CYP3A4, CYP2C9, and P-gp inhibitor) and fluvoxamine (a CYP1A2 and CYP2C9 inhibitor) were

administered at 400 mg and 100 mg, respectively. These data were compared to the PK parameters obtained in a traditional, non-microdosing DDI study. The CYP substrates represent lead compounds, whereas the inhibitors represent coadministered marketed drugs/perpetrators. The results showed that alterations in PK due to inhibition in the microdosing study were quantitatively comparable to those observed in the regular studies. Another example of human cassette microdosing was published by Maeda et al. [177] and describes the simultaneous administration of atorvastatin, pravastatin (OATP substrates), and midazolam (a 3A4 substrate) as a microdose. The PK of the substrates was determined before and after administration of separate, regular (pharmacologically active) doses of rifampicin (an OATP inhibitor) and itraconazole (a CYP3A4 inhibitor) at 600 and 200 mg, respectively. The AUC exposure was significantly increased for the microdosed OATP (pravastatin) and 3A4 (midazolam) substrates in the presence of its corresponding inhibitor. The atorvastatin AUC exposure significantly increased only in the presence of the OATP inhibitor rifampicin but not in the presence of the CYP3AA4 inhibitor itraconazole, suggesting that hepatic elimination of the compound is predominantly driven by hepatic uptake by OATPs at a microdose level. For microdose cassette dosing, no interactions are expected among the compounds in the cassette due to the very low levels of the compounds.

Microdosing studies have also been utilized to evaluate DDIs due to food [178] or transporters [179]; however, these interactions are not discussed in more detail here as this section is focused on DDIs due to metabolic enzymes.

Due to the nature of microdosing studies, various highly sensitive analytical tools (e.g., accelerator mass spectrometry (AMS), positron emission tomography (PET), and liquid chromatography-tandem mass spectrometry (LC-MS/MS)) are utilized to quantify the low drug concentration levels [180, 181]. For the purpose of assessing DDI studies at the lead optimization stage, LC-MS/MS appears to be the most practical. LC-MS/MS is most widely available, is relatively cost-effective, and allows for faster and more straightforward sample processing and analysis. In addition, the sample shipment is less challenging than that for PET studies, which requires that the PET facilities be in close proximity to the research laboratory because of the generally short half-life of positron emitting nuclides. Unlike AMS and PET, LC-MS/MS enables dosing of multiple compounds simultaneously (cassette dosing) and the use of non-radiolabeled material. This is particular important at the lead optimization stage when a radiolabeled compound is typically not available. While LC-MS/MS is less sensitive than AMS and PET, an evaluation of the lower limits of quantitation of 31 diverse drugs suggests that LC-MS/MS is sensitive enough to quantify plasma levels of most non-radiolabeled drugs in microdosing studies to a degree that basic pharmacokinetic parameters can be determined [182]. In practice, LC-MS/MS has already been successfully utilized as an analytical tool in various microdosing studies, although there are reported instances where it failed. Compounds with very low bioavailability or a high volume of distribution might be challenging to analyze via LC-MS/MS [183, 184].

At this point, only a very small amount of published data exists for determining DDIs of potential victim drugs by human microdosing, and the data is currently

limited to marketed drugs with no examples of exploratory drugs [175]. More caveats might emerge and a better understanding obtained of the prediction accuracy as more study results become available. However, this approach appears to be a feasible, safe, and complimentary tool that can add valuable information at the lead optimization stage if there are limitations or low confidence in other predictive methods [171]. In this case, microdosing can help to prioritize and select lead compounds and/or make "go/no-go decisions" efficiently and confidently.

6.3.7 Summary

Evaluating the in vitro propensity of a compound to inhibit or induce CYPs in vitro allows for identification of potential DDI liabilities and SARs. However, in vitro potency alone does not reflect the potential risk of a DDI manifesting in vivo. Achieving an assessment of clinical significance of DDI is an important aspect of drug discovery and development. Many tools exist to predict clinical DDI using in vitro data, ranging from simple "rule-of-thumb" assessments such as $[I]/K_i$ to complex PBPK simulation packages such as SimCYP®. These in vitro tools are used to obtain clear data on DDI potential over conducting in vivo animal studies, which can lead to variable results due to species differences in enzymes or physiology. In addition, while much less explored, microdosing in human can be considered in certain instances as a valuable tool at the lead discovery stage to predict the risk of drugs being victims of DDI. The evaluation of DDIs is an iterative process throughout drug discovery and development with different approaches appropriate at different stages and continuous refinement of the simulations as new data becomes available.

6.4 Integrated Approaches to Assess Brain Penetration

The brain is separated from the systemic circulation by two main barriers: the blood-brain barrier (BBB) and the blood-cerebrospinal-fluid barrier (BCSFB). The BBB is composed of cerebral endothelial cells that differ from those in the rest of the body by the presence of extensive tight junctions, the absence of fenestrations, and the sparse pinocytotic vesicular transport. The BCSFB is formed by a continuous layer of polarized epithelial cells that line the choroid plexus in the brain ventricles. Both the BBB and BCSFB exhibit very low paracellular permeability and express multiple drug transporters. These characteristics restrict the entry of hydrophilic compounds or efflux transport substrates into the brain [185]. In this section we will introduce the concepts related to brain penetration from the perspective of small molecule drug discovery and discuss how to effectively address BBB issues in lead optimization.

6.4.1 Pharmacokinetics of Brain Drug Delivery

For drugs that target the central nervous system (CNS), compounds with good brain penetration should be selected during the drug discovery phase. Drug brain penetration can be quantified by two parameters: the time required to reach equilibrium between the brain and plasma concentrations and the extent of brain penetration. These concepts are analogous to the rate and extent of oral absorption [186]. The time to reach equilibrium is defined by the half-life needed to reach equilibrium between brain and plasma concentrations [187]. The extent of brain penetration is defined as the ratio of concentrations between free drug in the brain and free drug in the plasma at distributional equilibrium, $K_{p,uu}$ [188].

6.4.1.1 Time to Equilibrium

An empirical approach to identifying compounds with a quick onset of action involves the screening of compounds in in vivo studies. In order to understand the theoretical basis of this practice and develop a rational approach to design a compound with a quick onset of action, one needs to understand the kinetics involved in reaching equilibrium between brain and plasma concentrations. The intrinsic brain equilibrium half-life ($t_{1/2eq,in}$), defined as the time required for the free brain concentration to reach 50% of free plasma concentration, is used to quantitate how quickly a compound can enter into the brain [187]. The $t_{1/2eq,in}$ value is calculated according to Eq. 6.16

$$t_{1/2\mathrm{eq,in}} = \frac{V_\mathrm{b} \ln 2}{\mathrm{PS} \cdot f_{\mathrm{u,brain}}} \tag{6.16}$$

where V_b represents the physiological volume of brain tissue, PS is the permeability-surface area product, and $f_{u,brain}$ is the unbound fraction in the brain tissue [187]. This equation demonstrates that a combination of BBB permeability and brain tissue binding determines the time to reach brain equilibrium, which is supported by experimental observations. Theobromine has a low to moderate PS (23 mL/h/kg) and a high $f_{u,brain}$ (0.61), resulting in a PS·$f_{u,brain}$ of 14 mL/h/kg. In contrast, fluoxetine has a high PS (619 mL/h/kg) and a low $f_{u,brain}$ (0.00094), resulting in a PS·$f_{u,brain}$ of 0.6 mL/h/kg. Consistent with a higher PS·$f_{u,brain}$ product, the observed $t_{1/2eq,in}$ for theobromine (~0.1 h) was shorter than that of fluoxetine (~1 h) [187]. Similar conclusions were made by Syvanen et al. [188].

BBB permeability and brain tissue binding are likely correlated [187]. For example, lipophilic compounds tend to have high BBB permeability and brain tissue binding, while hydrophilic compounds have low permeability and tissue binding. For many CNS drug-like molecules, plasma concentrations can quickly equilibrate with brain concentrations despite substantial variability in BBB permeability. In a study reported by Liu et al. [187], the brain concentration of six out of

seven model compounds equilibrated with plasma concentration within 2 h post-subcutaneous dose. Similar conclusions were drawn from a brain microdialysis study in which compounds with much different BBB permeability values were quickly able to reach brain equilibrium [189]. As a result of these data, a lead compound should not be eliminated as a candidate compound for quick brain penetration solely on the basis of low BBB permeability.

6.4.1.2 Extent of Brain Penetration

$K_{p,uu}$ is a measure of the level of free brain concentration relative to the free plasma concentration; therefore, understanding the factors governing $K_{p,uu}$ is important in drug design. In general, these factors can be identified through a compartment-based pharmacokinetic analysis. For CNS pharmacokinetics specifically, a simplified physiologically based three-compartment model that incorporates the plasma, brain, and CSF can be used.

According to this three-compartment model (Fig. 6.9), Eq. 6.17 can be derived at steady state when the plasma, brain, and CSF concentrations remain constant. Cl_{uptake} and Cl_{efflux} are the active uptake clearance and efflux transport clearance, respectively, at the BBB. Cl_{bulk} is the clearance due to brain interstitial fluid bulk flow and $Cl_{metabolism}$ is the brain metabolic clearance. According to Eq. 6.17, the extent of brain penetration, $K_{p,uu}$, can be augmented by either increasing $Cl_{diffusion}$ or Cl_{uptake} or reducing Cl_{efflux}, Cl_{bulk}, or $Cl_{metabolism}$. The plot is from Liu et al. [190].

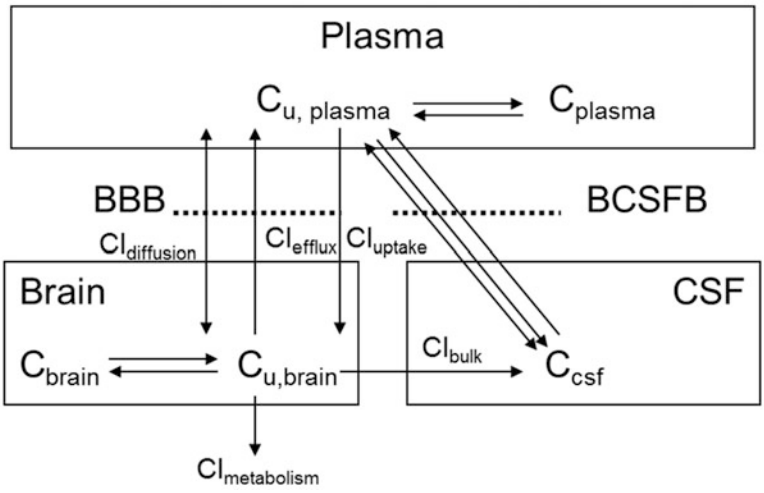


Fig. 6.9 Three-compartment model for CNS drug disposition

$$K_{p,uu} = \frac{Cl_{diffusion} + Cl_{uptake}}{Cl_{diffusion} + Cl_{efflux} + Cl_{bulk} + Cl_{metabolism}} \quad (6.17)$$

Cl_{uptake} can be enhanced by designing a compound to be a substrate of brain uptake transporters. For example, the large neutral amino acid transporter 1 transports L-DOPA and gabapentin across the BBB. Although L-DOPA has been available for several decades, the same success in increasing brain penetration has rarely been replicated in other drugs, except for its close-in analogs. Effective in vitro approaches have yet to be developed to screen brain uptake transporter substrates that deliver drugs through the uptake transporters at the BBB for compounds that are not closely related to endogenous substances. However, as explained below, it is more feasible to design lipophilic compounds (high $Cl_{diffusion}$) without significant efflux transport (low Cl_{efflux}) than to design compounds as uptake transporter substrates (high Cl_{up}).

Cl_{bulk} can play an important role in decreasing $K_{p,uu}$ for low permeability compounds. Cl_{bulk} is estimated to span the range of 0.2–0.3 μL/min/g [191]. Take the example of mannitol, a compound of low permeability with a $Cl_{diffusion}$ value of less than 1 μL/min/g. Bulk flow becomes significant compared to its permeability, resulting in a low $K_{p,uu}$ (0.01). Cl_{bulk}, however, is not an important factor for typical CNS lead compounds, which generally have moderate to high $Cl_{diffusion}$. For example, $Cl_{diffusion}$ for caffeine is approximately 13 μL/min/g. In this case, Cl_{bulk} is much lower than the permeability and has an insignificant effect on caffeine's $K_{p,uu}$ (1.0) [192].

Brain metabolism, $Cl_{metabolism}$, can also play a significant role in reducing $K_{p,uu}$. Metabolizing enzymes such as monoamine oxidases (MAOs), flavin-containing monooxygenases (FMOs), cytochrome P450s, and glucuronosyltransferases have been identified in brain endothelial cells and brain tissue [193–196]. Hence, the metabolic stability of a compound in brain tissue needs to be examined in early drug discovery. If a compound is not stable in brain tissue, its impact on brain penetration assessment, such as brain/plasma ratios, and free brain concentrations need to be investigated.

For most CNS compounds, the uptake drug transport, bulk flow within the brain tissue, and the metabolism in the brain is insignificant compared to the diffusion process. In typical CNS drug discovery programs, the main mechanism that impairs brain penetration of small molecules is efflux transport mediated by drug transporters at the BBB, which is quantified by efflux clearance, Cl_{efflux}. In this situation, Eq. 6.17 can be simplified to Eq. 6.18.

$$K_{p,uu} = \frac{1}{1 + Cl_{efflux}/Cl_{diffusion}} \quad (6.18)$$

It is clear from Eq. 6.18 that compounds with high diffusional permeability (high $Cl_{diffusion}$) are desirable so that the impact of the efflux transport does not reduce $K_{p,uu}$ significantly.

6.4.2 Drug Transporters at the BBB

Efflux transporters such as P-glycoprotein (P-gp, gene symbol Abcb1), breast cancer resistance protein (Bcrp, Abcg2), multidrug resistance-associated proteins (Mrp, Abcc), and several organic anion transport polypeptides (Oatp, Slco) as well as the organic anion transporter (Oat3, Slc22a8) have been identified at the BBB and/or the BCSFB in preclinical species and humans [55, 197, 198]. Yousif et al. [199] examined the gene profile and expression for Mdr1a, Mdr1b, Bcrp, Mrp1–5, and Oatp1a4 (Oatp2) in rat brain and found that the gene profiles of only Mdr1a, Bcrp, Mrp4, and Oatp1a4 were similar to those of endothelium markers, indicating the presence of these transporters at the BBB. In the past few years, significant progress has been made in using a proteomic approach to assess the transporter expression at the endothelia from various species. Teresaki and coworkers determined the absolute drug transport proteins at the BBB using LC-MS/MS [200–202]. Figure 6.10 shows the relationship between monkey and human and between mouse and human transporter protein expression levels. The protein expression level of MDR1 was not significantly different between brain capillaries of human and monkey (left panels). In contrast, MDR1 expression in human was 43% of that in mouse (right panels). The lower protein expression of MDR1 in human and monkey brain capillaries would suggest a higher brain distribution of MDR1 substrates in human and monkey than in mice. In contrast, the protein expression of breast cancer resistance protein (bcrp)/ABCG2, which is also a drug efflux transporter at the BBB, was 1.7-fold greater in monkey but 1.9-fold lower in mouse compared with human brain capillaries. Considering the variability of the observed data, the functional importance of different transporter expressions among various species remains to be examined. However, quantitative-targeted proteomic analysis provides clear molecular evidence for species differences in the BBB, which is important when predicting drug permeability across the human BBB from animal and/or in vitro data.

Although many drug transporters at the BBB have been reported in the literature, majority of the data reveals that only P-gp and in some cases Bcrp are functionally important in limiting drug distribution to the brain. The functional activity of drug efflux transporters at the BBB is normally quantified by the brain-to-plasma ratio in knockout (KO) animals versus the brain-to-plasma ratio in wild-type (WT) animals, abbreviated as the KO/WT K_p ratio.

Figure 6.11 shows the importance of P-gp in limiting brain penetration of its substrates and, to a lesser extent, the importance of Bcrp in limiting the brain penetration of its substrates. Citalopram is not a substrate of P-gp or Bcrp and its KO/WT K_p ratio is within or near twofold error of unity. Amprenavir, digoxin, loperamide, quinidine, and verapamil are P-gp substrates, and their KO/WT K_p ratios are much greater than unity in the P-gp KO mice (blue bars); however, in Bcrp KO mice (brown bars), their KO/WT K_p values are near unity. In this data set, sulfasalazine is the only Bcrp substrate, but its brain drug level is below the quantitation of the assay in the Bcrp KO mice. Therefore, it is difficult to assess

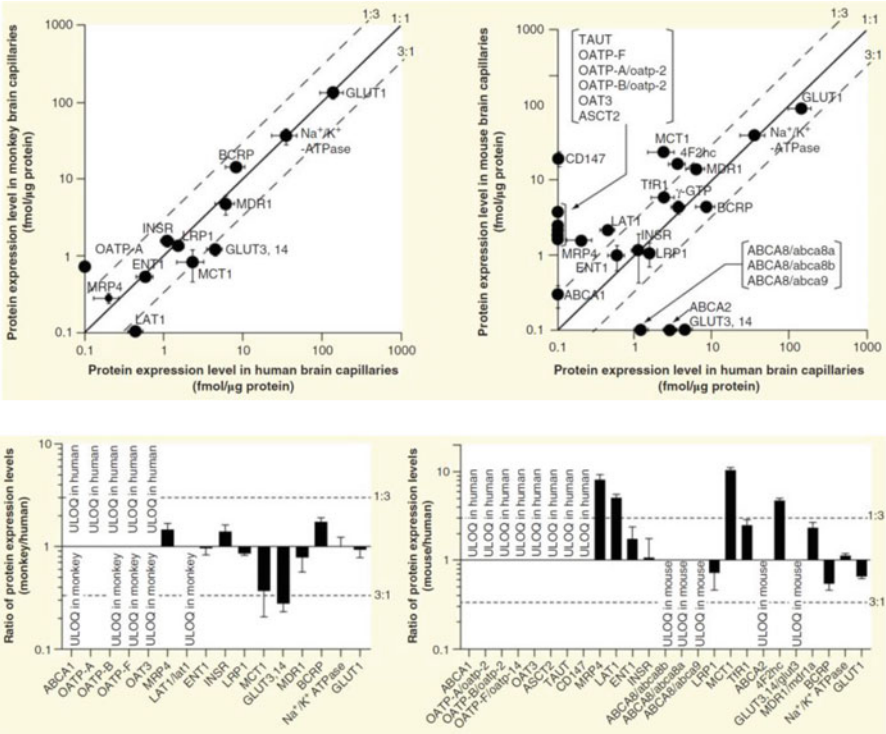


Fig. 6.10 Comparison of protein expression levels in brain capillaries between human and monkey and human and mouse. (**a**) Human-monkey and (**b**) human-mouse. *Upper panels*: comparison of absolute protein expression levels of membrane proteins between humans and animals. The *solid line* passing through the origin represents the line of unity, and the *broken lines* represent threefold differences. Each point represents the mean ± SD. The molecules on the *horizontal* (mouse, monkey) or *vertical* (human) *axis* are below the limits of quantification. *Lower panels*: ratio of expression levels of membrane proteins in animals to those in humans. The *broken lines* represent threefold differences. Each bar represents the mean ± SD. The molecules were ordered according to their expression levels. ULOQ means that the expression was under the limit of quantification in the indicated brain capillaries. *INSR* insulin receptor, *TfR* transferrin receptor. Plots are from Ohtsuki et al. [203]

the functional importance of Bcrp at the BBB. Elacridar, imatinib, and prazosin are dual P-gp and Bcrp substrates. For these substrates, knocking out Bcrp alone has little impact on brain penetration, but knocking out P-gp alone has a clear effect on the brain penetration for some of these compounds such as elacridar and imatinib. The greatest effect, however, is from knocking out both P-gp and Bcrp. This observation is consistent with many studies reported in the literature [205–207].

P-gp is considered to be the most important efflux drug transporter at the BBB, and therefore, any CNS drug that is also a P-gp substrate will have a reduced therapeutic window. The reduced therapeutic window occurs because P-gp-mediated efflux at the BBB normally cannot be saturated and a high plasma free

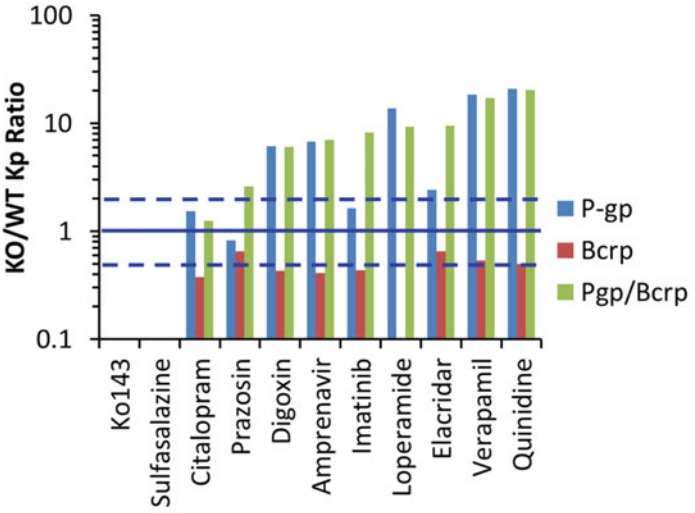


Fig. 6.11 Effect of P-gp and Bcrp on brain penetration in mice. The *solid line* represents unity. The *broken lines* represent twofold error from the line of unity. The concentrations of Ko143 and sulfasalazine are below the quantitation of the assay. Data are from Liu et al. [204]

concentration is necessary to compensate for the efflux transport and drive the free concentration in the brain to the efficacious level. In this situation, the higher plasma concentration can increase the risk of peripheral toxicities. An ideal CNS drug, therefore, is not a good substrate for P-gp efflux; however, scarce literature data are available to define the level of P-gp-mediated transport that is acceptable for drug candidates. In a comprehensive study, P-gp transport of the 32 most prescribed CNS drugs were examined in mdr1a/1b KO and WT mice. In all, 22% of the compounds showed an efflux ratio of unity, 72% had values between 1 and 3, and 6% had values between 3 and 10 [208]. These results indicate that the majority (92%) of CNS drugs tested show no to weak P-gp-mediated transport. These data support the conclusion that "good" P-gp substrates should be avoided as CNS drugs. On the other hand, P-gp-mediated drug transport per se would not be the sole reason to terminate the development of a candidate if a large therapeutic window is projected in humans.

Several examples indicate the benefits of developing P-gp substrates as peripheral targeted drugs to reduce CNS side effects. First-generation H1 antagonists such as diphenhydramine, triprolidine, and hydroxyzine produce histamine blockade at H1 receptors in the CNS and frequently cause somnolence or other CNS adverse effects. However, second-generation H1 antagonists such as cetirizine, loratadine, fexofenadine, and desloratadine produce relatively little somnolence or other CNS side effects at recommended doses. Chen et al. [209] demonstrated that the first generation of H1 antagonists are non-P-gp substrates and the second generation of H1 antagonists are P-gp substrates. The first generation of these drugs, therefore,

displays high brain concentrations, while low brain concentrations are seen for the second-generation drugs. Similar observations were made by several other groups [210].

The literature is not consistent on species differences for P-gp activity. One study showed a difference in P-gp ATPase binding affinity between rhesus monkey, dog, and human [211]. Further, the K_m values of diltiazem exhibited approximately 16.5-fold differences among human, monkey, canine, rat, and mouse P-gp-transfected cell lines [212]. Yamazaki et al. [213] reported different efflux ratios between mouse and human P-gp-transfected cells, suggesting species differences for P-gp activity. In contrast, a study using a set of 3300 compounds demonstrated a 93% overlap between mouse and human P-gp-mediated transport [214]. Thus, significant mouse-human differences in P-gp activity may be a rare phenomenon.

6.4.3 Integrated Approaches in Assessment of Brain Drug Delivery

6.4.3.1 In Silico Methods

There are generally two types of in silico models for the BBB: one is used to predict the brain-to-blood ratio, K_p, and the other is a rule-based model for CNS drugs. Most BBB in silico models were developed to predict K_p. The main limitation of these models is that K_p is not a good parameter for characterizing brain penetration, as K_p is determined by both $K_{p,uu}$ and plasma and brain tissue binding. For example, K_p for the 32 most prescribed CNS drugs ranged from 0.1 to 24 in mice. A compound having a K_p value as low as 0.1, such as sulpiride, can still be a successful CNS drug, demonstrating the difficulty in assessing brain penetration on the basis of K_p alone [208]. To address the limitation of the K_p model, Gratton et al. [215] and Liu et al. [216] developed a BBB, permeability-surface area product (PS) model using the data generated by the in situ brain perfusion method. The logPS model may be used in conjunction with in vivo PS. An observed permeability that is substantially lower or higher than the predicted value indicates that efflux or uptake transporters modulate brain penetration for the tested compound. For example, in a study of BBB permeability, the PS values of the uptake transporter substrates phenylalanine and levodopa were underpredicted, and the PS values of P-gp substrates, digoxin, CP-141938, and quinidine, were overpredicted [216].

In practice, rule-based models are more useful as they can be easily understood and used by medicinal chemists in drug design. Rule-based models are based on the observation that many brain-penetrant compounds exhibit different physicochemical properties than non-brain-penetrant compounds. Although these rule-based models have their own limitations, they are useful in defining the chemical space based upon known CNS drugs or drugs with CNS activities. These rules are similar to Lipinski's rule of five, but are generally more stringent. A compound is likely to penetrate into the brain if its molecular weight is less than 500 Da, it has fewer than

two to six hydrogen bond acceptors, it has fewer than two to three hydrogen bond donors, and its *c*log*P* or log*D* is in the range of 2–5. In addition, its polar surface area (PSA) should be in the range of 40–90 $Å^2$ and its p*K*a less than 10 [217–220]. These properties are based on strong scientific rationale and can be either directly observed from the chemical structure or readily calculated using commonly available software, and therefore, the rule-based model is widely used in CNS drug design.

6.4.3.2 In Vitro Methods

Brain capillary endothelial cells (primary cell cultures and derived cell lines) have been used as models to study CNS penetration for many years, particularly to explore mechanistic questions involving drug transport [221]. However, these brain-derived systems are challenging to use because of culture variability and the need to repeatedly isolate cells. They also exhibit increased paracellular permeability, which has limited their broad use as a tool to study BBB permeability. Recently, BBB endothelial cells derived from human pluripotent stem cells have been isolated and shown to exhibit many BBB attributes, including low paracellular permeability; however, the utility of this model in predicting BBB drug transport remains an active area of investigation [222]. Typically, cell lines from noncerebral origins are used as in vitro models for prediction of BBB transport. MDCK and LLCPK1 cell monolayers that stably express transporter proteins such as P-gp and Bcrp are the most common systems because they efficiently form tight junctions, are easy to culture, and are predictive of the extent of BBB efflux. Since these cell lines do not originate from cerebral endothelial cells, however, they do not recapitulate the transport characteristics of the BBB other than those of the specific transfected transporter. It is, therefore, important to consider what question is being explored when selecting a noncerebral cell line to characterize CNS penetration of a drug. An ideal in vitro model should have similar paracellular permeability and transporter characteristics as the BBB and should be easily set up for routine drug screening. More research is needed to develop such an in vitro BBB model.

6.4.3.3 In Vivo Methods

Several methods are used to estimate $K_{p,uu}$. Brain microdialysis is a direct approach to determine free brain concentration. However, the utility of microdialysis in the drug discovery setting is limited because the method requires extensive resources and is not easily applied to highly lipophilic compounds. In drug discovery, $K_{p,uu}$ can be readily estimated using Eq. 6.19

$$K_{p.uu} = \frac{f_{u,brain}}{f_{u,plasma}} \cdot K_p \tag{6.19}$$

where $f_{u,plasma}$ and $f_{u,brain}$ are the plasma and brain unbound fraction, respectively, and K_p is the total brain-to-plasma ratio. The $f_{u,plasma}$ and $f_{u,brain}$ values can be estimated using in vitro equilibrium dialysis approaches with plasma and brain tissue homogenate, respectively, and K_p can be determined from in vivo studies [223]. A good correlation exists between the $K_{p,uu}$ determined using microdialysis and the $K_{p,uu}$ estimated from Eq. 6.4 [224]. A potential caveat in using brain tissue homogenate to estimate $f_{u,brain}$ is that homogenization may change binding properties by unmasking binding sites that are not accessible to a drug in vivo. These concerns may be addressed by using a brain slice approach in which the brain structure remains intact [225, 226].

A cassette dosing approach has been developed to increase throughput for determining K_p in in vivo studies [204, 227]. Although drug transporter substrates and inhibitors may incidentally exist in one cassette and the brain penetration for the drug transporter substrates could be modified by the inhibitors, we hypothesized that if cassette dosing is conducted at a low dose of 1–3 mg/kg, the possibility of drug-drug interactions at the BBB is probably low. To test this hypothesis, we selected a set of 11 compounds including known potent P-gp and Bcrp inhibitors and typical P-gp and Bcrp substrates to create the "worst" scenario of potential drug-drug interactions at the BBB; we observed no difference in the K_p values between individual and cassette dosing in mice (Fig. 6.12).

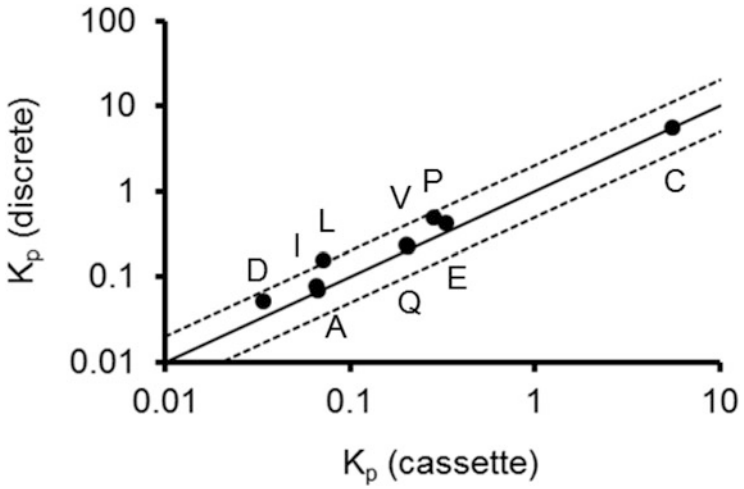


Fig. 6.12 The relationship between K_p determined from discrete dosing and cassette dosing of nine compounds. The *solid* and *dotted lines* represent unity and twofold error. *A* amprenavir, *C* citalopram, *D* digoxin, *E* elacridar, *I* imatinib, *L* loperamide, *P* prazosin, *Q* quinidine, *V* verapamil. The brain concentrations of sulfasalazine and Ko143 and the plasma concentration of Ko143 were below the lower limit of quantitation. These results demonstrate that drug-drug interactions at the BBB are unlikely to occur at a subcutaneous dose of 1–3 mg/kg, and they support the use of a cassette dosing approach to assess brain penetration in drug discovery. The plot is from Liu et al. [204]

$K_{p,uu}$ may also be estimated in drug discovery from CSF drug concentration, assuming that the CSF drug concentration represents the free brain concentration. Shen et al. [228] observed that CSF concentration approximates free brain concentration for moderate to high permeability compounds, but this relationship does not necessarily hold true for low permeability compounds. Our results indicate that CSF concentration is typically between plasma free concentration and brain free concentration [227, 229]. Although the cellular location of P-gp suggests that it pumps substrates from plasma into the CSF [230], the in vivo functional importance of the transporter at the BCSFB seems limited. The free brain/CSF concentration ratios of three typical P-gp substrates, loperamide, verapamil, and quinidine, in P-gp KO and competent mice were 1.5, 1.9, and 3.6, respectively, which are much less than the $K_{p,KO}/K_{p,WT}$ ratios of 9.3, 17, and 36, respectively [208]. However, other transporters, such as Mrp1, do not play a significant role at the BBB but are important at the BCSFB [231]. Therefore, in drug discovery settings in which in vitro or in silico data have demonstrated that compounds are not substrates for efflux transporters, plasma free or CSF concentrations provide a simple way to estimate $K_{p,uu}$. This approach is useful in the lead optimization of highly lipophilic compounds when measurement of the free fraction is difficult and the unbound brain concentration cannot be calculated from the observed total brain concentration.

6.4.4 Summary

The optimization of brain penetration in lead optimization needs to consider the time to reach equilibrium and the extent of brain penetration. For CNS drugs whose indications require a quick brain penetration, a short time to reach brain equilibrium is essential. This can be achieved by screening compounds for a combination of high permeability and low brain tissue binding. For all CNS projects, compounds with high predicted human $K_{p,uu}$ should be selected by screening out very poorly permeable compounds and, more importantly, efflux transporter substrates. Many drug transporters are expressed at the BBB; however, the available data point to the importance of P-gp, and in some cases Bcrp, in limiting the brain penetration of its substrates in vivo. For other drug transporters at the BBB, more research is needed to reveal their in vivo significance. CNS drug discovery screens should, therefore, be used to eliminate good P-gp and Bcrp substrates; however, special consideration should be given to weak or moderate P-gp and Bcrp substrates as potential CNS drugs if a large safety margin exists.

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Chapter 7
Developability Assessment of Clinical Candidates

Shobha N. Bhattachar, Jeffrey S. Tan, and David M. Bender

Abstract The role of the developability (aka preformulation) scientist at the discovery development interface has been extensively discussed in the literature. In response to shifting trends in discovery and the continued push to shorten timelines and reduce costs, the engagement of the developability scientist on discovery teams has steadily moved upstream over the past two decades. In this new and continually changing role, the developability scientist has the opportunity to influence the selection of chemistry scaffolds entering the lead optimization phase and subsequently the selection of developable compounds for clinical testing. In its current state, developability assessment of clinical candidates is an assessment of the physicochemical and biopharmaceutical properties of the compound, carried out with due consideration to the patient in question, the clinical testing plan, and the commercial landscape. This chapter describes the dynamic and integrated nature of this assessment, along with a description of the in silico, in vitro, and in vivo tools used, and illustrative case studies. Key areas of focus include:

(a) Solid form design and selection.
(b) Characterization of the physicochemical properties associated with the solid form, such as solubility, stability, and dissolution properties.
(c) Absorption modeling, including the definition of clinical product performance criteria and the need (if any) for absorption enhancement.
(d) Assessment of absorption enhancement potential using technology platforms that lend themselves to commercial development (including in vivo evaluation where relevant).
(e) The assembly of a comprehensive data package that includes an assessment of potential risks to clinical and commercial development.

Keywords Developability risk assessment • Absorption modeling • Physicochemical properties • Patient centricity • Preformulation • Maximum absorbable dose

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7.1 Introduction

The term "developability" has been steadily gaining acceptance in the pharmaceutical industry over the past 5–8 years [1]. It takes its roots from the older and more well-known concept of "preformulation" but is different in that it is a far more comprehensive assessment of the development potential of a compound into a drug product that meets specific and well-defined criteria.

The term "preformulation" has long been used to describe activities governing the assessment of physicochemical properties of drug substances, with a view to inform formulation development. Formulation development for new molecular entities (specifically small molecules) has generally been accepted to mean immediate release of solid oral dosage forms. Accordingly, for a number of years, the standard preformulation package has been a detailed assessment of measured pKa, log P, solubility and stability in aqueous buffers, and solid form properties including crystallinity, thermal, and vapor sorption properties [2]. Historically, in addition to assembling the preformulation package, the role of the preformulation (developability) scientist has been to profile the physicochemical properties of compounds entering the discovery funnel and also to apply their knowledge to provide as-needed advice on formulations for pharmacology and ADME studies.

Over the years, shifts in discovery paradigms have driven gradual but big changes in the business of preformulation and the role of the development scientist [3]. The advent of high-throughput biological screening in the late 1980s has been widely known to bias compound selection toward more potent compounds (aka the high-affinity trap) that have subsequently been found to pose significant druggability and developability challenges due to molecular size, lipophilicity, etc. [4]. In a move to address these issues and to stay abreast of discovery efforts, computational tools and high-throughput physicochemical property screens have been developed and used as tools, along with preset criteria, to filter out compounds with undesirable properties from progressing through the discovery flow schemes [5]. As a result, the gap between discovery and development has narrowed significantly. However, preformulation scientists have largely operated with a rule-based mindset, generating valuable data to inform the downstream organization of development risks, without directly facilitating or influencing the discovery engine.

The growing complexity of biological targets, the continued push to increase productivity, the influence of increasing partnerships, and in-licensing and out-licensing activities have ushered in a new development paradigm. In concept, this new paradigm is built on the foundation of critical drug product attributes that are derived from the patient/caregiver profile and is a comprehensive assessment encompassing the clinical and commercial development potential of a compound in terms of synthesis of the drug substance, physical properties as they pertain to isolation, handling, product performance, stability, absorption, the toxicology formulation, and drug product parameters. Lastly, but equally important, the assessment is also designed to provide the program team and the downstream development organization with relevant information on developability and risk profile as it relates to impact on timelines, flexibility, and costs.

7.2 Components of Developability Assessment

The various components of developability assessment are described in the paragraphs below. The screens and tools used to address these components are not new and have been previously described in the literature. However, the distinctive difference between the integrated approaches described in this chapter and a "rule-based" approach is that the integrated approach applies these components in the holistic context of the project and not based on simple rules set for individual parameters. This approach looks at developability as it pertains to early clinical development, but with a longer-term line of sight to commercial development. Thus it takes into account the required patient/product profile, knowledge of the target (first in class/best in class), risk tolerance based on business drivers, and impact of recommendations on downstream activities (see Sect. 7.6 for details).

It is important to note that while the integrated approach provides a fair degree of flexibility based on scientific judgment, it operates on the premise that all assessments are ultimately tied to the clinical and commercial development potential of the compound and risks associated therein. Accordingly, the integrated approach specifies critical attributes (Table 7.1) that *must* be satisfied for a compound to be selected for clinical development.

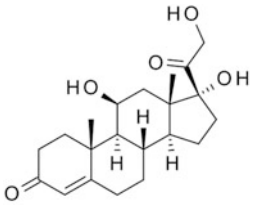
7.2.1 Synthetic Complexity of Drug Substance

Consideration of the synthetic complexity of a molecule is an important aspect of drug design, as this parameter can have a significant impact on the cost to produce the drug substance as well as on development timelines. While interest in this area has grown over the past several decades, the topic has remained somewhat controversial, due at least in part to the fact that there remains a degree of uncertainty in what the term complexity means. For example, a molecule may be inherently complex while still being readily accessible, as is the case with corticosteroids which are typically manufactured starting from raw materials that are derived from natural sources. Synthetic complexity can also change over time, as a result of novel synthetic designs as well as advances in organic synthesis. For example, when the molecule strychnine was first synthesized by Woodward in 1954, it required a total

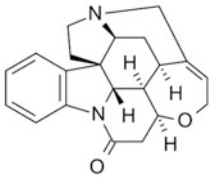
Table 7.1 Critical attributes of a developable compound

1. Has no physical or chemical stability issue that would preclude development and when formulated using reasonable means (Sect. 7.2)
2. Has a maximum absorbable dose that will allow exploration of the full clinical dose range (Sect. 7.2)
3. Has demonstrated technical feasibility for an enabled formulation if needed for absorption (Sect. 7.2)
4. Absorption parameters are well characterized and understood (Sect. 7.4)

of 30 steps [6]. Since this original publication, a number of other researchers have published improved routes to this molecule, the most recent of which was reported in 2012, in which the total number of synthetic steps had been reduced to six [7].



hydrocortisone



strychnine

The costs associated with the production of a pharmaceutical product as referred to as cost of products sold (COPS) or more commonly cost of goods sold (COGS). Based on data collected between 1975 and 2007, these costs represent a significant percentage of total sales (mean COGS/sales = 35.95%) [8]. As molecules enter commercial development, an assessment of COPS becomes increasingly important to ensure that these costs are appropriate for the therapeutic class. For example, COPS for a novel oncology asset are significantly less important than for a glucose-lowering agent, in which the cost per day of therapy that the current market will support is much lower. Costs to produce drug substance are even more significant during clinical development, when routes used to produce drug substance have likely not been optimized for large-scale production. Development timelines are arguably more important than costs during this phase of development. In many cases, project teams find themselves working in very crowded space with competitors, making it is very important to complete clinical development and reach the market as soon as possible. As a result, it has become increasingly important to begin to assess complexity of chemical scaffolds under investigation during the discovery phase to ensure that molecules brought forward carry with them an appropriate amount of complexity relative to the competitive landscape as well as to the therapeutic area to which they are targeted.

A number of different approaches have been used to estimate complexity. Early in the discovery and development process, a simple "bucketing" method may be appropriate, in which the complexity of new molecules is simply classified as high, medium, or low. This method, while not detailed, does provide some general guidance to project leaders and company stakeholders as to the high-level implications of the complexity of a given molecule in development. This type of assessment is best considered in the context of other historical projects and would, therefore, be expected to be different across different companies.

Complexity	Costs	Timelines
High	Above average	Above average
Medium	Average	Average
Low	Below average	Below average

The bucketing approach relies on the knowledge of the individual(s) conducting the assessment and, as a result, is highly subjective. However, given even a small set of parameters (number of synthetic steps, number of chiral centers, method of isolation, etc.), an assessment can be made that should provide a reasonable estimate of the challenges likely to be encountered when first preparing the drug substance during clinical development.

In an ideal setting, computational tools could be used to assess complexity, which would remove subjectivity and bias that arises from the knowledge and backgrounds of individual chemists. Such QSAR models of complexity have been explored for several decades. The first significant report of the development of a synthetic complexity index using graph theory was published in 1981 by Bertz at Bell Laboratories [9]. Bertz's complexity index C(η,ε) is still in use and is reported for all chemical structures in PubChem. Work in this area has continued. Recently, Böttcher reported [10] on the development of a new model based on an additive approach. It utilizes the evaluation of the microenvironment of each individual atom, ion, and molecule and summation of the properties of each atom into an additive complexity score. Other more empirically based computational approaches have been reported such as those by Barone and Chanon [11]. These models assess complexity of a chemical structure by counting structural features (i.e., number of heteroatoms, rings, chiral centers, etc.) and multiplying them by optimized weighting factors. In another approach, Kjell et al. reported on the use of a model of process mass intensity (PMI) as a surrogate for complexity [12]. PMI is defined as the mass of materials consumed divided by the mass of product. A synthetic scheme with a lower PMI would be considered a more efficient one than a corresponding route with a higher PMI. In a somewhat hybrid version of these approaches, authors from Merck have recently reported the results of a crowdsourcing approach. In this study, a total of 386 chemists were asked to assess 2681 molecules taken from a combination of public and internal structure databases. This manual assessment was then used to construct a QSAR model of complexity in which additional structures could be assessed. While there was considerable disagreement among different chemists, the authors demonstrated that it was possible to utilize this dataset to build a self-consistent QSAR model of complexity.

There will likely be a continued interest in the area of complexity determination. Based on work in the current literature, a combination of different approaches may ultimately be needed to fully assess complexity of new molecules. Due to the increasingly complex nature of new biological targets as well as the compounds designed to target them, this work will continue to play an important role in the selection and development of new medicines.

7.2.2 Physicochemical Properties

Generally, physicochemical properties include pKa, log P, solid-state properties, solubility, and stability. As needed, additional assessments such as counterion analysis (to confirm stoichiometry of salts) or Karl Fischer analysis (to confirm hydration state) may be carried out to gain a deeper understanding of the solid form of the compound. For a detailed treatment of the individual properties and structure-property relationships, the reader is referred to sources in the literature [13]. The importance of a deep understanding of these fundamental aspects cannot be overestimated as it is essential for appropriate planning of studies and application of results.

The means used to assess some of these properties may include in silico tools at an initial stage, followed by in vitro screens of increasing depth as compounds advance through the discovery flow scheme. The goal of these activities is to initially triage compounds to support discovery efforts, followed by more rigorous but phase-appropriate assessments to support clinical studies and toxicological assessments as smaller numbers of compounds progress toward candidate selection.

In simple terms, the overarching goal of assessing physicochemical properties is to understand (a) the dissolution and solubility of the compound under bio-relevant conditions; (b) the solid form properties as they relate to isolation, handling, and storage of drug substance; and (c) the impact of properties on drug product attributes including formulation options. Ultimately, this understanding is directly tied to the critical attributes of the compound that must be met for developability.

Table 7.2 lists the material requirements for assessing these properties, the tiered manner in which they can be assessed, and the information that can be gleaned from the data. Figure 7.1 describes the relationship between physicochemical properties of drug substance, biopharmaceutical properties, and drug product performance.

For orally absorbed compounds, the primary goal is to ensure that absorption is not a limiting factor for achieving the desired plasma exposures in clinical studies (and beyond). This depends on the solubility (dissolved concentration in the gastrointestinal tract) and permeability of the compound across the intestinal membrane. Compounds that have aqueous solubility greater than 2 mg/mL across the pH range of 2–8 and human intestinal permeability greater than 1.25×10^{-4} cm/s may be deemed highly absorbable provided the solubility is associated with a developable solid (crystalline) form of the compound. (Developability criteria for solid forms in this context will be described in detail later in this section.) For compounds that do not fit this definition, a more detailed assessment of the absorption potential has to be made, taking the projected clinical dose range into context. These details are discussed in Sects. 7.3 and 7.4.

The secondary goals include the physical and chemical attributes of the compound relevant to manufacturing of drug substance and drug product. Assessment of physicochemical properties using a judgment-based integrated approach therefore is focused on achieving the primary goal while striking a reasonable risk-benefit balance with the secondary goals. To some extent, the definition of

Table 7.2 Developability assessment: physicochemical properties

Property	Minimum material need (mg)	Place in flow scheme	Implications and comments
pKa	1–2 mg	Tier 1: in silico Tier 2: measured (as needed; UV spectral shift [14] and capillary electrophoresis [15] commonly used)	If compound has basic pKa(s) $\geq$4 or acidic pKa (s) $\leq$7: Compounds with basic pKa (s) may have large variability in oral dose PK in dogs depending on intrinsic solubility and dosing conditions. Gastric pH may impact oral absorption. Extent of impact will depend on the intrinsic solubility and pKa(s) Potential for salts to improve physical properties and/or dissolution rate
Log P and Log D	1 mg	Tier 1: in silico Tier 2: measured (as needed; miniature "shake flask" [16] or HPLC ([17, 18]) are commonly used)	Log P $\geq$~3 may be accompanied by solubility limitations and poor druggability [19] Log P range, in combination with solid form properties, can have significant impact on enablement potential of poorly soluble compounds [20]
Crystallinity/ phase purity	Tier 1: 1 mg Tier 2: 5–10 mg (non-destructive) Tier 3: 5 mg	Tier 1: polarized light microscopy (PLM) (in combination with aqueous solubility) Tier 2: powder X-ray diffractometry (PXRD) Tier 3: differential scanning calorimetry and thermogravimetric analysis (DSC and TGA) [21]	Tier 1 (PLM) Birefringence under PLM generally characteristic of crystalline solid. However, additional characterization of crystalline state will be required as compound advances through flow scheme If measured aqueous solubility is associated with non-birefringent (amorphous) solid, solubility could drop ~$\geq$10x upon crystallization of solid Tier 2 (PXRD) Characterizing crystallinity and solid form of compound Useful for assessing form changes associated with solubility values When run as a variable temperature or variable humidity measurement, can be used along with thermal and/or vapor sorption data

(continued)

Table 7.2 (continued)

Property	Minimum material need (mg)	Place in flow scheme	Implications and comments
			to understand form changes Tier 3: DSC and TGA When used along with PXRD and PLM, provides valuable information on solid form, form transitions upon heating and thermodynamic aspects Compounds with melting points >200C and/or high heats of fusion may point to aqueous solubility limitations TGA (with MS) provides valuable information on residual solvents, solvates/hydrates, and decomposition temperature
Solubility	5–50 mg	Tier 1: in silico or high-throughput measurement Tier 2: thermodynamic (plate based automated or more manual screens) [22]	A variety of screens available for Tier 1 assessment, mostly to guide SAR and/or to bin compounds into high/med/low solubility bins [23] Tier 2 should be designed to understand bio-relevant solubility, pH-solubility relationships, solid form-solubility relationship, and preliminary information on supersaturation potential [24]
Stability	5–10 mg	Tier 1: in silico Tier 2: solution stability Tier 3: solid form stability, forced degradation, degradation mechanism, and excipient compatibility	Autoxidation potential assessment based on calculations of bond dissociations energies [25–27] Solution stability (chemical): generally covering bio-relevant pH range and oxidizing agents. Incubating at 40C with sample time points through 24 h useful. May add light conditions. Informs bio-relevant, handling, and storage aspects Solid form stability: chemical can be coupled with physical (PXRD, SSNMR, or other) to inform drug product formulation and manufacturing

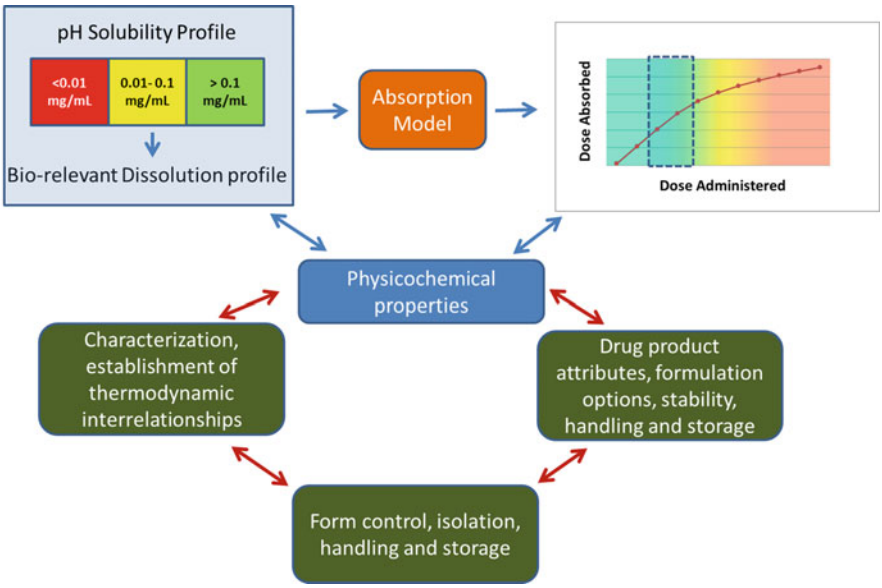


Fig. 7.1 Relationship between physicochemical properties of drug substance, biopharmaceutical properties, and drug product performance

developability for the secondary parameters is a function of the goals for the clinical program, the patient profile, and business considerations. These aspects will be discussed in detail in Sect. 7.6.

7.2.3 Solid Form Criteria for Developability

The solid form parameters described here pertain to the isolation, handling, storage and formulation aspects of the drug substance, and implications on product manufacturing and performance. As with the rest of this chapter, the discussion is focused on the application of these parameters to developability assessment. In order to gain a more fundamental understanding of the basic scientific aspects that are essential for working in this field, the reader is encouraged to refer to the abundant literature on these topics [28–30].

7.2.3.1 Crystallinity: Polymorph Landscape and Associated Thermodynamic Interrelationships

The basic goal of solid form screens in the lead optimization phase is to find a stable, well-behaved form of the compound for development. Solid form screens are generally designed to promote crystallization of compounds from slurries of

various solvents and solvent-anti-solvent mixtures. The crystalline hits obtained from such a screen are analyzed initially by microscopy and then powder X-ray diffractometry (PXRD) in order to obtain preliminary information on the solid form. The first evidence of the existence of polymorphic forms generally comes from PXRD patterns, with thermogravimetry (TG) and TG with mass spectroscopy (TG-MS) typically used to determine their solvation/hydration states. The thermodynamic relationships between forms are generally elucidated from melting point and heat of fusion data obtained from differential scanning calorimetry (DSC) experiments. As a general rule, for monotropic systems, the highest melting form is the most desirable as it is the most thermodynamically stable form. For enantiotropic systems, it is important to determine the transition temperature and whether this temperature is conducive to reproducibly isolating and handling the form that would be thermodynamically stable under ambient conditions. Solid form landscapes and the thermodynamic interrelationships between forms can be complex with far-reaching implications on the regulatory submissions [31], manufacturing process [32], and safeguarding intellectual property. However, all activities leading up to selecting developable solid form(s) must always be done with a clear understanding of the impact of the form properties on the absorption parameters of the compound (see Sects. 7.3. and 7.4 for details) .

7.2.3.2 Hydrated and Solvated Forms

Solvated forms are generally not acceptable for development. The occurrence of solvates in preliminary screens is typically a strong indication that the compound is a prolific solvate former. Organic solvates are generally unacceptable for clinical use and also limit the crystallization design space. In addition, solvated forms tend to be more soluble in aqueous systems than non-solvated forms, and therefore, it is both essential to find the means to isolate non-solvated forms and to assess absorption parameters based on bio-relevant solubilities of these non-solvated forms. Hydrated forms are generally less soluble than anhydrous crystalline forms of compounds. The biggest risks with hydrated forms, however, have to do with their physical stability under standard conditions of isolation, handling, and storage of drug substance and drug product. Therefore, the stoichiometry of hydrates and the potential for interconversion between the desired and undesired hydration states must be tested with appropriate rigor for any changes in the water activity range of 0.2 to 0.8. If there is any evidence of instability, the risks and benefits of recommending such hydrates for development must be carefully evaluated and addressed. For example, a compound that may form a hydrate at critical water activity levels >0.6 may be physically stable for reasonable durations of time as drug in capsule when exposed to 40 °C/75% RH conditions but may convert (to varying extents) to the hydrate when subjected to a fluid bed granulation process that uses water as the granulation fluid. When using a judgment-based approach, the risks versus benefits of recommending forms that do not meet these criteria must be appropriately assessed and well understood.

7.2.3.3 Isolation Mechanism

The preferred means to isolate the desired form of the drug substance is filtration. Crystalline forms of compounds are generally amenable to isolation by filtration, but oily liquids and amorphous materials that cannot be crystallized may be isolated by evaporation or spray drying processes or through adsorption into inert porous silica or other matrices [33, 34]. When non-filtration processes are necessary for isolation, the development organization needs to be appropriately informed of equipment needs, cost implications, and impact on scale and drug product presentation. In addition, impact of the utilization of solid matrices on the physicochemical and biopharmaceutical properties of the compound need to be properly assessed and understood.

7.2.3.4 Chemical Stability

For the purpose of developability assessment, the potential for a compound to undergo photodegradation, autoxidation, or degradation in aqueous or organic systems must be well understood. For compounds that are unstable, the mechanism of degradation, known genetic toxicity of degradants, implications on isolation, handling and storage, and impact on packaging configurations (including in-use and shelf-life stability) must be thoroughly assessed. If it is established that the risk of degradation is significant, the regulatory implications on the impurity profile of the drug substance and risk of degradation on practical time scales despite special protection (light, oxygen, and humidity control) must be thoroughly evaluated. Any special needs and restrictions must be fully communicated with the development organization, in order to facilitate appropriate considerations for development decisions based on business priorities for the compound/product.

7.2.3.5 Particle Morphology

While particle morphology is not a primary criterion in the solid form selection process, particles with large aspect ratios can pose significant challenges with isolation of drug substance by filtration and also in solid dosage form development due to poor flow properties.

7.2.3.6 Particle Size Specifications

For orally administered solids that have an aqueous solubility <1 mg/mL, particle size has a direct impact on dissolution and therefore the rate and extent of absorption [35]. Details of dissolution rate on absorption are discussed in Sect. 7.3.2, but basically, particle size specifications must be set with due consideration given to the

desired absorption profile and the resulting pharmacokinetic profiles in the clinic. When particle size specifications are narrow and restrictive, impact on manufacturing operations such as crystallization and milling processes, special facilities that may be needed, limitations on scale, material specifications, robust processes to meet the material specifications, etc. must be appropriately assessed and its implications communicated to the development organization.

7.2.4 Solid Form Selection for Absorption Enhancement

7.2.4.1 Salts and Co-crystals

Crystalline solids of neutral forms of compounds are generally the simplest and therefore the most preferred forms when viewed in the context of drug substance isolation and handling and drug product formulation processes. In some instances, salt or co-crystal forms may be selected over neutral forms due to favorable solid-state properties. However, as is often the case, a more important goal with solid form selection is to find a crystalline salt or co-crystal form with improved absorption parameters of the compound for clinical development, relative to the neutral form of the compound. Whenever possible therefore, the risks versus benefits of a developable crystalline salt or co-crystal form should be assessed [29] relative to more expensive and complex systems for absorption enablement. For compounds that have ionizable functional groups such that they have at least one basic pKa ≥ 4 or an acidic pKa ≤ 7, salt formation is an attractive means to enhance the rate and extent of absorption and to minimize gastric pH effects on the rate and extent of absorption. These beneficial effects of salts are often the result of reduced energy barriers to dissolution relative to the neutral forms of the compound [36, 37]. In addition, the fact that dissolution of salts is often driven by their microenvironment pH, the dissolution process is less sensitive to bulk fluid pH conditions [38]. The importance of this latter attribute of salts is further explained in Sect. 7.3.2. Salt formation can also be an effective method to counter solid-state stability observed for the neutral compound.

The solubility and dissolution behavior of salts are more complex than those of neutral forms of the compound as will be described in Sect. 7.3.2. In addition to the physical properties that apply to neutral forms of compounds (as described in the paragraphs that follow), salts are also susceptible to disproportionation within solid dosage forms. The propensity to disproportionate when combined with standard formulation excipients and implications thereof must be carefully studied and addressed [39–41] before selecting salt forms to go into development. This becomes even more important if the salt form is critical for the dissolution properties necessary for absorption [42] where a drug product with disproportionated salt can lead to a subtherapeutic absorption. In assessing salts therefore, sufficient time and material must be allocated in order to gain a thorough understanding of their physicochemical and biopharmaceutical properties.

Lastly, in selecting counterions for salt formation, due consideration must be given to the safety of the counterions, impact of its molecular mass on the dose, and dosage form of the drug product. For example, 1-hydroxy-2-naphthoic acid is acceptable as a counterion for the long-acting inhaled drug salmeterol due to the low, twice daily dose of 50 μg of this drug in the inhaled product. However, the toxicology of this acid at higher doses may preclude its use in drug products that may have to be dosed in milligram quantities (Lilly internal data). Similarly, maleic acid is acceptable in low-dose products such as enalapril maleate, chlorpheniramine maleate, and prochlorperazine maleate but carries renal safety issues [43] that may make it unsuited for drugs that require doses greater than ~100 mg. Therefore, in general, while the absorption profile and solid form properties of the selected salt form are always the main focus, a toxicology opinion of the selected counterion must always be obtained as safety overrides all other aspects of salt form selection.

For compounds that do not have ionizable functional groups, co-crystal formation is an option for improving solid-state, stability, and/or dissolution properties of compounds [44, 45]. For a comprehensive description of standard co-formers used to make co-crystals, screening and characterization techniques, phase diagrams, merits, limitations, and recent examples, the reader is referred to reviews by Brittain and Williams et al. [46, 47]. In addition, the reader is also referred to the recent regulatory guidance on this topic [48].

In some instances, the dissolved concentrations of salt or co-crystal forms may result in very high degrees of supersaturation relative to their solubility in the intestinal luminal milieu. In order to stabilize the supersaturation that is thus achieved, and facilitate the absorption enhancement that these forms were designed to produce, these high energy solid forms of compounds are formulated as blends with functional excipients [49, 50]. Generally, these functional excipients serve to inhibit precipitation of the supersaturated solution and/or increase the microenvironment solubility and include polymers, complexing agents, or pH-modulating agents [51]. For compounds that lend themselves to this approach, this form of absorption enablement is a cheaper and simpler alternative to amorphous solid dispersions. However, as part of the developability assessment, it is very important to make sure that these solid forms and functional excipients that may be essential for dissolution enhancement (and absorption) have acceptable physical and chemical compatibility with the compound in question.

7.2.4.2 Amorphous Solid Dispersions

Over the past decade, amorphous solid dispersions have been extensively discussed as a means to enhance the apparent solubility (and thus absorption) of compounds formulated as solid oral dosage forms [52–54]. They are generally produced by spray drying a solution of the active drug and polymer, such that the resulting powder is a solid solution of amorphous drug homogeneously dispersed in the polymer matrix. In some instances, a surfactant or other agent is also included in the mix in order to further enhance the solubilization of the compound and/or stabilization of the

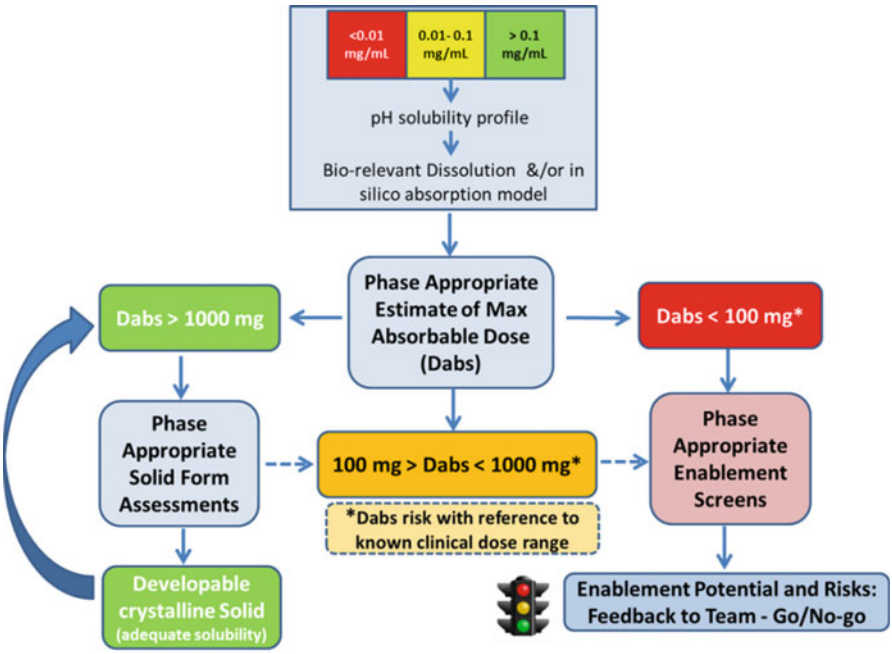


Fig. 7.2 Illustration of solid form and absorption potential screening in lead optimization

system. The polymer(s) in these systems serve to stabilize the drug substance in an amorphous state for a reasonable duration of time to support manufacturing and product shelf life when stored under appropriate conditions of temperature and humidity. They can also function to varying degrees as solubilizing agents and/or as precipitation inhibitors that sustain supersaturation of the dissolved compound in the aqueous environment of the gut, thus maximizing the potential for absorption.

While spray drying is still the mainstream technology for producing solid dispersions at this time, hot-melt extrusion technologies are developing as a viable alternative when compound and polymer properties are amenable to the process [55].

If it has been determined that enablement through amorphous solid dispersions is essential to achieve the target absorption profile required for the clinical development of a given compound (Fig. 7.2), then the feasibility of developing a viable amorphous solid dispersion system that would be suited for the intended drug product must be fully investigated through appropriate screens. This includes screening for polymers and other excipients used in the system to ensure an optimal degree of interaction (and compatibility) with the drug substance, such that there is no crystallization and/or phase separation during handling and storage. It must also be confirmed that the glass transition temperature of the resulting system is sufficiently high to ensure that there is no risk of crystallization during the product's shelf life [56, 57]. In addition, the mechanism through which the polymer (and any additional excipients) impacts dissolution and supersaturation in vivo must also be

well characterized and understood [58]. Finally, these systems must be optimized for the largest possible drug loads so as to strike a suitable balance with product stability and dissolution performance [46]. Typical drug loads in these systems range from 20–30%, while 2 and 50% would be considered to be the lower and upper limits based on stability and dose size.

7.2.5 *Integrated Developability Risk Assessment and Feedback to Discovery Teams*

The solid form and formulation platform selection activities to support clinical (and commercial) development must progress hand in hand with other activities in the lead optimization phase. As the pharmacology, ADME properties, and toxicology of a given compound become better understood and the discovery team increases its level of investment in the compound, the critical developability attributes of the compound must be assessed in a commensurate manner. Figure 7.2 illustrates the interrelationship between solid form development activities and absorption potential assessment screens. Absorption potential assessment is discussed in detail in Sect. 7.4. As data on the solid form properties and absorption potential is gathered, challenges for development must be communicated with discovery teams and the downstream development organization, such that risks and benefits can be weighed appropriately against the competitive landscape and any unmet medical needs. If the challenges are such that the compound is deemed to be not developable by reasonable means (Table 7.1), it is important to inform the discovery team in order to either impact the SAR activities or to aid with the selection of appropriate compounds for development.

Thus the screens used in developability assessment should be designed such that they answer critical questions using the minimum amount of material and time and are performed in a staged and phase-appropriate manner, so as to maintain an active feedback loop to discovery. If compound properties point in the direction of enablement screens, an increased compound requirement must be anticipated and the discovery teams informed so appropriate funds and chemistry resources can be planned. Data generated from these screens should be interpreted in the context of the larger goals of the project and clinical development plans and proper judgment applied in decision-making.

7.2.6 *Clinical and Commercial Formulations*

Clinical and commercial formulations are described in detail in Chap. 10. This section carries a very brief overview of developability risk assessment of compounds in the context of clinical and commercial formulations.

Every organization has its own strategy for clinical and commercial formulation design and development. But in general, clinical formulations are simple in composition and in the process of "manufacture." In view of the fact that attrition in Phase 1 (and in Phase 2) is relatively high, the goal of these studies is to quickly test the clinical hypothesis, ADME parameters, and safety margins to the desired clinical exposures. As these initial studies are small and of short duration, the demand for drug product is generally small enough such that it can be met using simple formulations that can be produced through manual compounding operations in the clinical pharmacy or manufactured using simple processes in a GMP manufacturing facility. Key considerations influencing the drug product presentation and the means to produce the supplies for these studies include geographical location(s) of clinical testing site(s), healthy volunteers versus patients, and clinical study design aspects such as duration of studies and any seamless transitions to the next phase.

In instances where special formulation, handling, and packaging (e.g., humidity and/or light conditions) are necessary to accommodate stability issues, the risk of degradation of the drug substance and drug product and the implications thereof need to be evaluated and fully understood. When milling or other means of particle size control are essential for product performance, the implications on the manufacturing process and the handling properties of the milled material (in terms of stickiness, tendency to agglomerate, flow properties, etc.) need to be evaluated and appropriately addressed. If the compound belongs to a special containment class due to occupational exposure hazards, the choice of manufacturing sites that are equipped to handle the material might be limited and therefore factored into development plans. In addition, the development organization needs to be informed if any of these properties pose potential risks to commercial development.

For orally administered drugs, clinical formulations include simple drug in bottle, drug in suspension or solution, neat powder in capsule, dry blend powder in capsule, and, less commonly, tablets and powder or granules in a sachet. Transdermal formulations are typically simple gels or solutions with or without occlusion by a patch. Formulations such as orally disintegrating tablets, modified release tablets, and buccal and sublingual tablets may be used in small clinical studies to answer specific questions pertaining to drug product design, absorption parameters, and the resulting pharmacokinetic profiles essential for the desired clinical outcome. Details on developing these and other formulations are discussed in chapter 14.

7.3 Drug Product Performance

7.3.1 Product Performance Criteria in the Context of PK-PD

The PK-PD relationship of the compound is the projected relationship between plasma (or other target tissue) drug exposure and the pharmacodynamic response. These projected relationships are generally based on clearance and volume of

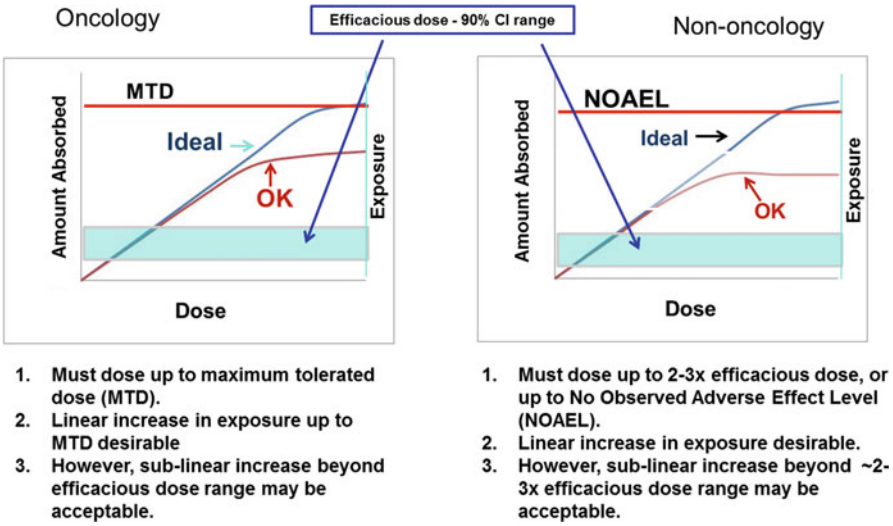


Fig. 7.3 Desirable relationships between administered dose and amount absorbed and/or plasma exposure

distribution estimated from preclinical data and come with a certain level of uncertainty. As a result, the dose that is projected to result in efficacious plasma exposure is typically not a well-defined value, but a range defined by the assumptions of the underlying models. In addition, in Phase 1 studies, it is standard practice to explore doses beyond the projected efficacious dose range to assess safety and tolerability (while staying within the limits enforced by regulatory agencies). Typically, this amounts to a minimum of three- to fivefold over the upper end of this dose range. It is the responsibility of the development scientist to have some understanding of how these estimated ranges are arrived and, more importantly, to make sure that the drug product will have an acceptable absorption profile (i.e., rate and extent of absorption) that will cover this dose range with adequate separation of exposures across the doses. This concept is illustrated in Fig. 7.3.

7.3.2 Solubility and In Vitro Dissolution

Solubility is an extremely important physicochemical property as it has a direct bearing on the absorption potential of compounds. It is a very simple concept on the surface but prone to lead the investigator to wrong conclusions unless proper techniques are used for measurements and proper principles applied for interpretation of results. For details on solubility and solubility measurement as they pertain to pharmaceuticals, the reader is referred to reviews on the topic [59].

In actual practice, the challenge that the developability scientist faces is that due to the nature of the discovery workflow, solubility measurements and the

judgment-based decisions made from the results take on a tiered approach that spans the entire lead optimization phase. The earliest assessment of solubility generally comes from computational predictions. In our experience, these predictions are best suited for flagging compounds with low solubility in aqueous buffers. Once material actually becomes available, measured solubility data is usually collected using high-throughput assays. Results from the high-throughput assays generally do not come with adequate (or any) information on the associated solid form and impurities of the compound, and so, they should be used with an appropriate level of caution. This is especially true when the initial measured solubility value is in the 0.01 to ~0.1 mg/mL range because it can sometimes fuel false hopes among chemists who are striving hard to improve the solubility of their compounds. Material made in discovery is often amorphous, partially crystalline, and metastable crystalline form or an organic solvate. Until the compounds progress through the flow scheme and are made in gram quantities for additional testing to become a potential clinical candidate, they do not warrant extensive solid form characterization. However, consistent with the Tier 1 approach outlined in Table 7.2, simple assessments such as PLM might still be beneficial. When solubility is measured using more refined techniques and with developable crystalline solids, there is a high propensity for the values to be far lower relative to initial results. Thus as a rough rule of thumb, judgment on solubility results from initial measurements should be based on tolerance for a tenfold drop in the value [60].

There is abundant discussion in the literature on the use of bio-relevant media for solubility measurements [61]. While there is general acceptance that Dressman's recipes [62] are well researched and most relevant, there are also numerous examples of the use of alternate recipes with isolated examples of success in understanding in vivo solubility [63]. In reality, simulated fluids are valuable but do not always mimic the mechanism of solubilization and/or supersaturation that occurs in vivo [64]. In addition, there is large interindividual variability in the actual composition of gastric and intestinal fluids [65], pH, fluid volumes, transit times, etc. Therefore, when the solubility of a compound is highly sensitive to bile-salt concentration in simulated fluids, it is extremely risky to rely heavily on a single value such as fasted- or fed-state simulated intestinal fluid (FaSSIF or FeSSIF) solubility unless it is supported by robust in vivo data (described in Sect. 7.4).

7.3.2.1 In Vitro Dissolution

While solubility is a fundamental parameter for absorption and is used as a key input in the most basic estimations of maximum absorbable dose [66], actual dissolved drug concentration at the absorption site that is achieved in a bio-relevant time frame is often different from equilibrium concentrations (i.e., solubility). More generally, dissolution, supersaturation, and precipitation (for basic compounds, solution formulations, etc.) will impact actual dissolved drug concentrations at the absorption site. For compounds that have the potential to become clinical candidates, therefore, in vitro measurement of dissolved drug

concentrations in a simulated bio-relevant setting (under dynamic or non-sink conditions) provides essential information for absorption modeling [67].

In vitro dissolution testing in developability assessment is centered on understanding the biopharmaceutical properties of the drug substance and/or drug product. Accordingly, these tests are generally conducted under non-sink conditions that simulate stomach and intestinal fluid composition, pH, volume, mixing, etc. The compound being tested in these systems has the "opportunity" to dissolve in the simulated gastric environment before it enters the absorption environment. At a basic level, standard fasted conditions of pH, compositions, etc. are simulated. Thereafter, physiologically relevant changes to these parameters are made, and dissolution performance under these conditions is further evaluated.

There is no standard guidance on conducting these dissolution studies. The ORBITO project conducted an extensive study of the literature on this topic and published a comprehensive review that describes the pros and cons of the existing tools and practices [68]. It is a generally accepted fact that regardless of how the experiments are conducted, the aim is to understand the mechanism of dissolution and the impact of bio-relevant boundary conditions on the system. This information ultimately feeds into drug product design, where the goal is to ensure that the rate and extent of absorption of the compound under testing conditions described in the protocols will meet the needs of the clinical program.

In our experience, the "two-step" dissolution system [69] and the artificial stomach and duodenum (ASD) [70] are well suited for biopharmaceutical assessment in the lead optimization phase. Variations of these models include the pH dilution model [71] and the gastrointestinal simulator (GIS) [72]. The two-step dissolution system has a greater throughput and provides an initial read on the potential for supersaturation of compounds. The ASD has a lower throughput but serves as a tool for a better understanding of dissolution/supersaturation/precipitation phenomena as a function of various physiological conditions. One limitation of both systems, however, is that for highly permeable compounds, they might be more biased toward precipitation relative to in vivo conditions.

Despite the advances in bio-relevant dissolution testing and in silico absorption modeling technologies, predicting absorption in humans might sometimes require some form of in vivo confirmation to assist with clinical formulation platform selection [73].

7.4 Absorption Modeling

7.4.1 Basic Principles and Commonly Used Tools

Absorption modeling is an important tool for the developability scientist as it allows for the translation of physicochemical properties such as solubility and permeability to in vivo performance measures such as the amount or fraction of drug absorbed

and the instantaneous systemic drug concentration. The level of detail of the model output dictates the complexity of the model. Absorption models consist of simple mathematical expressions such as the maximum absorbable dose (MAD) equation [66] to sophisticated compartmental models [74] such as that utilized in the GastroPlus™ commercial software package (Simulations Plus Inc., Lancaster, CA). Both provide an estimate of a molecule's absorption potential but differ in the level of detail used in describing the mechanisms involved in the absorption process and as a result what one can learn from applying the model. According to the MAD equation, the maximum absorbable dose is defined as

$$\mathrm{MAD} = \mathrm{S} \times K_{\mathrm{a}} \times \mathrm{SIWV} \times \mathrm{SITT}$$

where S is the intestinal solubility, K_{a} the permeability, SIWV the small intestinal water volume, and SITT the small intestinal transit time. While simplistic, the MAD equation takes into account some of the key components contributing to absorption albeit with gross approximations. When interpreted in conjunction with estimates of clinically efficacious dose and the intended clinical testing dose range, it can provide a preliminary assessment of the absorption potential of a molecule. Furthermore the simplicity of the model allows for a high-throughput assessment of absorption alongside the optimization of druggable properties in the discovery engine. If sufficiently reliable in silico global or preferably SAR specific models are available for S and $\mathrm{K_a}$, it is also possible to consider including absorption potential as a parameter in de novo design. The limitations of solubility measurements during early discovery as described elsewhere in this chapter as well as the uncertainty in accuracy of in silico models utilized should be fully integrated into the interpretation of data from such a high-throughput absorption assessment system.

While the MAD equation can be a powerful screening tool to assess absorption early in discovery, its utility becomes limiting in activities related to the design and development of a drug product for optimal clinical performance, manufacturability, and commercialization. The limitations of the MAD equation can be effectively summarized as limited mechanistic details in its integration of physicochemical and physiological properties. These shortcomings have been addressed by various improvements such as compartmental absorption transit models, microscopic mass-balanced absorption models [75], bile-salt solubilization models [76], and ultimately the implementation of the GastroPlus ACAT model which integrates many of these advances into an easy-to-use commercial software package. The reader is referred to numerous in-depth reviews for details of the various mechanisms that are currently represented in absorption models, their strengths and shortcomings, and their complex interdependence on one another [77]. At a high level, the GastroPlus ACAT model can be summarized in Fig. 7.4.

In addition to being highly mechanistic, the true utility of such models comes from their integration with pharmacokinetic models (non-compartmental, multi-compartmental, and physiologically based), metabolism models, and multispecies physiological models, thus providing the developability scientist the ability to test

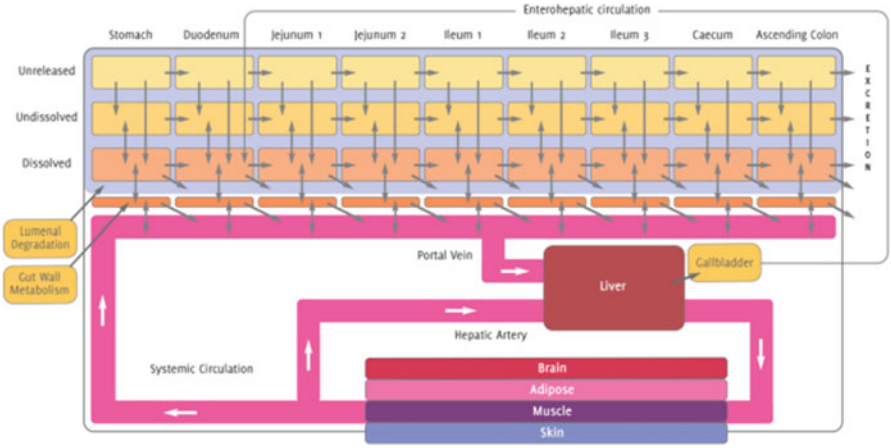


Fig. 7.4 GastroPlus advanced compartmental absorption and transit (ACAT) model

hypotheses related to drug product performance against in vivo preclinical and clinical data.

A key area of absorption modeling for the developability scientist is in evaluating the effects of defining the solid-state and its associating properties such as particle size which affects the solid's surface area and the kinetics of dissolution. While the MAD equation assumes the equilibrium solubility is realized instantaneously, in the in vivo setting, the intestinal concentrations achieved from an oral dosage form are initially limited by the dissolution of solid as described by the Nernst-Brunner equation:

$$\frac{\mathrm{d}C}{\mathrm{d}t} = \frac{DS}{Vh}(C_s - C)$$

where D is the diffusion coefficient (diffusivity), S is the surface area of solids, V is the volume of the dissolution media, h is the diffusion layer thickness, C_s is the solubility, and C is the concentration at time t. Absorption modeling allows for sensitivity analysis of in vivo exposure changes to drug product particle size changes. This is an important assessment as a small particle size requirement optimal for absorption and product performance may be suboptimal in flow properties critical for manufacturability. Similarly one can remove a development constraint by realizing a drug product's absorption has little or no particle size sensitivity in which case the particle size specification is driven solely by manufacturability. Absorption modeling can also provide an assessment of relative bioavailability changes from modifications to the drug product unit formula through the development of mechanism-based IVIVx models [78, 79].

7.4.2 *Absorption Parameters from Modeling*

While in vitro solubility and permeability measurements can provide an early assessment of absorption and performance of a drug or drug product, it is nevertheless an important exercise to confirm that these absorption parameters are in vivo relevant and that clinical performance can be predicted with high confidence. In the case of solubility, it has been observed that solubility measured in bio-relevant simulated fluids generally is representative, but there are instances where there are significant differences in comparison to measurements made in extracted bio-fluids [80]. As such, it is important to confirm absorption parameters using in silico tools such as GastroPlus models. This can be achieved by building an absorption model with in vitro parameters and comparing the predicted results with preclinical in vivo data. For cases where predictions from an absorption model using in vitro absorption parameters agree well with preclinical in vivo data, it can be concluded that the in vitro data are representative and can be used to predict clinical performance with confidence. If on the other hand there is poor agreement, the initial in vitro absorption parameters can be optimized to obtain maximum agreement between predicted and preclinical in vivo data. This set of optimized in vivo parameters can now be used to predict clinical performance. This general scheme is summarized in Fig. 7.5. Two case studies are included for illustration.

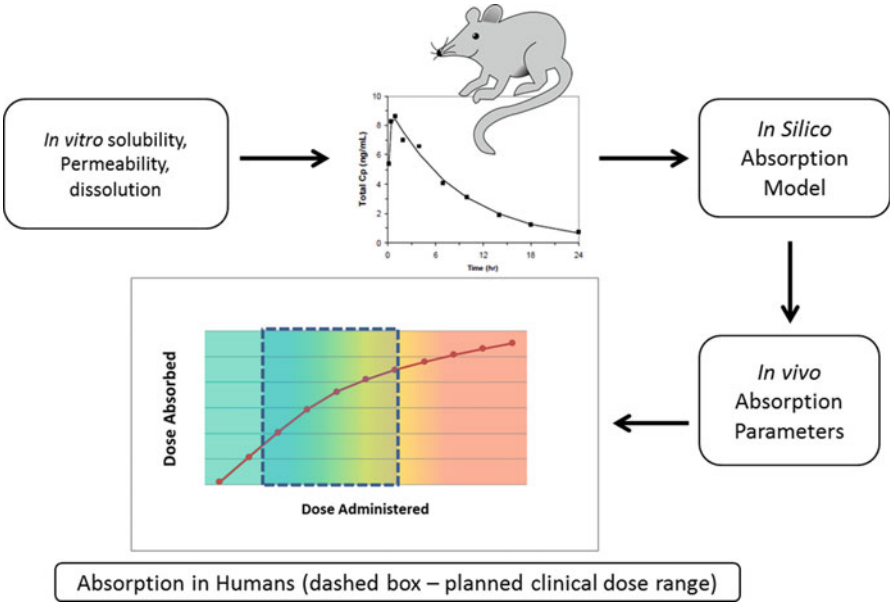


Fig. 7.5 In silico absorption modeling and in vivo absorption parameters

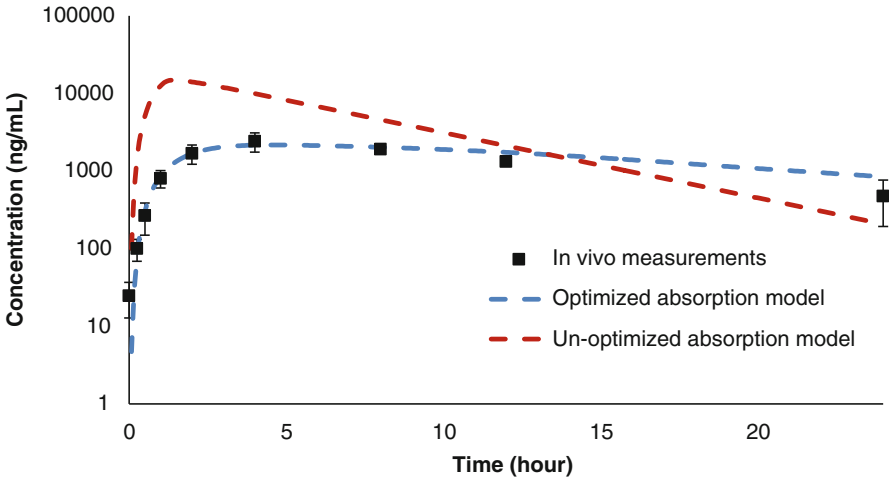


Fig. 7.6 Case Study 1: In silico predicted and actual in vivo PK profiles of molecule A in rat

7.4.2.1 Case Study 1

Molecule A is a weak acid with measured pKa of 4.74. Solubility in bio-relevant media was measured to be high (0.1 mg/mL in SGF and 0.393 mg/mL in FaSSIF). Passive permeability was predicted to be high (2.99×10^{-4} cm/s).

Figure 7.6 compares predicted concentration-time (Cp-time) profile from models using in vitro solubility measurements ("un-optimized absorption model") and optimized solubility values ("optimized absorption model") with measured in vivo Cp-time profiles in rats. It was observed that measured stomach and intestinal solubilities of 0.1 mg/mL and 0.393 mg/mL, respectively, overpredict observed measured in vivo concentrations ($R^2 = 0.523$). An optimized absorption model was built using stomach and intestinal solubility of 0.001 mg/mL and 0.025 mg/mL, respectively. Passive permeability remains unchanged. This model provided good agreement with measured in vivo data ($R^2 = 0.935$).

Given an absorption model for rat, one can readily utilize the model to predict performance in human by applying human physiological parameters. Such a model can be utilized to answer a range of clinical performance questions. In this case an early assessment of Fa vs dose was conducted to evaluate absorption potential. Figure 7.7 shows relative absorption as measured by the ratio of amounts absorbed from both optimized and un-optimized absorption models. Relative clinical absorption is defined as

$$\frac{AUC_i^{0-t}}{AUC_0^{0-t}} = \frac{\frac{Fa_i \times (1-\epsilon) \times Dose_i}{Cl}}{\frac{Fa_0 \times (1-\epsilon) \times Dose_0}{Cl}} = \frac{Fa_i \times Dose_i}{Fa_0 \times Dose_0}$$

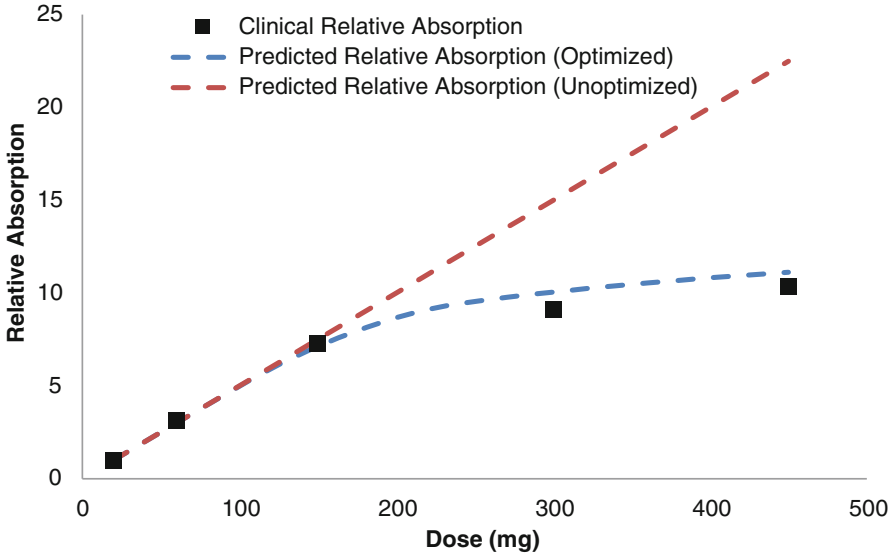


Fig. 7.7 Case Study 1: Predicted and actual relative absorption of compound A in humans based on optimized and un-optimized models

Also shown is the relative absorption as measured by the ratio of AUC^{0-t} from clinical data. As can be seen, the optimized solubility model agrees best with the clinical data. The optimized absorption model predicted an exposure plateau to occur between 100 and 150 mg doses due to solubility-limited absorption. This is reflected by the clinical data at the 150 mg dose. Conversely the un-optimized model predicts dose-linear exposure up to and beyond 450 mg.

7.4.2.2 Case Study 2

Molecule B is a weak base with low solubility in bio-relevant media (2 mg/mL in 0.01 N HCl and 0.018 mg/mL in FaSSIF). The passive permeability of this compound is predicted to be moderate-high (1.15×10^{-4} cm/s). The oral bioavailability of molecule B in rats and dogs as estimated from this study was 64% and 60%, respectively. An absorption model built using the in vitro solubility and permeability values did not provide a good fit to the in vivo Cp-time data. Therefore, the smallest possible optimization of both parameters was performed.

Figure 7.8 shows an overlay of the experimental Cp-time profile with the simulations obtained from the initial ("un-optimized") and optimized permeability and solubility ("optimized") from rats and dogs. The optimal absorption parameters are 2 mg/mL and 0.031 mg/mL for the gastric and intestinal solubility, respectively, and 3.8×10^{-4} cm/s for the permeability.

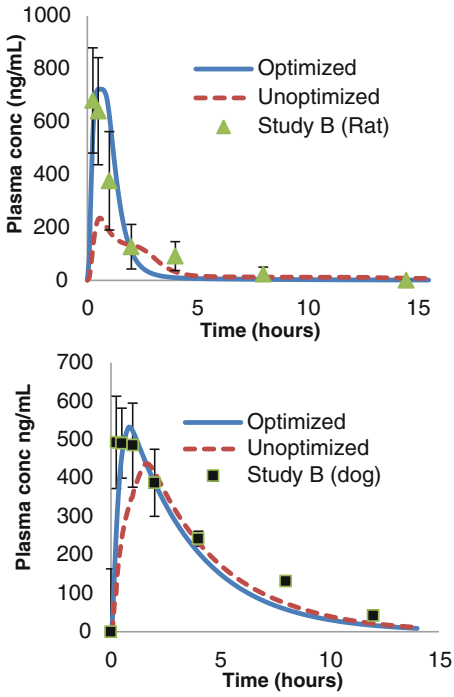


Fig. 7.8 Case Study 2: In silico predicted and actual in vivo PK profiles of molecule B in rat and dog

As was conducted previously, the optimal preclinical absorption model was utilized to predict absorption in humans for a given dose range.

Figure 7.9 shows the predicted relative absorption of molecule B in humans as a function of dose using both the optimized and un-optimized absorption models. Also shown in the plot is the relative clinical $AUC^{0\text{-}t}$ of the compound as a function of dose. As can be seen, the optimized absorption model is the best predictor of the dose-exposure relationship observed in clinical data.

7.5 Toxicology Formulation

The topic of toxicology formulations has been covered in detail in the literature [81]. In the context of developability assessment, toxicology formulations are assessed in terms of their acceptability for long-term toxicology studies and the level of complexity associated with the formulation. For example, formulations that use solid dispersions of the drug substance would be considered more complex and expensive, requiring longer lead times and additional material (to compensate for manufacturing losses) relative to crystalline material that can be dosed as aqueous suspensions.

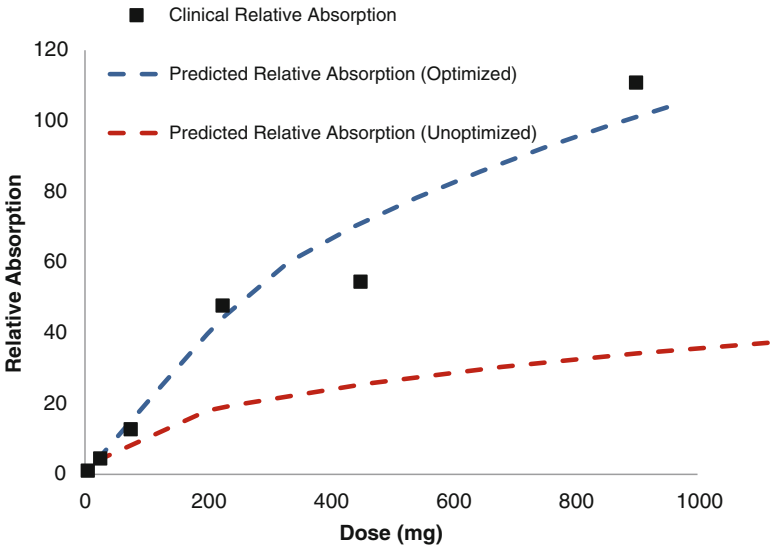


Fig. 7.9 Case Study 2: Predicted and actual relative absorption of compound B in humans based on optimized and un-optimized models

7.6 Developability Summary

Most companies require a comprehensive summary of the developability assessment of new molecular entities when they are nominated as candidates for the clinical development. Integrated developability assessment, the subject of this chapter, includes the following major components and their interrelationships: (a) drug substance and drug product parameters, (b) patient-centered design parameters, and (c) business parameters.

7.6.1 Drug Substance and Drug Product Parameters

These have been described in sufficient detail in Sects. 7.2 and 7.3. In the overall developability summary, these assessments must be organized in a manner that captures all the information in a manner that is meaningful to both the discovery and development organizations. Table 7.3 shows one such format. Each subcomponent of this assessment may further be scored depending on how they compare with what might be considered "standard" and/or the potential impact of the parameters on cost, timelines, and flexibility of clinical and commercial development activities.

Table 7.3 Drug substance and drug product developability summary

Parameter	Assessed in terms of
Drug substance	Synthetic complexity, cost, demand, and containment class
Physical properties	Solid form properties as they pertain to isolation, handling, and storage
Drug product design	Efficacious dose, Phase 1 dose range and biopharmaceutical properties
Drug product manufacturing	Product platform, manufacturing and packaging, any special facilities
Toxicology formulation	"Standard" versus "nonstandard" formulations

7.6.2 Patient-Centered Design Parameters

This parameter refers to the design parameters of the drug product as they relate to the disease state, patient, and caregiver profiles. For example, if a given drug product is intended for use in mild cognitive impairment, the patient population might generally be expected to be able to self-administer the drug as a conventional orally dosed tablet of reasonable dose. Twice daily dosing, while not preferred, might still be acceptable. However, if the compound is intended for use in severe dementia associated with Alzheimer's disease, the patient might have swallowing difficulties and also be dependent on a caregiver in a nursing home to administer the drug. Thus once daily dosing of a very small swallowable tablet, an orally disintegrating tablet, or a transdermal patch might be a requirement for the product to be commercially viable. Generally speaking, patient-centered design parameters must be taken into account in building lead optimization flow schemes and setting the critical attributes for a clinical candidate.

7.6.3 Business Parameters

These include a complex matrix of parameters such as the extent of understanding of the biological target (i.e., novel versus validated), the competitive landscape, priority in the company's pipeline, potential development costs, etc. Generally, novel targets that are of high priority to the company are geared toward meeting the near-term goals of clinical target validation through target engagement and demonstration of a pharmacodynamic response. Such "first-in-class" assets often enter development as long as the clinical development criteria are met, even though commercial viability may be uncertain.

When viewed in the context of all three parameters described above, the compounds nominated as clinical candidates fall into four major categories as shown in Fig. 7.10.

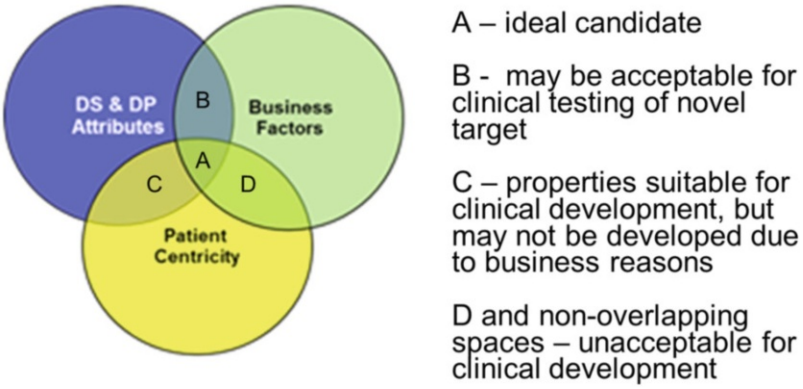


Fig. 7.10 Schematic depiction of clinical candidates based on drug substance and drug product (DS and DP) attributes, patient centricity, and business factors

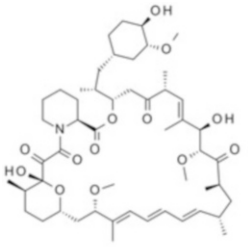
7.7 Case Studies/Illustrative Hypothetical Scenarios

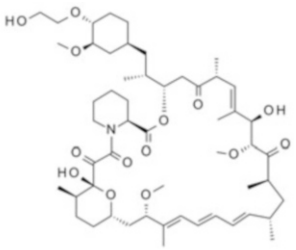
The concepts presented in this chapter are best exemplified by examining the development histories of molecules that have progressed through clinical development.

7.7.1 mTOR Inhibitors Rapamune® (Sirolimus) and Afinitor® (Everolimus)

The introduction of inhibitors of the mammalian target of rapamycin (mTOR) over the last several decades highlights the role of developability concepts in the development of novel medicines. In 1999, the first commercial formulation of sirolimus (Rapamune) became available. The compound was formulated as an oral solution of 1 mg/mL in Phosal 50 PG and polysorbate 80. This formulation was required to overcome the very poor physical properties of the compound (logP 4.3, aqueous solubility 2.6 μg/mL), which limited oral absorption. Despite the use of this solubilizing formulation, the compound still showed high variability in patients, with some subjects having ~8× higher exposure than others. Oral bioavailability of the solution formulation was estimated to be ~14% [82]. From a patient standpoint, this formulation also suffered from having an unpleasant taste and required refrigerated storage and protection from light. In addition to the inconvenience these requirements impart to the patient, it is arguably more concerning that any noncompliance with the recommended storage conditions could lead to degradation of the compound. Failure to follow the prescribed dosing regimen could also negatively impact the compound's efficacy. With reference to Fig. 7.10, this drug product presentation would put Rapamune in the D category.

To provide an alternate dosage form, an oral tablet formulation of sirolimus was later developed utilizing NanoCrystal® technology from Elan Corporation, which greatly improved patient convenience in terms of taste and ease of administration and storage.


Rapamune® (sirolimus)

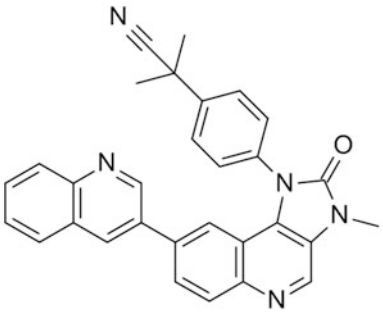

Afinitor® (everolimus)

Given these clear hurdles to development, at first glance it may be surprising that this compound advanced into clinical development at all. The fact that a molecule such as sirolimus was taken to market, despite having significant development challenges, highlights the influence of important business drivers and a recognition that the compound filled an unmet medical need. The compound was able to advance, despite the added cost and complexity required for its development.

Research has continued in the development of additional inhibitors of this pathway. Everolimus (Afinitor) is a synthetic derivative of rapamycin, which was designed to have improved pharmacokinetic properties relative to sirolimus, in part through improved solubility, which would provide more consistent oral bioavailability. The compound was developed as an oral tablet consisting of a solid dispersion (SDD) with HPMC. In addition to the tablet formulation, everolimus is also available as a tablet for oral suspension (Afinitor Disperz), for use in pediatric patients.

7.7.2 BEZ-235 (PI3K/mTOR Inhibitor)

Another example of a compound developed as an inhibitor of the phosphatidylinositol 3-kinase/mTOR (PI3K/mTOR) pathway is represented by BEZ-235 (dactolisib), which first entered clinical development for the treatment of cancer in 2006. The compound was shown to be well tolerated and was found to elicit partial responses in some patients. However, the PK data showed significant intersubject variability as well as low oral bioavailability when the compound was dosed as a simple powder filled into hard gelatin capsules. An MTD was not established during this study, due to an observed plateau in exposure. This poor in vivo behavior was attributed to the compound's poor aqueous solubility, especially at pH>4. As a result, clinical development was delayed to allow for additional formulation development to identify a more suitable formulation.



BEZ-235

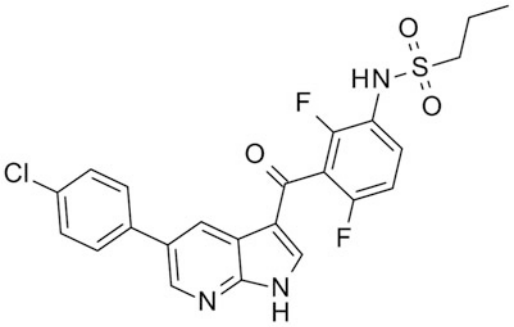
In a subsequent Ph1/1b study, several formulations, including the original gelatin capsule formulation, two solid dispersion capsule formulations, and a solid dispersion sachet, were all evaluated. The sachet formulation outperformed the others in terms of variability and permitted the identification of an MTD of 1600 mg. Additionally, BID dosing was explored in this study, which provided an equivalent AUC and lower C_{max} when compared to QD dosing. However, this change to the dosing schedule did not produce changes to the tolerability or efficacy of the compound. In 2015, it was reported that the compound was no longer in active clinical development.

The example of BEZ-235 highlights the critical role of assessing developability of compounds prior to their entry into clinical development. The results obtained with this molecule may not be surprising given the selection of a powder in capsule formulation platform for a poorly soluble compound with a high target dose range. While the innovator ultimately responded by bringing forward several enabled formulations, this was likely done at considerable cost in terms of dollars and several years of development time and resources. This investigation also required additional clinical studies to optimize the formulation and corresponding pharmacokinetic profile.

7.7.3 BRAF Inhibitors (Vemurafenib: Zelboraf)

Following the discovery that the BRAF gene was mutated in a number of different types of cancer [83], this target became a major focus of drug discovery and development. While a large number of structural scaffolds had been identified as part of kinase programs across the industry, none of them possessed the structural requirements for inhibition of BRAF. A scaffold-based screening approach was used to identify novel chemical space that showed modest activity for this target, and these compounds served as the starting point for SAR efforts [84]. From these efforts, the compound vemurafenib was identified as a candidate for clinical

development [85]. The compound possessed sufficient potency and selectivity and was shown to demonstrate acceptable efficacy and toxicology in preclinical species to warrant further development. The compound formally entered the clinic in 2006.



vemurafenib (Zelboraf)

The physical properties of the compound were not ideal, but given the other attributes listed above, as well as a strong scientific rationale that this mechanism of action should be explored for the treatment of cancer, a sufficient justification to move forward existed. To accelerate the start of Phase 1 clinical studies, the drug product selected for first-in-human studies consisted of a blended powder in capsule, with excipients that were selected to increase oral bioavailability. Dosing started at 200 mg per day and was dose escalated to a top dose of 1600 mg/twice per day. After observing a plateau in exposure, a reformulation effort was undertaken which resulted in the development of an amorphous drug product, which provided a sixfold increase in bioavailability relative to the crystalline formulated capsule [86, 87]. This reformulation work was carried out in a very efficient manner and only required a ~6-month suspension of clinical dosing. Significant efforts in modeling and simulation were utilized to predict therapeutic plasma levels based on translation of in vitro assays and animal efficacy models, which supported the hypothesis that the amorphous formulation would provide the desired exposure to drive efficacy. Vemurafenib was ultimately approved by the FDA in 2011, only 5 years after filing of the investigational new drug application (IND) [88]. This rapid clinical development could only be accomplished through close collaboration between cross-functional partners. This example highlights the type of speed and efficiency that can be obtained when strong business drivers overlap with science to deliver a profound benefit to patients.

7.8 Conclusion

Developability assessment of clinical candidates has evolved in the recent past into a multifaceted function. As a key component of the discovery development interface (DDI), it serves the dual role of supporting discovery projects by providing essential preclinical formulation support to run in vivo studies while tracking the trajectory of physicochemical and biopharmaceutical properties of compounds progressing through flow schemes. Overlaying these functions, however, is the seminal role it plays in influencing the selection of compounds entering clinical development, based on business priorities, commercial viability, and custom-defined criteria for drug product attributes based on the target patient population. On the technical front, it involves solid form selection and physical properties design and formulation platforms selection guided by in vitro and in silico tools to predict in vivo performance. All of these activities are performed in a tiered and phase-appropriate manner, to drive sound decision-making that culminates in the selection of a compound that lends itself to clinical and commercial development. The need for continued improvement of the tools and technologies, along with strategies to enhance speed and efficiency in the discovery and development timelines, offer ample opportunities for innovation in this field.

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Chapter 8
Lead Optimization, Preclinical Toxicology

Marcus H. Andrews and Vincent L. Reynolds

Abstract Nonclinical Toxicology During "Lead Optimization".

The major deliverable from a "lead optimization" (LO) tox package will be a high-quality candidate compound, suitably characterized to enable judgment-based selection of clinical candidates destined for further development and preparation for initial clinical investigation. For small molecules, the LO phase of development typically represents the first opportunity to characterize the novel chemistry using an integrated approach that collectively scrutinizes a molecule's overall "druggability," with a focus on characterization of all the physical chemistry properties that may influence drug disposition, safety and tolerability, and dose prediction (with the underlying assumption that the hypothetical biological mechanism of action remains intact).

In keeping with the 3R principles, modern safety assessment continues to explore the potential risks and liabilities associated with the chemical structure via various predictive in silico screens that tackle both intrinsic toxicophore identification, in addition to structural similarity assessment of chemical moieties appearing in other structures with known adverse event profiles, and a battery of cell-based profiling assays that enable characterization of tolerability based on chemical properties, in addition to bespoke cell models that afford characterization of functional risk (e.g., induced pluripotent cell lines for different target organ systems). Collectively, these data are used to better inform investigators on the potential in vivo risks which may manifest in the preliminary multidose studies, which are designed to not only corroborate the in vitro predictive assessments but also identify the degree of monitorability (and subsequently, manageability) of on- and/or off-target toxicities associated with different drug exposures, in the context of a developable clinical dosing range.

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8.1 Overview

The successful development of a drug requires a careful and continuous multifaceted assessment of the risk-benefit profile of the agent(s) under consideration. Toxicology studies in the lead optimization (LO) phase of drug development are conducted to provide essential information on the "risk" side of this ratio. It is important to appreciate that there is no predetermined value that defines a priori an acceptable level of risk. Rather, pharmaceutical development requires the consideration of a range of variables such as the intended indication; preexisting medical conditions, morbidities, and the potential for concomitant medications in the intended patient population; synthetic complexity and chemical stability of the pharmaceutical agent; and comparative benefits and risks relative to existing standards of care. These and other factors are collectively used to determine the degree of risk that is acceptable at each stage of development.

Toxicology assessments, then, are an important component in a larger palette of factors that are considered when accepting or rejecting molecules for development as pharmaceutical agents. Blomme and Will [1] put forward an interesting historical perspective describing the change in industry attitudes from the time when LO safety assessment was still in its infancy to the current philosophy of "kill early, kill often." This approach deliberately front-loads toxicity evaluations of compounds into LO development workflows and provides differentiation by safety through empirical testing. It is now well recognized that current practice of including toxicity assessments in LO contributes directly to reductions in later attrition due to safety concerns which may manifest themselves after drug candidates progress into clinical trials [2].

LO toxicology has evolved to include sophisticated strategies which include predictive modeling and structural similarity assessments (e.g., tools described in Chap. 6) along with screening studies and successive repeat-dose toxicity studies of increasing rigor that allow cautious and staged exploration of chemical scaffolds to guide informed decisions about whether specific compounds should be either discontinued or carried further in development.

Before proceeding forward with the conduct of nonclinical safety assessment studies, it is important for the LO toxicologist to pause and appreciate the wealth of existing information that may already be available regarding potential toxicity liabilities with the drug target under study. Although definitive information regarding doses, exposures, and various commonly accepted toxicology endpoints will in most cases be lacking at this early stage of development, there may nevertheless be a substantial amount of information on adverse target-related effects that can be reasonably expected to occur. In addition to the predictive modeling which can directly support medicinal chemistry structural design (see earlier chapter), this

information can be used to tailor the design of safety assessment studies so that potential target-mediated toxicities receive an additional measure of attention and scrutiny throughout development.

A characteristic feature of the LO phase of drug development is that there are often numerous molecules under evaluation at any given time. To complicate the challenge of connecting structure with activity with pharmacological outcome (both positive and negative), there may be only a very small amount (as little as a few milligrams) of any given molecule available for study. This is particularly true during the very early phases of LO. Thus, the LO toxicologist is immediately faced with the logistical challenge of generating a broad array of key data needed to guide decision-making, but often in the face of having only very limited amounts of test material available for use. Consequently, nonclinical safety assessment studies in the LO phase almost invariably rely on a series of successive studies which increase in complexity (and test material requirements) as compounds proceed further along in LO development. Throughout the LO phase, the overall goal is to gather a sufficient body of evidence to support not only the "druggability" of the target but also the suitability of one or more molecules to continue further in development.

Without the availability of historical precedents from previous efforts to use as a guide, toxicology information early in LO for new targets and/or chemical classes may rely on a range of in silico evaluations, in vitro screens, or cell surrogate profiling (e.g., assessments of cytolethality, phospholipidosis, and other endpoints of potential concern explored through in silico evaluation and in vitro test systems; see earlier chapter for a more detailed description of this technology). It is readily recognized that the output from these studies clearly cannot provide a comprehensive assessment on potential toxicity concerns. However, these early screens can be used effectively to support a rank ordering of multiple compounds and thereby assist in prioritizing those compounds with desirable properties which should be selected for further study as well as identifying undesirable compounds for which development activities should be discontinued.

As compounds with undesirable properties are identified and filtered out/excluded, the LO toxicologist can focus a greater degree of scrutiny on a smaller number of compounds which may be still under consideration. At this point, in vivo screening studies with abbreviated dosing schedules can be conducted. Results from the initial in vivo screening studies can provide critically important toxicity data in a range of tissues which may be helpful in identifying any acute on- or off-target toxicities as well as essential pharmacokinetic data to understand whether the study molecule possesses the requisite absorption, distribution, metabolism, and excretion (ADME) properties to warrant continued investment.

An important logistical point to consider when planning the initial in vivo toxicology studies is the high likelihood that the availability of test compound may be very limited, particularly for molecules that have complex or difficult synthetic routes. This can affect decisions on the choice of the test system, the dosing duration, and dose selection for the initial in vivo screening pilot studies. The test system for the initial in vivo screening pilots is almost always a rodent, which provides two distinct advantages. First, this provides an opportunity to protect higher-order species from compounds with uncharacterized (and therefore

potentially high) toxicity. Second, studies in rats or mice require smaller amounts of test compound than comparable studies in nonrodents (see Tables 8.1, 8.2, 8.3, and 8.4 for a listing of test material requirements for screening pilot studies in rats with different dosing durations and dose levels). When considering the optimal dosing duration, the intended pharmacology of the molecule may dictate the need for lengthier durations for these initial studies. For example, a very short dosing duration for agents that are intended to act via changes in gene expression (e.g., nuclear hormone receptors) may not be adequate to detect slowly developing adverse changes associated with excessive pharmacology. Finally, the dose levels to be studied may be defined by test compound availability. To some extent, the limitations in test material availability may be mitigated by conducting the initial in vivo pilot studies with small group sizes (e.g., $n = 3$/sex/group), by conducting the study in only one sex, and by collecting toxicokinetic (TK) data via dried blood spot analysis (which requires only 20–40 μL of blood per sample) from the main study animals instead of adding a satellite TK group. Despite efforts to minimize the amounts of compounds needed, it should be expected that the design of the initial in vivo toxicology screening pilot studies will be defined in most cases by balancing what is feasible based on test material availability against what is desirable based on the pharmacology and expected toxicities of the molecule. This balancing act generally results in a development plan in which the initial in vivo screening pilots are done in rodents treated for somewhere in the range of 4–7 days. Importantly, the frequent necessity for a compromise between logistics and optimal study designs underscores the value of maintaining close communications with others (particularly the chemists) on the development team so that overall team needs can be met.

Historically, it has been shown that a significant amount of attrition due to toxicology occurs in the preclinical phase which includes the aggregate of all nonclinical studies conducted prior to the start of Phase 1 clinical trials. Therefore, one of the key benefits of the LO toxicology workflow is the flexibility it allows. The initial short-duration in vivo screens in rodents can then be followed up either with longer pilot toxicology studies for more promising compounds or with more short-duration studies on other compounds if the lead compound is deemed to be unsuitable for further development. Overall, the primary goal of the LO toxicology work is to produce a data package which identifies candidate molecules which are worthy of continued advancement toward clinical development. A secondary goal is to guide dose selection for the definitive nonclinical safety assessment studies that will be needed later to support those clinical trials. Concomitant with the above-described in vivo toxicology assessments, additional toxicity profiling work may be done to identify potential genetic toxicity liabilities or other toxicity problems known or suspected to be associated with the pharmacologic target under study.

In summary, LO toxicologists frequently work from a commonly used "template" of studies. However, this should not obscure their thinking at any point. They should ensure that any nonclinical safety assessment work is truly "fit for purpose" and focused on guiding decision-making. Departures from a template approach should be taken whenever dictated by properties of the target or compounds under study.

Table 8.1 Typical test material requirements supporting abbreviated (4-day) repeat-dose pilot studies in rats with different dose levels

# Animals	Dose	Animal Wt	Dose Vol	Conc	Duration	Material		
(Tox + TK)	(mg/kg)	(kg)	(mL/kg)	(mg/mL)	(days)	(g)		
5	100	0.300	10.0	10.0	4	0.6		
5	300	0.300	10.0	30.0	4	1.8		
5	1000	0.300	10.0	100.0	4	6.0		
								Potency
							Total + 20%	**100**
					Total:	8.4	10.1	**10.08**

Animal groups are divided by toxicity ($n = 3$) and toxicokinetic ($n = 2$) cohorts. These studies are often conducted in one sex (usually female rats as they are compound sparing due to lower body weight). If dry blood spot sampling for toxicokinetic evaluations is used, only toxicity cohort animals may be required. Overestimates (e.g., +20%) are often factored into the calculation in order to take into account variations in animal body weight losses during formulation and dosing. Compound potency is assumed to be 100%

Table 8.2 Typical test material requirements supporting mid-/late-LO phase (14-day) repeat-dose pilot studies in rats with different dose levels

# Animals	Dose	Animal Wt	Dose Vol	Conc	Duration	Material		
(Tox + TK)	(mg/kg)	(kg)	(mL/kg)	(mg/mL)	(days)	(g)		
10	100	0.300	10.0	10.0	14	4.2		
10	300	0.300	10.0	30.0	14	12.6		
10	1000	0.300	10.0	100.0	14	42.0		
3	100	0.300	10.0	10.0	15	1.4		
3	300	0.300	10.0	30.0	15	4.1		
3	1000	0.300	10.0	100.0	15	13.5		Potency
							Total + 20%	**100**
					Total:	77.7	93.2	**93.24**

Animal groups are divided by toxicity ($n = 5$/sex/group) and toxicokinetic ($n = 3$ females/sex/group) cohorts. The sizes of the dosing groups may vary, depending on specific scientific needs, and this will affect the test material requirements. In cases where collection of toxicokinetic data from male animals is desired, the test article requirements would increase

Table 8.3 Test material requirements for dose-escalation and toxicokinetic studies in nonrodents (animal weights in table refer to typical Beagle dog)

# Animals	Dose	Animal Wt	Dose Vol	Conc	Duration	Material		
	(mg/kg)	(kg)	(mL/kg)	(mg/mL)	(days)	(g)		
2	3	12.0	5.0	0.6	1	0.1		
2	10	12.0	5.0	2.0	1	0.2		
2	30	12.0	5.0	6.0	1	0.7		
2	100	12.0	5.0	20.0	1	2.4		
2	300	12.0	5.0	60.0	1	7.2		Potency
							Total + 20%	**100**
					Total:	10.6	12.8	**12.76**

Animal cohorts of $n = 1$/sex/dose group, and when possible (pending tolerability), doses may be escalated across a fully logarithmic dose range to establish a toxicokinetic exposure profile. If the nonrodent test system is a nonhuman primate (with cynomolgus monkeys being the most commonly used strain of monkey), the test material requirements would be decreased since cynomolgus monkeys have a lower body weight (generally in the range of 3–4 kg)

Table 8.4 Test material requirements for nonrodent in vivo pilot studies (example requirements here based on dog) with different dosing durations and dose levels

# Animals	Dose	Animal Wt	Dose Vol	Conc	Duration	Material		
	(mg/kg)	(kg)	(mL/kg)	(mg/mL)	(days)	(g)		
2	100	12.0	5.0	20.0	7	16.8		
2	300	12.0	5.0	60.0	7	50.4		
2	600	12.0	5.0	120.0	7	100.8		Potency
							Total + 20%	**100**
					Total:	168.0	201.6	**201.60**

Animal cohorts of $n = 1$/sex/dose group (samples for toxicokinetic assessment may be collected from the toxicity cohort animals)

8.2 The LO Toxicology Workflow

A priority of the LO workflow is to simultaneously reduce the levels of uncertainty and the numbers of molecules under study pertaining to the pharmacological modulation of the drug target of interest. Different institutions have different approaches to LO toxicology which are shaped largely by their prior experience, logistics, and target-related concerns that may require special attention. However, the overall workflow for LO toxicology across the pharmaceutical industry is driven by the common dual themes of reducing or resolving uncertainties related to safety while at the same time moving from screening studies with numerous compounds to more intensive toxicology studies with a much smaller number of compounds (see Fig. 8.1). Ultimately, a successful LO toxicology effort will support the identification of one or a few compounds with which safety uncertainties have been sufficiently resolved that a decision to proceed forward into human testing can be supported.

For simplicity, we have divided the LO workflow into three distinct periods of development (early/mid/late), each of which containing a discreet set of goals and activities. Early LO challenges a project team to "place the right bet" with their chemical scaffold. The focus is not so much about predicting toxicity (see Chap. 6), but rather on how to avoid it. During early LO, iterative evaluations of structure-activity relationships are predicated on the premise that minimizing intrinsic chemical features associated with toxicities will increase the likelihood of producing a successful clinical candidate. As efforts progress into mid-stage LO, attention gradually shifts to exploring the breadth of possibilities within a more limited zone of chemical space. It is during this time that lead optimization really earns its name, as the majority of issues that threaten the identification of candidate molecules are resolved in this mid-stage phase. Because toxicology is a key driver of attrition, a series of pilot toxicity studies with multiple compounds is not uncommon during mid-stage LO. Finally, the goal of late-stage LO activities will be to refine toxicity profile of the candidate molecule not only to increase the level of confidence that the molecule can advance safely in human trials but also to guide decisions on study design and dose selection for the Phase 1-enabling nonclinical safety assessment studies.

8.2.1 Early-Stage LO Toxicology Activities

While the earlier pre-lead phases of drug discovery focus on testing biological hypotheses with targets and molecules from chemical scaffolds which may or may not ultimately be "druggable," the LO phase represents a period when at least one molecule from at least one chemical scaffold has been identified which demonstrates—at least in principle—that the intended drug target can be pharmacologically modulated. With this promising information, confidence is bolstered that the

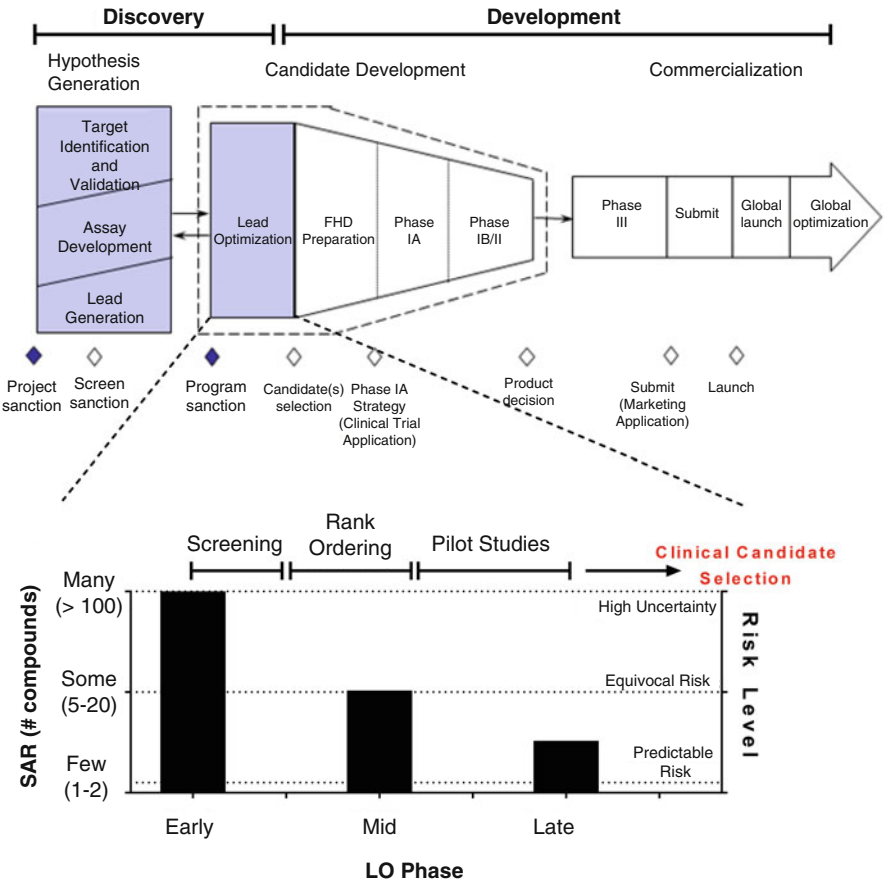

Fig. 8.1 A general depiction of the drug development paradigm (typical of small molecules), including conceptual interpretation of the progression of risk assessment occurring during lead optimization. Three key points to consider are the (1) ability to provide a detailed risk assessment of target- and/or compound-related risk is inversely related to the chemical space being considered; (2) the number of compounds should dramatically reduce in number as data is generated to either create or support hypotheses pertaining to the target, indication, and chemistry; and (3) the ultimate goal of the LO is to produce a clinical candidate compound suitable for further GLP testing, based on studies which identify target organs and putative margins of safety

desired pharmacology can be harnessed to bring therapeutic benefit to a defined patient population. This confidence, in turn, justifies the further investment of effort and resources from biology, chemistry, drug disposition, toxicology, and others in development activities where the end goal is to identify molecules which meet or exceed the internally established requirements of a clinical candidate. Despite this confidence, however, uncertainty remains high, molecular options are still numerous, and the amounts of individual molecules available for study are low. Thus, the

LO toxicologist must address multiple and often shifting challenges in order to help the drug development team.

In the time period immediately following identification of a lead molecule, the medicinal chemistry efforts typically focus on exploring a range of structure-activity relationships (SARs) in an effort to identify molecules which retain the desired pharmacologic properties, but which also possess improvements in a range of other properties (e.g., potency, solubility, chemical stability, etc.) which collectively increase the likelihood that druggable molecules can be identified. As the SAR is explored, a series of molecules from one or more chemical scaffolds will emerge, and the LO toxicologist will be asked to assist in "filtering" the stream of molecules to remove compounds with unacceptable liabilities. This filtration process is an essential feature of the broader range of LO activities and is particularly applicable to LO toxicology efforts. It can be readily appreciated that conducting a full range of toxicology studies and tests on every molecule in the stream emanating from the medicinal chemistry laboratories would be prohibitive on both a cost and logistical basis. Moreover, conducting in vivo toxicology studies across the full range of molecules comprising a developing SAR would contradict the 3Rs principle which calls for reducing, refining, and replacing the number of toxicology studies done in animals (Russell, 1959 (as reprinted 1992); see also Tannenbaum and Bennett [3]). In an effort to minimize these concerns while at the same time allowing early safety assessment to assist LO teams, the careful and judicious selection of compounds for toxicology screening studies has developed and become well recognized as an important and effective way to filter undesirable molecules from a SAR. Consequently, LO toxicology has evolved from being largely absent during the LO phase to being a key player where early identification of molecules potentially unacceptable or unmanageable toxicity liabilities brings great value to teams by allowing them to focus their energies and resources on other compounds with a higher probability of success in later phases of development.

One of the challenges mentioned previously was that the amount of test material available for use in toxicology assessment during this period of development may be quite scant. Because only very limited amounts of any given molecule might be available for study, the early-stage LO toxicology evaluations generally involve in vitro screens or evaluations using cell-free test systems (see Chap. 6). Although there are no specific requirements that prescribe exactly what toxicity screens should be performed, many pharmaceutical companies rely on a relatively standard battery of core assays and study types to define their pre-LO and LO toxicology activities. The list of toxicity evaluations is generally defined by two rather flexible considerations: a list of assays that the particular institution has relied upon in the past and any additional tests that are designed to address specific concerns that may be raised by existing information on the target. Thus, the design of the LO toxicology plan is largely tempered—for better or worse—by institutional experience within individual institutions themselves (not by overarching regulatory guidelines) and by the amount of investment in money and time that is deemed appropriate to frame the clinical relevance and manageability of compound- or target-related toxicities. Early LO toxicology efforts are, not surprisingly, most

often oriented toward in vitro screens, which can capitalize on the technologies described earlier in this volume: as described in Chap. 6, the in vitro test systems are an ideal starting point as they generally require only very small amounts of the test drugs (as little as 4 mg dry test material per assay) and can be set up to provide high-throughput testing of large numbers of molecules. The range of screens included in the workflow will vary across different institutions as well as across different development teams within the same institution, depending on the specific needs and concerns at hand. As Stark and Steger-Hartmann [4] observed, the "extent of screening largely depends on the need for differentiation between candidates as well as the need for early de-risking of specific liabilities."

It should be recognized and accepted that the in vitro test systems themselves may have notable inherent deficiencies (e.g., inability to take into account absorption parameters, metabolic differences, etc.) compared to intact in vivo mammalian test systems. The in vitro results can nevertheless provide great value to development teams by rank-ordering compounds and guiding prioritization decisions to determine which molecules should be terminated and which should be continued in development.

8.2.2 Mid-Stage LO Toxicology Activities

As drug development teams move from early- to mid-stage LO in developing and assessing their emerging SAR, the number of compounds requiring toxicology evaluations will (hopefully!) begin to diminish. Some molecules will have been de-prioritized by the earlier in vitro toxicology screens, whereas other compounds may have been found to be unsuitable because of pharmacodynamic insufficiencies, chemical instability, synthetic complexity, or other reasons. Regardless of the reason (s), the de-prioritization of some compounds means that more intensive evaluations of smaller numbers of other molecules can now be undertaken. With this movement into the mid-stage of LO development, the toxicologic scrutiny can increase as molecules can now start to be evaluated in the initial in vivo repeat-dose toxicology studies.

Repeat-dose toxicology studies are often viewed as the centerpiece of nonclinical safety assessment. Goals of repeat-dose studies include target organ identification, establishment of a dose-response (or exposure-response) relationship, differentiation between on-target toxicities (i.e., excessive pharmacology) and off-target toxicities, determination of kinetic parameters, and the development of biomarkers of toxicity that can be leveraged to improve the efficiency of safety assessment for other compounds under study. When approaching the first set of repeat-dose studies, the LO toxicologist will face several key decisions which will have a substantial impact on subsequent nonclinical safety assessment. These include selection of the rodent and nonrodent species that will serve as the test systems, dose selection, and biomarkers which can be leveraged to identify hyperpharmacology or to define and validate change that can be used in a premonitory way to enhance clinical safety through the early detection of incipient adverse

effects. Of these considerations, biomarker selection should be leveraged by an examination of the intended pharmacology and will often overlap with the typical endpoints collected in pilot studies (see Tables 8.5 and 8.6); as these are likely to be disease specific, general strategies for selection will not be discussed here.

Table 8.5 Typical pathology assessment parameters applied to rodent LO toxicology studies

	Abbreviated rat multidose		Pilot LO toxicology
General design principles	No organ or terminal body weights are collected		Organ weights—collected only if control animals are included in study and $n \geq 2$ Descriptive statistics (mean, sd, % change) if controls are included in study and $n > 2$ Collect pretreatment and end-of-treatment hematology, clin chem, coagulation, and urinalysis
Group size	$N = 3$/sex/group; may use only one sex		$N = 5$/sex/group; both males and females used
Duration	4 days		14 days
Study endpoints			
Hematology	Blood cell morphology Absolute differential leukocyte count (not percent) Erythrocyte count Hematocrit Hemoglobin concentration Mean corpuscular hemoglobin	Mean corpuscular hemoglobin concentration Mean corpuscular volume Platelet count Absolute reticulocyte count (not percent) Total leukocyte count	In addition to the endpoints listed for abbreviated studies: Bone marrow smear
Coagulation	N/A		Prothrombin time Activated partial thromboplastin time
Clinical chemistry	Alanine aminotransferase Albumin Albumin/globulin ratio Alkaline phosphatase Aspartate aminotransferase Blood urea nitrogen Calcium Chloride Cholesterol Creatine kinase Creatinine	Gamma-glutamyl transferase Globulin Glucose Inorganic phosphorus Potassium Sodium Total bilirubin Total protein Triglycerides	

(continued)

Table 8.5 (continued)

	Abbreviated rat multidose		Pilot LO toxicology	
Urinalysis	Total protein	Specific gravity		
Organ weights	Typically not collected due to low numbers of animals		Adrenal glands Brain Heart Epididymis Kidney Liver	Ovaries Prostate Spleen Testes Thymus Uterus
Histopathology	Adrenal glands Bone sternum Brain stem Cerebellum Cerebrum Heart Ileum Injection/infusion site (if appropriate) Jejunum Kidney Liver Lung Muscle		In addition to the endpoints listed for abbreviated studies: Epididymis Eyes Nerve (sciatic) Ovaries Prostate Testes Thyroid Uterus Vagina	

Table 8.6 Typical pathology assessment parameters applied to nonrodent LO toxicology studies

	Large animal dose escalation		Pilot LO toxicology
General design principles	No organ or terminal body weights are collected Pathology parameters (typically collect pre-study, before each dose, and 48 h after each dose) No histopathology performed (dose range considerations for the pilot study are based on overt clinical observations/tolerability as well as evidence of exposure separation between doses)		Organ weights—collected only if control animals are included in study and $n \geq 2$ Descriptive statistics (mean, sd, % change) if controls are included in study and $n > 2$ Collect pretreatment and end-of treatment hematology, clin chem, coagulation, and urinalysis
Group size	Generally 1/sex/dose; M and F used		Group size generally varies from 1 to 3/sex/dose; M and F used
Duration	Staggered group design, duration depends on washout period (contingent on compound's physical-chemical properties)		7 days
Study endpoints			
Hematology	Blood cell morphology Absolute differential leukocyte count (not percent) Erythrocyte count Hematocrit	Mean corpuscular hemoglobin concentration Mean corpuscular volume Platelet count Absolute	In addition to the endpoints listed for abbreviated studies: Bone marrow smear

(continued)

Table 8.6 (continued)

	Large animal dose escalation		Pilot LO toxicology	
	Hemoglobin concentration Mean corpuscular hemoglobin	reticulocyte count (not percent) Total leukocyte count		
Coagulation	N/A		Prothrombin time Activated partial thromboplastin time	
Clinical chemistry	Alanine aminotransferase Albumin Albumin/globulin ratio Alkaline phosphatase Aspartate aminotransferase Blood urea nitrogen Calcium Chloride Cholesterol Creatine kinase	Creatinine Gamma-glutamyl transferase Globulin Glucose Inorganic phosphorus Potassium Sodium Total bilirubin Total protein Triglycerides	Same clinical chemistry battery collected as in dose-escalation studies	
Urinalysis	Total protein Specific gravity		In addition to the endpoints listed for abbreviated studies:	
			Bone marrow smear color Clarity pH Protein	Glucose Occult blood Ketone Bilirubin Urobilinogen Microscopic examination
Organ weights	Typically not collected due to low numbers of animals			
Histopathology			Adrenal glands Bone sternum Brain stem Cerebellum Cerebrum Colon Epididymis (dog) Eyes Heart Ileum Injection/infusion site (if appropriate) Jejunum Kidney Liver Lung	Muscle, quadriceps femoris (dog) muscle, pectoral (monkey) Nerve, sciatic ovaries (dog) Pancreas Prostate (dog) Spleen Stomach Testes (dog) Thymus Thyroids Uterus (dog) Vagina (dog)

8.2.2.1 Species Selection for Mid-Stage Toxicology Studies

Perhaps the decision with the most impact on future nonclinical activities is the choice of the mammalian species to be used for the in vivo repeat-dose toxicology studies [5]. While regulatory guidelines are silent regarding which species should be used for LO toxicology studies, the International Committee on Harmonization (ICH) guidelines are clear that the later definitive toxicology studies needed to support regulatory filings and clinical trials must be completed in both rodents and nonrodents ICH Guidelines M3(R2). As with other regulatory requirements, the guidance is not overly prescriptive and leaves the species selection decision with the drug sponsor who will ultimately be accountable for the collection and interpretation of data. Interestingly, toxicology data sets based on rodent and nonrodent species are referred to in ICH M3(R2) (link to ICH M3(R2)) without any detailed historical context on why this expectation exists apart from ensuring justification of the appropriateness of species selection. Additional detail on test species selection is included in the discussion around general principles of study design (including dose justification, dosing route, and frequency of administration) in the European guidance on repeat-dose toxicology studies (Doc. Ref. EMEA/CHMP/SWP/488313/2007). This guidance refers to selection from "the usual spectrum of laboratory animals used for toxicity testing" which should naturally hold some degree of clinically translatable value, in terms of general safety profile and capacity to respond to the intended pharmacological effects of the substances being tested. This guidance does propose that repeat-dose toxicity studies should be carried out in two species, including both rodent and nonrodent unless a sound argument can be made justifying the use of only one nonclinical test system.

The recommendations appearing in the guidance documents mentioned above apply specifically to the definitive safety assessment studies required to enable clinical investigation. The data collected during the LO phase are generally less important to the regulatory reviewers, but hold greater appeal to project teams who are focused on defining and ranking multiple compounds' developability as potential clinical candidates. There are, of course, exceptions where submission of LO toxicology data is requested by a regulatory agency. For example, if later good laboratory practice (GLP) toxicology studies fail to characterize fully the toxicology profile by not establishing the maximum tolerated dose level, then earlier pilot data may be requested by regulatory reviewers to ensure that dose selection in the GLP toxicology studies was justified and that the GLP studies themselves are indeed valid.

Confidence in candidate molecule selection (i.e., confidence that the candidate will successfully "pass" through a GLP toxicology package that is supportive of initial clinical investigation) is increased when based on critical toxicology endpoints evaluated in multiple species. Indeed, regulatory guidelines specify the need for toxicology studies in both rodent and nonrodent test systems as a prerequisite for human clinical trials. Therefore, in order to guide the design of subsequent definitive toxicology studies, it is advisable that pilot in vivo studies of some form be

conducted in both rodents and nonrodents at some point during LO. The most commonly used rodents are rats and mice, and the most commonly used nonrodents are dogs and monkeys. Other options for in vivo mammalian test systems may be considered, if justified.

The LO toxicologist should make a carefully reasoned decision on which rodent species to be used, as it is very likely that this species will continue to be used to support future repeat-dose toxicology studies throughout the later development of the molecule. In the mid-stage of LO development, it is not uncommon for repeat-dose toxicity assessments to rely primarily (if not solely) on a rodent test system, usually the rat. However, a decision regarding the nonrodent must also be made. There are several critically important points that must be considered when selecting the mammalian species for repeat-dose toxicology studies.

- Kinetic differences. Differences in absorption, distribution, or excretion pathways across different mammalian species may result in exposure limitations that preclude the ability to establish adequate margins of safety needed to ensure human safety in clinical trials. For the LO toxicologist, the estimation of exposure margins is complicated by the fact that human exposures—in particular, the exposures needed for adequate efficacy—have not yet been determined. Thus, the LO toxicologist must rely on PK-PD modeling to provide estimates of efficacious exposures in humans.
- The potential for metabolic differences. The nonclinical metabolic profile of the molecule being studied should include the metabolites that are expected to form in humans. Not only should the metabolites be present, but they should also be present at levels that "cover" the human exposure to these metabolites. It is recognized that, because human trials would not yet have been conducted during the LO phase of development, definitive human metabolite profiles would not be established. However, in vitro evaluation of early metabolite profiling data from human and nonhuman microsomes or liver slices may help guide the selection of species for the toxicology studies. The level of confidence emerging from the in vitro metabolite work may be enhanced by including metabolite profiling in nonclinical pharmacokinetic studies to provide in vivo data which may also be informative.
- Unique species sensitivities. Some nonclinical test systems are known to be particularly sensitive to certain classes of drugs or to certain components which may be present in the formulation. For example, hamsters have been used in the development of some agents with potent peroxisome proliferation activity since the liver of hamster is less prone to injury from this class of compounds than the livers of rats or mice. Dogs are known to have a high incidence of anaphylactic reactions to Cremophor©, an ingredient used in some formulations [6]. If an alternative formulation cannot be identified, then the dog would not be a suitable choice as the nonrodent test system for toxicology studies.
- Pharmacologic responsiveness. At least one (and preferably both) of the nonclinical test species used in safety assessment studies should be responsive to the pharmacologic activity of the test molecule. A clear understanding of

on-target versus off-target effects of a molecule is often invaluable to the medicinal chemists on the development team. On-target toxicities (i.e., adverse effects resulting from excessive pharmacology) may be ameliorated by relaxing the pharmacologic potency/engagement for the intended target. Off-target toxicities, on the other hand, can often be minimized by increasing the potency of the molecule for the intended pharmacologic properties.

- Class-specific regulatory requirements. Occasionally, members of a new drug class will be found to cause a dose-limiting toxicity (DLT) in a nonclinical mammalian test system that is not deemed to be relevant to humans. In such cases, the DLT can limit the ability to test nonclinical doses that are high enough to establish a sufficient safety margin to support clinical development. For example, some molecules in PPARα/γ dual agonist class of insulin sensitizers were found to be poorly tolerated by dogs. The adverse findings in dogs were not predictive of effects seen in human clinical trials with earlier members of this class. Consequently, the US FDA issued advice to the pharmaceutical industry that monkeys would be a more suitable choice as the nonrodent for toxicology studies with these agents. Subsequently, the FDA acknowledged that this shift may have been a mistake and that what was initially viewed as an example of a unique species sensitivity actually anticipated important clinical safety issues. The LO toxicologist should, therefore, maintain an awareness of the evolving external and regulatory landscape and be prepared to make appropriate adjustments.
- Sensitivities regarding nonhuman primates (NHPs). Recently, there has been a gradually increasing desire to minimize the use of NHPs for nonclinical safety assessment studies. Where possible, it is recommended to use dogs rather than NHPs as the nonrodent test system. The dog provides other advantages that warrant consideration in species selection independent of these societal pressures, which include ease of dosing using typical routes of administration, simplified animal husbandry and housing needs, easily interpretable clinical observations, and accumulation of rich class-specific data sets which contribute to evaluation/comparison of toxicities with newer generation compounds.

8.2.2.2 Dose Selection and Other Study Protocol Considerations in Mid-Stage Lead Optimization

Another important decision for mid-stage LO toxicology work is dose selection. Because the availability of test compound may be limited, dose selection for these studies frequently requires a compromise that balances several competing concerns. The LO toxicologist does have the ability, however, to make some adjustments to the mid-stage LO study protocols that can provide more flexibility when selecting doses.

A well-established paradigm common to many toxicology studies supporting clinical development calls for at least three dose levels as well as a concurrent control group. The high dose should define the maximum tolerated dose (MTD), the mid-dose should provoke an intermediate (or "graded") toxic response, and the low

dose should be a no-observed-adverse-effect level (NOAEL). Inter-dose intervals may be based on maintaining a defined degree of separation between doses (e.g., log or half-log intervals), on multiples of doses associated with efficacy in nonclinical models, or on some other rationale. Because the high dose usually requires the majority of drug used in a toxicology study (for a study with three dose levels separated by half-log intervals, approximately 70% of the compound would go to the high dose group), it is particularly important at this stage of development to recognize and appreciate several points related to high dose selection. In the absence of any other considerations, the high dose for repeat-dose toxicology studies is usually the so-called limit dose of 1000 mg/kg (ICH M3 (R2); see also Organization for Economic Cooperation and Development (OECD) Test Guideline No. 408, No. 408, No. 409, and No. 452).

In mid-stage LO, however, there are virtually no constraints on how to design a toxicology study. This means that unconventional study designs and dose levels may be appropriate, as long as the scientific hypothesis can still be challenged. The opportunity to explore fully the best study design (conventional or not) should be embraced by the LO toxicologist. This may mean considering study designs including multiple dose groups in order to refine a dose-response curve as well as inclusion of nonstandard endpoints to address specific molecule- or class-specific concerns. As discussed previously, the data generated in mid−/late LO are critical to building confidence that the best candidate molecule is selected for the requisite nonclinical safety assessment studies needed to support the initial clinical investigation. In many cases at this stage of development, compound availability prohibits administration of a limit dose, and even the establishment of an MTD may be difficult, if not impossible. However, the LO toxicologist should always be looking forward to future studies and recognize that identification of an MTD will decrease the upper limit of the dosing range and substantially reduce the amount of test drug needed for future toxicology studies.

The dosing route for nonclinical safety assessment studies should in almost all cases be selected to match the intended route of administration in humans. Occasionally, exceptions to this general rule may be justifiable. For example, if an adverse gastroenteropathy occurs following oral dosing, then a parenteral dosing route might be used to investigate whether the GI toxicity occurred as a result of a local effect on the enterocytes (i.e., from a luminal exposure) versus a systemic exposure via the circulatory system following absorption.

When pursuing the goal of obtaining the maximum amount of toxicology data with only a minimum amount of test compound, the LO toxicologist must be both innovative and thrifty in the design of the mid-stage LO studies. Studies can be staged, with rodent studies (which require less test material) preceding nonrodent studies. For compounds that fail to give satisfactory results in the rodents, a termination decision may be appropriate which would preclude the need to conduct a nonrodent study at that point. The size of the study can be controlled by limiting the number of animals used. Smaller studies may require group sizes of only three/sex/group. Using only one sex for the initial tox studies would also decrease the size of the study with a concomitant reduction in the test material needed. If a validated

bioanalytical assay exists such that toxicokinetics (TK) can be assessed using very small blood volumes by dried blood spot (DBS) analysis, then perhaps TK could be measured in the main study animals rather than a separate group of rodents dedicated solely for TK. Finally, the dosing duration can be adjusted in response to limited compound availability. However, the dosing duration should be scientifically justified. A duration that is too short may not allow adequate time for the development of histologic indications of adversity or for specific toxicities known or suspected to be associated with the target. On the other hand, dosing durations that are unnecessarily lengthy can cause significant logistical problems for the chemical synthesis group.

One final point regarding dose selection for mid-stage LO studies deserves mention. If the dose-response and toxicity profiles show promise that the target organ toxicities are manageable and are likely to be accompanied by adequate exposure margins relative to projected human exposures, then further development activities with that particular molecule may be considered. Establishing a no-observed-adverse-effect level (NOAEL) is needed in order to calculate a safety margin, which even at this stage may affect early clinical study design to some extent (assuming human dose predictions are available). On the other hand, if the initial repeat-dose toxicology study data indicate that unacceptable findings occurred with inadequate exposure multiples, then a termination decision for the molecule may be made.

8.2.2.3 Nonrodent Studies in Mid-Stage LO

As noted previously, repeat-dose studies in rodents are often sufficient to support decision-making in the mid-stage of LO. Studies in nonrodents frequently require a substantial amount of test compound that simply presents too great of a logistical hurdle at that point for the development team. However, there are some situations where safety data in a nonrodent is desirable. It is generally not good practice to assume that dose-exposure profiles and kinetic parameters will be similar across different species. Consequently, data from rodent studies should not be used to guide expectations for study outcomes in nonrodents. In situations where nonrodent data are desired in mid-stage LO, a dose-escalation pilot study in the nonrodent may be considered. These studies are generally conducted such that one animal of each sex receives single ascending doses (e.g., doses of 1, 3, 10, 30, 100, 300 mg/kg or even higher doses if deemed necessary). A twice-weekly dosing schedule is often sufficient, as it would provide a time interval of several days separating the administration of successive doses. A longer time interval between doses should be used if there is reason to believe that the molecule has a long half-life of elimination. Endpoints on these studies typically include assessments of survival and tolerability, hematology, serum chemistry, and toxicokinetics. Necropsies and histopathology evaluations are not usually included due to the discontinuous dosing schedule. In situations where the availability of test material is particularly limited, the nonrodent dose-escalation study may be deferred to a later stage in LO.

8.2.3 Late-Stage LO Toxicology Activities

As drug development teams approach the latter stages of LO activities, two goals emerge that are of paramount concern for the LO toxicologist. First, any remaining safety concerns must be adequately addressed in order to bolster confidence in the future developability of the molecule or class of molecules under study. Second, a toxicology data set must be generated that will provide guidance for the optimal design of the Phase 1-enabling nonclinical safety assessment studies. These two concerns generally call for toxicology studies that are more robust than the screening work and pilot studies conducted earlier in LO. The need for more intensive toxicology studies might seem initially to present a logistical hurdle for the development team. However, the great majority of potential drug candidate molecules should have been filtered out for one reason or another (e.g., inadequate efficacy, poor chemical stability, inferior salt forms, formulation difficulties, etc.) in earlier stages of LO, leaving only a few molecules still under consideration. Thus, the workflow at this point should have evolved from the rapid screening of multiple compounds and chemical scaffolds that was characteristic of early LO to more rigorous studies with only a very few compounds. This shift allows the LO toxicologist to focus and bring a more intense level of scrutiny to the molecule(s) before the development team recommends triggering the substantial expenditures that will be needed to prepare a drug candidate molecule for entry into clinical trials.

8.2.3.1 Species Selection for Late-Stage Toxicology Studies

The final LO pilot toxicology studies most commonly include repeat-dose studies in rodents and nonrodents (please refer back to Sect. 8.2.2.1). The need for both rodent and nonrodent test systems is indirectly mandated by the ICH M3(R2) guideline (ICH 2009). As noted above, the ICH M3(R2) guideline is, strictly speaking, applicable only to nonclinical safety assessment studies needed to support clinical investigations. However, the need for rodent and nonrodent toxicology studies to support Phase 1 clinical trials effectively means LO pilot toxicology work should assess the potential for adverse changes in the same nonclinical test systems that will subsequently be used later in the development. While it is a common practice to defer studies in nonrodents from early- and mid-stage LO activities, a repeat-dose study in the nonrodent test system is generally viewed as an essential component of late-stage LO.

An exception to the need for both rodent and nonrodent toxicology studies in late-stage LO and even during clinical development may arise in some cases with biotechnology-derived pharmaceutical agents. For example, in a situation where when the epitope for monoclonal antibody drug candidate is expressed in only one nonclinical species and unintended cross-reactivity with nontarget tissues is not expected, then toxicology studies in a second test system may not be appropriate (ICH S6(R1)).

8.2.3.2 Dose Selection

Once the test systems have been determined, the single most important consideration for late-stage LO toxicology studies is dose selection (see Fig. 8.2). Careful thought is recommended when selecting not only the low dose but also the high dose. The departure point when selecting the low dose should be to consider the projected human efficacy exposure. Although there may be a substantial degree of uncertainty with human dose and exposure projections for molecules that are still in the LO phase of development, these projections nonetheless assume great importance as they become firm numerical values upon which nonclinical dose selection and future clinical planning will be based.

Using the projected human exposure as a denominator, the low dose should be set to define a no-observed-adverse-effect level (NOAEL) that will meet previously agreed upon criteria regarding the magnitude of the safety margins (i.e., the ratio of the exposures in the nonclinical studies to the projected human exposure) that will be needed to support continued development. If the low dose is set too low, the likelihood of establishing a NOAEL increases, but the safety margin with an excessively low NOAEL may not be adequate to support continued development. On the other hand, overzealous efforts to define unnecessarily high safety margins may result in the selection of a low dose that is high enough to provoke adverse findings.

In late-stage LO, an increased level of care and attention should be directed at selection of the high dose in toxicology pilot studies. At this point in development,

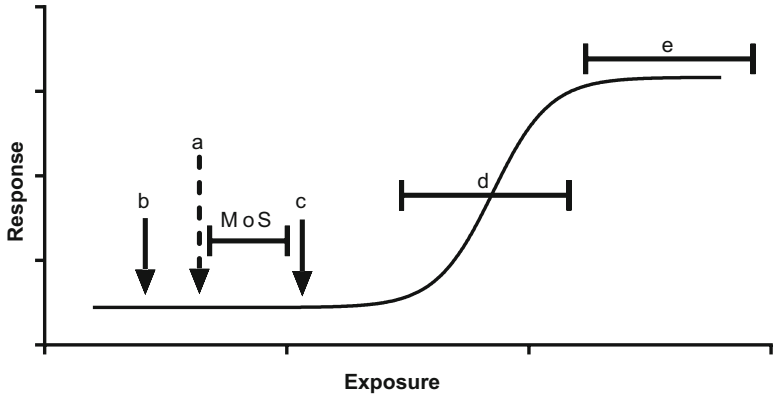

Fig. 8.2 Graphical depiction of a typical dose-setting strategy for LO toxicology studies. (**a**) Represents the predicted clinically efficacious exposure estimated by PK-PD modeling. Adverse effects at or below this exposure (i.e., at exposures lower than the predicted clinical dose range) will likely result in termination of further development of this compound for most indications (with oncology indications being a notable exception). (**b**) Optional low dose group to establish a "no-observed effect level" (NOEL). The exposure here may be too low to support continued development. (**c**) Typical low dose group, needed to establish an exposure multiple (i.e., margin of safety) to support continued development. (**d**) Ideal mid-dose range, which will likely be associated with some graded or intermediate degree of toxicity. Characterization of these findings (i.e., monitorability, clinical translatability, likelihood of reversibility) will be key to support further clinical development. (**e**) High dose range to establish maximum tolerated dose (MTD)

identification of a maximum tolerated dose in the pilot studies allows the upper boundary of the dose range for subsequent toxicology studies to be "fixed." As a result, the breadth of the dose range under consideration for the Phase 1-enabling safety assessment studies can be narrowed which can greatly optimize their design.

There are no established regulatory requirements regarding the size of safety margins. Rather, the margins are set based on safety concerns inherently associated with the drug target, the intended patient population, the nature and severity of the adverse finding(s), the availability of premonitory markers that can be used to monitor for incipient adverse effects, and the likelihood that the anticipated toxicities would be reversible. For some toxicities (e.g., cardiac arrhythmias, CNS-mediated convulsions), margins should be based on maximum concentration (Cmax) parameters, whereas for other toxicities (e.g., organ toxicities more commonly associated with longer exposure durations), margins should be calculated using area-under-the-curve (AUC) values. For non-oncology drugs, safety margins of tenfold or greater are common. For oncology drugs, safety margins may be lower, and margins based on a NOAEL are often not feasible. However, a minimally toxic dose (MinTD) with a manageable toxicity profile should be determined.

At the other end of the dosing range, the high dose for late-stage LO toxicology studies may be guided by any of several considerations. First, if earlier screens suggest that an MTD exists [e.g., if a dose-limiting toxicity (DLT) was identified in earlier screens], then the high dose for the later-stage pilot toxicity study can be selected to determine whether the MTD might decrease with longer dosing durations. If prior data demonstrate that an exposure plateau occurs with higher doses due to absorption limitations or other reasons, then the high dose for the late-stage pilot study may be chosen as a dose at which further increases in exposure cannot be achieved, even when higher doses are administered. In some cases, an argument may be made that the high dose should be based on formulation limitations, particularly if the physical-chemical nature of the drug under development is such that the formulated test material becomes too viscous or is otherwise not capable of being delivered to the test species. However, it should be recognized that arguments based on formulation limitations may be met with skepticism by regulatory agencies, and development teams should try to avoid invoking this as a reason to limit high dose selection. Finally, in the absence of other reasons, the high dose in repeat-dose toxicology studies for most pharmaceutical agents can be based on the limit dose of 1000 mg/kg/day (ICH M3(R2); see also Test Guideline No. 407, 408, 409, and 452 from the Organization for Economic Cooperation and Development). However, in the LO time frame, it is often not possible to predict with any degree of certainty what the Phase II and Phase III clinical doses will be.

8.2.3.3 Dosing Duration and Dosing Route

For non-oncology drugs, daily dosing for 2 weeks in rodents and at least 1 week in nonrodents is common for several reasons. First, these durations are often sufficient to provide guidance on the design of 1-month repeat-dose toxicology studies which

are commonly used to support Phase 1 clinical trials for non-oncology drugs. In addition, a recent analysis of attrition in the pharmaceutical industry noted that many "stopping toxicities" identified in later toxicology studies could be presaged from 14-day studies, suggesting dose-ranging studies of this duration may minimize attrition of compounds after being nominated as clinical candidates [2]. However, while study durations of 7–14 days in late-stage LO are often sufficient, the LO toxicologist should remain alert to the specific pharmacologic, toxicologic, and kinetic characteristics which differentiate one class of compounds from another. These properties may dictate changes in the duration of dosing to ensure that the study is indeed "fit for purpose" and will provide data that can be used with confidence to support or reject a recommendation for continued development.

For agents with oncology indications, the dosing duration and dosing schedule (i.e., daily dosing or intermittent dosing) may be much more variable and should be customized to support the intended dosing schedule for the candidate molecule. This may require additional treatment groups to ensure that the various dosing schedules being contemplated by the Phase 1 medical staff are assessed.

The route of administration for the nonclinical studies with both non-oncology drug and oncology drugs should match the intended clinical dosing route. In all cases, the LO toxicologist should be communicating closely with the medical staff who will be designing and overseeing the clinical development plan so that the nonclinical study durations and dosing routes are ideally set to guide the subsequent definitive repeat-dose toxicology studies that will be needed to support that clinical plan.

8.2.3.4 Endpoints for Repeat-Dose Toxicology Studies During Late-Stage LO

Institutional variation in study endpoints is certainly to be expected, in particular when considering the variety of disease areas that are currently being investigated for potential novel pharmacological solutions. However, the list of endpoints evaluated in the vast majority of repeat-dose toxicology studies has been defined and codified in test guidelines issued by the OECD. A summary of these endpoints is presented in Tables 8.5 and 8.6, and the reader is referred to their website for additional information.

The listings in Tables 8.5 and 8.6 should not be viewed as exclusive. It is important that the LO toxicologist considers the specific biological effects that may be reasonably anticipated to accompany the intended pharmacology of the agents under study. Depending on the pharmacologic class, the inclusion of additional endpoints may be warranted. Accurate measurements of serum biomarkers with short half-lives (e.g., cardiac troponin I) may require changes to the clinical pathology sampling schedule. Additional tissue sections or specialized stains may be needed to resolve questions about specific target organ effects. Electron microscopy on some tissues may be needed to characterize ultrastructural changes. Collectively, the data from these various toxicity endpoints must be woven into a

cohesive account that provides a clear account of the risks that will need to be included in the overall risk-benefit analysis so that a decision to terminate or continue development of the molecule can be made with confidence.

8.2.4 Additional LO Toxicology Activities

There are several additional subdisciplines of toxicology that are commonly included in LO safety assessment. These include genetic toxicology, safety pharmacology, and profiling assays to assess the pharmacologic selectivity of the molecules under study.

8.2.4.1 Genetic Toxicology

Genotoxicity testing is done to assess the potential for a molecule to damage DNA by various mechanisms (ICH S2(R1)). It is well accepted that serious diseases such as cancer have been associated with agents that damage DNA [7]. Not surprisingly, therefore, the identification of a genotoxicity liability with a molecule can cause serious disruption of a drug development program. The sooner that mutagenicity risks can be assessed and discharged, the less costly and painful the disruption will be. Thus, it is prudent that LO toxicology assessments should include screening studies for genetic toxicity.

The three primary genotoxicity endpoints can be defined as follows:

1. Gene mutations (also called point mutations) include any submicroscopic changes in the primary DNA base-pair sequence.
2. Structural chromosomal aberrations (SCAs) are changes to the appearance of chromosomes when viewed microscopically. Compounds that cause SCAs are referred to as clastogens.
3. Numerical chromosome aberrations (NCAs) occur with the gain or loss of one or more entire chromosomes. Molecules that cause NCAs are referred to as aneugens.

Because there is no single assay that is capable of reliably detecting all three primary genotoxicity endpoints, assessments for genetic toxicity rely on a battery of several tests. There is no overarching requirement on which genetic toxicity screens should be included in the test battery, but the LO screens generally include a gene mutation study in bacteria (an "Ames assay") and an in vitro test in mammalian cells for SCAs (either a cytogenetics assay or a micronucleus test) in mammalian cells. Conducting in vivo genetic toxicology studies in LO is relatively unusual and would most likely be done only if previous work with the chemical scaffold and SAR indicated a need.

Many contract testing laboratories offer miniaturized versions of the standard genetic toxicology tests. While the timing of genotoxicity testing is flexible, their

relatively low cost, rapid turnaround times, and low test compound requirements (as little as 100 mg per assay) generally allow these studies to be placed in the workflow with other early-stage LO toxicology activities. Alternatively, these screens can be completed at the beginning of the mid-stage of LO. Regardless, it is recommended that the genotoxicity screens be completed prior to starting any in vivo repeat-dose pilot toxicology studies.

Collectively, the results from the test battery are used to develop a weight of evidence regarding the potential genetic toxicity hazard associated with a molecule. However, when dealing with the multiplicity of compounds that typifies the LO phase of drug development, the LO toxicologist most often recommends that development teams simply discontinue work on test compounds that are positive in any of the genotoxicity screens used. This is particularly true for non-oncology drugs where the tolerance for risks is low. For drugs intended for the treatment of advanced cancer, genotoxicity studies are not considered to be essential for clinical trials (ICH S9). However, some genetic toxicity testing may be desirable to help ensure workplace safety for manufacturing as well as for labeling during shipping. Many institutions will, at a minimum, complete bacterial mutagenicity testing on compounds as they move through LO.

8.2.4.2 Target Selectivity Screening

Small compounds are often evaluated in commercially available selectivity screens that assess the potential of the molecule to interact with a panel of different enzymes and receptors that are not intended targets for the agent under study. The list of enzymes and receptors in the panel may vary with different institutions depending on prior experience and immediate concerns, but even very extensive receptor pharmacology screening panels require only a very small amount of test material (as little as 10–25 mg). Because of their low test material requirements as well as their relatively low cost, target selectivity screening is often included in early-stage LO, but their actual timing is flexible. The results of these selectivity screens are very useful for identifying off-target activity that may be predictive of unintended (deleterious) activity and responses. Their value is underscored by a series of computational analyses on structure-activity relationships of compounds reported by Mestres et al. [8] who proposed that any given small molecule may demonstrate activity up to six targets. This analysis was taken further into a longitudinal assessment of "promiscuity progression" over time by Hu and colleagues [9] who plausibly assumed that small molecules can specifically interact with multiple targets. This clearly has implications on patient safety and the long-term prospects for a successful drug development program continuing all the way to submission of the New Drug Application (NDA).

Due to the negative impact cardiotoxicity has had on drug development, selectivity screens for small molecules almost invariably include specific assays designed to predict negative cardiovascular outcomes. The most common of these assays is aimed at predicting the notorious torsades de pointes (TdP) phenomenon,

an abnormal arrhythmia associated with prolonged QT interval that can lead to sudden death [10]. Several drug products have been withdrawn due to incidence of TdP, the first being terfenadine, which was recalled in February 1998 (http://www.fda.gov/ohrms/dockets/ac/98/briefingbook/1998-3454B1_03_WL50.pdf). A comprehensive of drugs associated with QT effects can be found online at https://crediblemeds.org. Drug-induced QT prolongation has been linked to blockage of the Kv11.1 alpha subunit of the potassium ion channel in cardiac tissue. An in vitro assay in human embryonic kidney HEK293 cells expressing the human ether-à-go-go-related gene (hERG) that codes for the protein associated with this ion channel is commercially available and is now routinely incorporated in drug target selectivity screens specifically to address potential risks for drug-induced arrhythmias [11]. When assessing hERG results, a good rule of thumb is that the hERG IC50 should be at least 30× greater than the projected Cmax for nonprotein-bound drug concentrations in humans.

8.3 Communications with the Development Team

While all pharmaceutical agents are intended to confer clinical benefit to a defined patient population, it is axiomatic that these same agents also carry risks. As drugs move from discovery through early development and into clinical trials, ethical considerations and regulatory requirements mandate the need to conduct nonclinical safety assessment studies prior to any testing in a human population. Consequently, the identification of unwanted side effects, toxicities, and risks must necessarily precede any evaluation of the potential clinical benefits. The LO toxicologist must therefore be keenly aware that the initial view of risks and benefits will be very heavily weighted toward the "risk" side of the ratio. It is important for the LO toxicologist to anticipate this situation and to be prepared to offer the proper background and context for medicinal chemists and other key stakeholders to help maintain a balanced perspective on emerging toxicology data. An overly lurid description of initial toxicology findings may incite unnecessary anxiety among stakeholders and internal customers, and the energies needed to manage these anxieties may become a significant distraction. A balanced—but still accurate—approach when communicating is recommended. A key tool to assist the LO toxicologist in this regard is the information that may exist in internal records or external literature which can help identify toxicities which are known or suspected to accompany the intended pharmacologic action (agonism or antagonism) at the drug target under study. Such information can be leveraged in advance to manage the expectations of other members of the development team as well as stakeholders and to help keep new and emerging toxicology findings in perspective.

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Part IV
Early Clinical Development of Drug Candidates

Chapter 9
Design of Clinical Studies in Early Development

Margaret S. Landis

Abstract There is growing philosophy in the realm of drug discovery that rapid feedback from strategic and tactical early clinical studies is one of the most vital components to optimizing the cycle of successful drug design. The current early development paradigms however do not always support the rapid relay of this vital clincal information to early drug discovery teams. Several industrial, government and academic initiatives are underway to improve the efficiency of the discovery to clinical information feedback loop, thereby increasing the number of drug therapies ultimately commercialized. Success will lie in early, proactive clinical biomarker and diagnostics identification and/or co-development, an early and sustained focus on predictive model development, application of early, adaptive clinical design strategies, and the use of fully integrated information technology (IT) and knowledge management (KM) systems.

Keywords Pharmaceutical industry initiative • Adaptive clinic trial • Translational medicine • Predictive model • Knowledge management system • Biomarker • Diagnostic

9.1 Introduction

The overall success rate for market authorization of drugs entering clinical trials for all indications is circa 10.4% [1]. The contemporary reasons cited for the overall poor success rate include unbalanced regulatory risk-benefit assessments, higher regulatory efficacy hurdles, commercial and financial decisions driving project termination, and the increased complexity and cost of clinical trials [2, 3]. According to the same reports, success in Phase 2 efficacy testing is the lowest of the three phases, at 32.4% ($n = 2268$). This success rate was critically lower than for either Phase 1 (64.5%, $n = 1918$) or Phase 3 (60.1%, $n = 975$). While multiple reasons

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have been cited for overall poor success rates, the most predominant causes of investigational drugs not surviving to marketed status are due to failures in achieving overall efficacy markers or diminished safety to efficacy ratios [4].

The antiquated, unintegrated, inefficient clinical trial processes that have been utilized by the industry for decades contribute to these failures. Clinical trials of old have utilized the distinct phases approach to progress candidates, where key development decisions are made on small patient cohorts, short time frame studies, and highly dichotomized data outcomes. These paradigms are often complicated by slow clinical data analysis and inefficient clinical data transfer and dissemination, where data is distributed to the research teams in a hierarchical fashion, severely limiting the value gained by collective knowledge discussions within the whole of the research teams. Most times, the basic researchers and clinical researchers are far removed in temporal and physical space, as well as working in separate information technology (IT) systems. More recently, the basic researchers and clinicians may even be employed by different companies, thereby furthering the divide and disconnection of the scientists and teams which need to collaborate for success.

There is a strong, emerging philosophy that strengthening the link between drug design teams in early drug research with early clinical investigation teams will help address the alarming clinical attrition rates. There are many opinions regarding detailed solutions for this problem. In fact, a number of key cross pharmaceutical industry initiatives aimed at addressing the potential sources of the "pipeline problem" have evolved mainly within the last two decades and are discussed further below.

9.2 Key Cross Pharmaceutical Industry Initiatives

Several initiatives aimed at facilitating the rapid, bidirectional flow of information from early clinical studies to teams involved in the cycle of drug design are currently in play. These include the US Food and Drug Administration (FDA) Critical Path Initiative, various aspects of the Pharmacological Audit Trail (PhAT), recommendations from the newly developed Biopharmaceutics Risk Assessment Roadmap (BioRAM) process, the National Institutes of Health's Bench-to-Bedside (B2B) funding program, as well as the whole of the translational medicine movement.

9.2.1 US Food and Drug Administration (FDA) Critical Path Initiative

The US Food and Drug Administration Critical Path Initiative (CPI) was launched in March 2004 and outlined a strategy to enable more beneficial medicinal products to be delivered to patient populations. A recently published, detailed report

Innovation or Stagnation: Challenge and Opportunity on the Critical Path to New Medical Products [5] systematically evaluated the disconnect between the vital scientific progress made in advanced basic biology, biomedicine, genomics, biomarker identification, bioinformatics, bioimaging, and material sciences in key disease areas (cancer, Alzheimer's disease, and diabetes) and the lack of effective medical treatments. The report corroborates the numerous contemporary critical analyses that cite the hurdle for demonstrating the medical utility (efficacy) of a new product for beneficial treatment of human disease as a *primary source* of "innumerable failures" in the drug development process. It called for a "collective action" to "modernize scientific and technical tools as well as harness information technology to evaluate and predict the safety, effectiveness, and manufacturability of medical products." The report recognized the need for enhanced information technology (IT) systems to improve data and information flow and recommended the creation of new tools to assess the effectiveness (efficacy) of new products more rapidly and at lower costs. The disconnect between "drug discovery" and "product development" must be remedied using new, advanced predictive biomodeling, improved biomarkers and new, more rapid and efficient clinical evaluation paradigms.

This focus on demonstration of drug efficacy and effectiveness is again highlighted in the second element of the "Three Dimensions of the Critical Path" under the "Demonstrating Medical Utility" heading (Fig. 9.1). Here, the clearly stated objective is boldly and simply defined as "Show that the product benefits people". This sub-initiative heading clearly outlines the vital importance of the preclinical to early clinical connection of drug discovery and development advancement. The report dictates that preclinical activities need to more efficiently "select appropriate design (devices) or candidate (drugs) with high probability of effectiveness" and links this to the corresponding clinical imperative to "show effectiveness in people".

Demonstrating medical utility of a drug asset includes the successful application of in vitro and in silico predictive tools during prototype design/discovery phase and successful employment of translational in vivo and in silico capabilities during preclinical development stages. Ultimately, this should lead to more successful human efficacy evaluations during the clinical development stage. The interconnectivity of the tools utilized during the prototype design/discovery stage must be integrated with the initial clinical planning and subsequent development stages. This approach is expected to provide a paradigm where "better tools are used to identify successful products and eliminate impending failures more efficiently and earlier in the development process". Critical Path research in government, academia, and industry will be able to provide these necessary "publicly available scientific and technical tools — including assays, standards, computer modeling techniques, biomarkers, and clinical trial end points". Future success of this approach may require all researchers to engage in more collaborative, noncompetitive sharing of tools and data [6], which may ultimately prove mutually beneficial in the collective scientific progress against human disease.

Dimension	Definition	Examples of Activities
Assessing Safety	Show that the product is adequately safe for each stage of development	• Preclinical: show that the product is safe enough for early human testing • Eliminate products with safety problems • Early Clinical: show that product is safe enough for commercial distribution
Demonstrating Medical Utility	Show that the product benefits people	• Preclinical: Select appropriate design (devices) or candidate (drugs) with high probability of effectivenessClinical: Show effectiveness in people
Industrialization	Go from lab concept or prototype to a manufacturable product	• Design a high-quality product o Physical design o Characterization o Specification • Develop mass production capacity o Manufacturing scale-up o Quality control

Fig. 9.1 The three dimensions of the FDA Critical Path Initiative (reproduced from Ref. 5)

9.2.2 The Pharmacological Audit Trail (PhAT)

Similar to the Critical Path recommendations that mandate the strong bidirectional flow of data, the Pharmacological Audit Trail (PhAT) [7] provides a template of key questions, milestones, and measures to address along the transition from drug candidate selection to preclinical evaluation and ultimately clinical trials. This framework includes information, tools, and knowledge from drug design and early research to design of key early clinical efficacy and safety markers and the guidance frames critical performance criteria to address along the development path. This framework poses categorical questions that directly address the proof of concept (POC) principle, which can ensure rigorous testing of the mechanistic hypotheses. The ultimate aim of the framework is to select the best drug candidate(s) for further evaluation and be able to provide an adequate "risk of failure" assessment. As compounds progress through the rationally designed framework, the "likelihood of drug attrition" should decrease as the "hierarchy of sequential questions are successfully answered". In planning for success, significant early investment in evaluation tools, such as biomarkers and genomics assays, should be considered early to lay the groundwork for successful evaluation and progression of the drug or therapy.

The early version of the Pharmacological Audit Trail included specific but basic information, including the physical-chemical aspects of the drug candidate, drug pharmacokinetics, pharmacodynamics, biochemical pathway modulation,

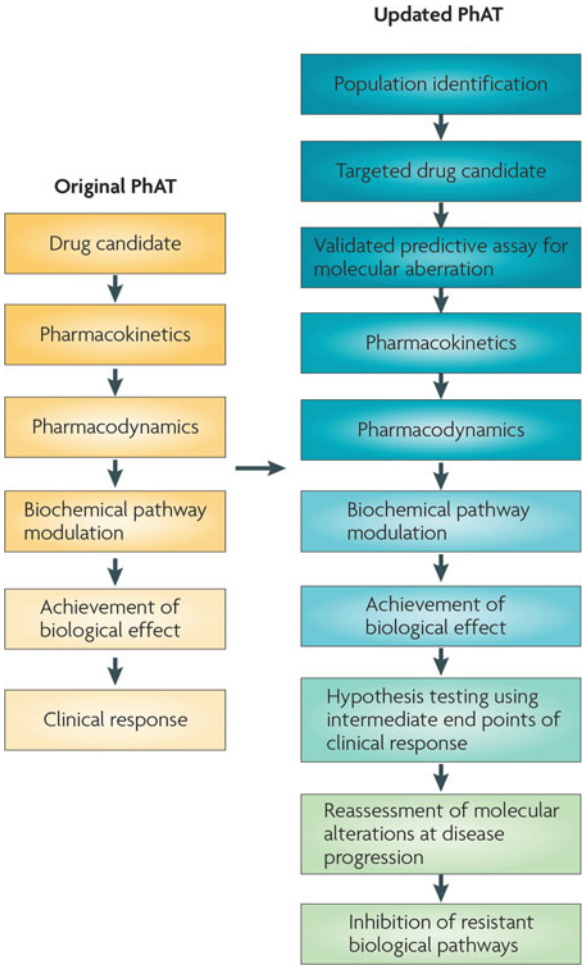

Fig. 9.2 Original and updated version of the Pharmacological Audit Trail (PhAT) (used with permission from Ref. 8)

achievement of biological effect, and associated clinical response [8]. Subsequently, the Pharmacological Audit Trail has been updated and expanded [9] to include additional important translational aspects. Key aspects are patient population identification, aspects of drug targeting, validation of predictive assay for molecular aberration, important and vital understanding of pharmacokinetic-pharmacodynamic (PK-PD) relationships, biochemical pathway modulation, achievement of biological effect, hypothesis testing using intermediate end points of clinical response, reassessment of molecular alterations at disease progression, inhibition of resistant biological pathways to further explore and understand drug attributes, and drug action, thereby ensuring a higher probability of success and reduced attrition of the drug candidate in subsequent clinical evaluations (Fig. 9.2).

9.2.3 National Institutes of Health (NIH) Bench-To-Bedside (B2B) Initiative

Sponsored by the National Institutes of Health (NIH), this multimillion-dollar funding source was created to "speed translation of promising laboratory discoveries into new medical treatments" [10, 11]. The initiative aims to address the gap in important contemporary discoveries in genomics, metabolomics, pharmacogenomics new disease target identification in key, underrepresented disease areas. Similar to the previous initiatives discussed, the ultimate goal continues to be enhancing the link between advances in basic biomedical research to positive clinical outcomes, which ultimately leads to transformative new medicines being available to humankind.

One of the key aspects of the initiative is focused on strengthening the bond between research scientists in laboratories (Bench) with clinical researchers evaluating efficacy in patients (Bedside). The Agency's ability to link *intramural* science efforts (research conducted at an NIH site) with *extramural* science efforts occurring globally at NIH-funded institutions is a vital keystone for the efficient and rapid flow of information. The strong multidisciplinary reach of the NIH is another critical caveat to the success of this approach and allows for a coordinated effort to be applied to the collaborative paradigm. It emphasizes and promotes the ideas that success lies not only in the progression and commercialization of the scientific advancements but must be supported by the corresponding organizational and network infrastructure. These facilitated interactions and common communication platforms enhance the flow of information, enable facile idea exchange, and promote critical aspects of scientific discussion, which vastly improves the potential and possibilities for any new drug candidate or therapy.

In order to funnel funding to the most critical areas of need, current initiatives are funded in the focus areas of AIDS, rare diseases, behavioral and social sciences, minority health and health disparities, women's health, rare diseases drug development, and pharmacogenomics. Award criteria focuses on scientific quality, potential for clinical trials, and potential for offering a new medical treatment or better understanding an important disease process.

9.2.4 Translational Medicine Paradigms

The concept of translational medicine was first introduced circa 2003 as a response to decades of investment in basic biomedical research failing to produce expected medical breakthroughs needed to treat or cure a variety of devastating human diseases, including Alzheimer's disease, diabetes, and cancer. A key review article states that "...translation, describes the transformation of knowledge through successive fields of research from a basic science discovery to public health impact—a complex process that requires both research (e.g., bench-work and

clinical trials) and non-research activities (e.g., implementation)" [12]. This excerpt again highlights that not all hurdles in the Bench-to-Bedside process lie in just the research areas. Support provided with facilitated interactions, organizational support, and information management are proposed to be critical in the successful process of translational interactions.

Several aspects of the current early development paradigms however do not always support the rapid relay of this information and collaboration of the early research teams involved in developing the drug and drug target landscape. In addition to limitations in practicalities of research, information technology, and organizational hurdles, the hurdles associated with building a collaborative translational team are "lack of qualified 'translationalists', career pathways and poor career incentives, the lack of alignment of clinical goals of the researcher, clinician, and developer and the lack of designated research time for research-clinicians" [13]. Program hurdles can also include intense competition for early resources, staff, and funding, a common environment encountered when there are many other research programs in play. Additionally, the bureaucracy associated with the execution of clinical trials is also often cited as a major impediment for effective translation [14].

Several articles [15–17] cite the need for more interdisciplinary collaboration in the critical periods of drug discovery and initial clinical trial planning and execution. Budge et al., in a recent article [13], invoke the use of new types of "collective intelligence" in the forms of open innovation and crowdsourcing measures to identify and facilitate the exchange of translational ideas. Gathering new and potentially beneficial ideas from different fields within biomedical research, science, clinical science, and engineering and tapping into the wisdom of practicing healthcare professionals is expected to deliver the "evidence-based innovation" needed to revitalize the process of translational medicine.

From the review of the pertinent literature, it is proposed that the key temporal phase of establishing these key connections lies in the drug discovery and nascent clinical development stages where basic researchers are in direct connection with early clinical scientists. Here, the teams are relatively smaller, and there is more nimble and decisive decision-making. Generally, the smaller, more focused teams experience less of a bureaucracy burden and are able to drive the planning of studies with scientific learning as the main goal. This early development space and time frame is thus theorized to be *the most dynamic and critical period* of translational power, and the basic science-to-practical clinical translation potential should be maximized through the use of collaborative team intelligence. The framework for the collaborative discussions should fully utilize the tools described in the Pharmacological Audit Trail [8] and the Biopharmaceutics Risk Assessment Roadmap [18].

9.2.5 *The Biopharmaceutics Risk Assessment Roadmap (BioRAM)*

This Roadmap [18] was recently developed as a multidisciplinary, systems-based scoring approach and focuses on the integration of all aspects of bioperformance to drug substance and drug product design. The goal of this framework is to provide guidance to determine the optimal and desired drug delivery rate, drug exposure profile, and pharmacodynamic response of a therapeutic entity. The Roadmap helps to optimize the development of the clinical (and ultimately commercial) drug product using iterative approaches that parallel and are ideally synergistic with the tenets of "Learn and Confirm" clinical trial approaches [19]. The BioRAM advocates the evolution and leveraging of in vitro, in vivo, and in silico modeling and simulation tools as key factors for achieving success. The BioRAM paradigm again highlights the importance of the basic science, but also the knowledge and data management components in a successful drug and clinical development process.

The Roadmap provides an integral platform that begins at the earliest drug discovery stages, when the program, drug, or therapy is initially conceived. It served as the collective knowledge database of the program and drug target that connects the early discovery/development information to clinical development (Fig. 9.3). The continued application of the Roadmap early in clinical study planning, where the

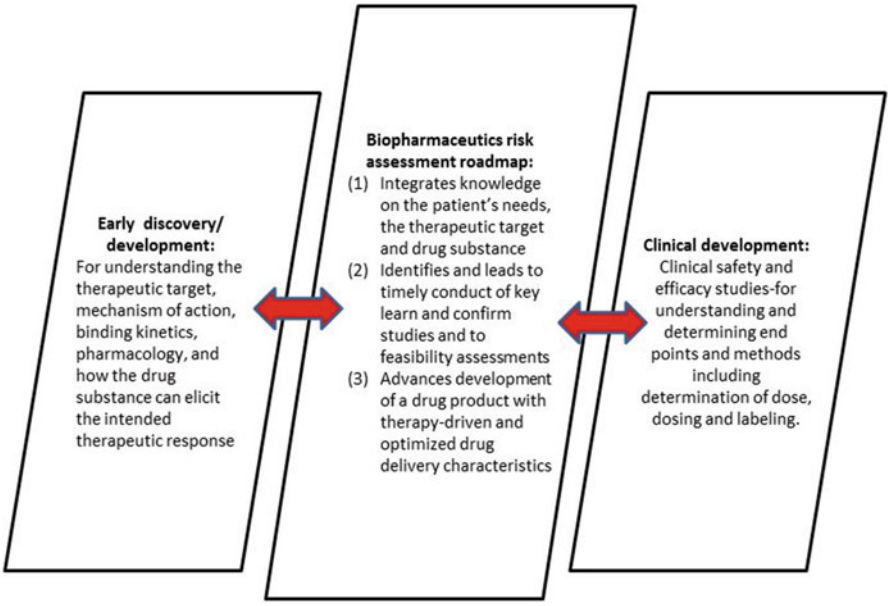

Fig. 9.3 How the BioRAM integrates early discovery/development with clinical development (reproduced from Ref. 18)

desired exposure profile may not be completely understood, will provide the ability to probe the mode of action of a drug substance using a number of different pharmacokinetic-pharmacodynamic (PK-PD) scenarios. This will lead to the identification of optimal delivery profiles that are desired for the most positive therapeutic outcome(s). This process can serve to identify the critical aspects of the drug and formulation as it is related to early efficacy and safety end points.

The current Roadmap details the examination of four basic and relatively common therapy-driven drug delivery scenarios. It provides a transparent rationale for decision-making, assures product quality in all stages of development and commercial production, and develops the robust linkages of drug target, drug substance, and drug product to clinical outcomes. Progressive application of the Roadmap in the later clinical development process can be used to optimize the drug product further, in a quality-by-design (QbD) approach to achieve most or all of the quality target product profile (QTPP)-driven specifications.

Implementation and consistent use of this BioRAM tool in the drug development process is key to delivering more positive clinical and commercial outcomes. It will also serve to build a framework for collective knowledge that can be utilized and shared across an organization and potentially across the industry. Common platforms, transparent frameworks, and rationale decision-making in drug and disease therapy development will enable more transformative medicines to reach the market and ultimately benefit the patient.

9.2.6 Other Related Pharmaceutical Industry Initiatives

The pipeline problem has spurred many initiatives to drive more productive investigation and translation of key discoveries to valuable and desperately needed disease therapies. Additional organizations, funding sources, and initiatives include:

- The National Institutes of Health (NIH) Roadmap, September 2004 [20]
- National Cancer Institute's (NCI) Specialized Programs of Research Excellence (SPOREs) [21]
- MdBio, a private nonprofit corporation that supports the growth of bioscience [22]
- The European Organization for the Treatment of Cancer (EORTC) initiatives [23]
- The National Translational Cancer Research Network (UK) [24]
- The programs and initiatives administered by National Institutes of Health National- Center for Advancing Translational Sciences [25] which include:
  - Bridging Interventional Development Gaps (BrIDGs) program
  - Clinical and Translational Science Awards (CTSA) program
  - Discovering New Therapeutic Uses for Existing Molecules (New Therapeutic Uses) program
  - Extracellular RNA (exRNA) Communication program
  - Genetic and Rare Disease Information Center (GARD)

- NIH/NCATS Global Rare Diseases Patient Registry Data Repository/GRDR® program
- Pfizer's Centers for Therapeutic Innovation (CTI) for NIH Researchers
- Rare Diseases Clinical Research Network (RDCRN)
- RNA interference (RNAi)
- Therapeutics for Rare and Neglected Diseases (TRND) program
- Tissue Chip for Drug Screening (Tissue Chip) initiative
- Toxicology in the Twenty-First Century (Tox21) initiative

9.3 Early Development Themes to Address Better Clinical Outcomes

The broad initiatives discussed above have highlighted the "pipeline problem" currently facing the pharmaceutical and biomedical industry. These initiatives have proposed detailed new process, roadmaps, and scoring systems to facilitate choosing the right drug candidates. A common theme across all these initiatives involves laying out a framework for understanding the mechanistic target and disease. Very often however, the in vitro, in vivo, and in silico tools needed to answer the key questions posed in these roadmaps may not exist or may have to be developed simultaneously as the drug asset is being investigated. This often leads to a scenario where there is intense program competition for limited resources. While the early discovery-development/preclinical space is challenging to manage from this perspective, it does offer the most potential to set the stage for clinical success. There are several key concurrent themes in the initiatives cited above that involve specific activities in the early drug discovery and development interface space which can improve the chances of better pharmacological to pharmaceutical translation and understanding.

9.3.1 Biomarker and Diagnostics Identification and Co-Development

As discussed in many of the initiatives above, access to reliable, validated clinical biomarkers and diagnostics for both safety and efficacy can be invaluable to the success of drug candidates in preclinical and clinical development. More specifically, the CPI opportunities report cites this aspect as one of the *key* solutions to address the Critical Path Initiative (CPI) "pipeline problem" [5].

For early research teams exploring relatively novel targets, the lack of validated biomarkers dictates that interdisciplinary teams must consider the prospect of co-development of biomarkers and diagnostic systems during the drug development process [26]. While the burden of this co-development approach has been discussed previously in terms of resources and project priorities, there is a general tenet that

"the more information known about the biology, the greater the strength of association between an analytical signal and clinical result, the more efficient and less risky the development process will be" [27]. Predictive-type biomarkers are cited as being highly valuable for clinical understanding as well as decision-making and serve to identify patients who will benefit most from the drug therapy. Successful examples, such as the *HER-2/neu* testing in breast carcinoma [28], have demonstrated that proactive or even concurrent investment into the development of the drug *and* biomarkers/diagnostics is the new development paradigm in the industry. It has been reported [29] that there is actually an increase in the FDA submission rate for diagnostics, including these types of predictive biomarker tests. Because diagnostics and devices have a completely different regulatory and approval path than drug therapeutics, they suffer more from a "front-end problem" [29] than traditional drug development processes. The development of these valuable tests and devices must compete within the program for the same resources and funding as the resources for actual drug development, so their importance can, unfortunately, often be downgraded in relation to the drug asset.

A synergistic addition to traditional diagnostic biomarkers in clinical trials may be the incorporation of electronic and mobile health monitoring devices. Incorporation of even simple devices that can confirm basic health related measures can provide feedback of real time patient status, safety and compliance. Ultimately these technologies have the potential to record individual pharmacokinetic or pharmacodynamic patient information to assess effects of target modulation. Incorporation of electronic monitoring devices in trials is becoming the new paradigm in clinical trial execution [30–33]. These nonsubjective, noninvasive observations and measurements rapidly gather and analyze data to detect early signs of efficacy, safety end points, or adverse events. Furthermore, they are generally considered more reliable than qualitative, human clinical assessments [34]. Real-time monitoring of various clinical signals can be funneled to research teams to inform and adapt early in vitro and in vivo and in silico models. These types of electronic monitoring data may ultimately uncover novel diagnostic and biomarker criteria. It is expected that a holistic systems-based knowledge platform will be needed to process and incorporate critical information for successful drug development in the current environment. Additionally, the availability of real-time patient data could greatly enable and accelerate the adaptive clinical trial paradigms that have been additionally proposed as a partial solution to the pipeline problem.

The emergence of the practice of conducting small, key, informative clinical methodology studies concomitantly to clinical drug evaluation often brings the basic research teams in more direct contact and discussion with the clinical development teams. With the objective of better understanding the biological target and pharmacological interaction of the drug candidate in the human body, detailed discussions of how to assess the key drug and drug target attributes can help facilitate the flow of information between biological researchers and clinical investigators. These exploratory-type clinical studies can address the clinical target "unknowns" and provide important guidance on how to conduct effective clinical trials on the actual drug assets, thereby, either improving their chances for

success or providing key, discriminating decision points for deprioritization of unacceptably risky drug assets. Such methodological clinical investigations can be evaluated in a Phase 0 exploratory approach and may include probe compound evaluations [35], investigational, noncommercial formulations of the drug asset [36], and/or screening of additional closely related potential drug candidates [37].

9.3.2 Early Focus on Predictive Model Development as a Key Success Factor

One of the key success factors highlighted in the translational initiatives mentioned previously was the focused integration of all aspects of the drug and drug target landscape, including the incorporation and co-development of correlations and models either prior to or during the drug development process. Traditionally, the establishment of structure-activity relationships (SAR), property correlations, and safety correlations represents the basis for some of the earliest and most basic predictive models. This process begins very early in the drug discovery process. For example, correlations of LogP to plasma protein binding [38] and correlations of LogD and aromaticity values to solubility [39] serve as the basis for predictions of expected physical-chemical properties for structurally related compounds within a given molecular series. These early correlations and trends often guide the decisions on potential molecules to further pursue in the course of lead drug candidate identification and selection in the early discovery phase.

Enhanced efforts are required in order to utilize all early experimental data and begin to build large correlation models and structure-property relationships. These models can ultimately be incorporated into full "systems" models [40], including building comprehensive, holistic platforms for the understanding of the drug target, the design of relevant chemical matter and dosage forms, understanding the intricacies of drug efficacy, and safety modeling [41]. As experimental data is acquired around the drug candidates and drug target, these models should be capable of "learning" such that all subsequent predictions are improved as more data is added. All early experimental data should be captured and fed into learning models to build the initial knowledge base around the drug and drug target [42]. As suggested by the BioRAM initiative discussed earlier in this chapter, the types of models that can be considered among the whole systems composite include but are not limited to biological modeling based on structural biology, pharmacological modeling including pharmacokinetic-pharmacodynamic relationships, and statistical modeling of most preclinical and clinical studies. Efforts made to support this model development in the early space may be a key success factor in the program progression and iterative design capabilities available for the development program.

Methodology studies may be used to bolster and test hypotheses related to the safety and efficacy of drug and drug targets. The results from these studies, if included in the systems model, will further develop the predictive modeling power

of the program. Therefore, investigational clinical methodology studies, potentially in the form of microdosing studies [43], may actually employ probe compounds as a method to obtain rapid clinical information on new targets and mechanisms without necessarily incurring a delay to optimize the compounds for oral administration [37]. Methodology studies may involve the use of several potential drug candidates [44]. These compounds will contain attributes that allow investigation of the methodological hypotheses and provide knowledge and learning to the larger knowledge base of the drug target landscape. This knowledge base can then be used adaptively by the research and clinical teams to address the key mechanistic questions posed in the Biopharmaceutics Roadmap and Pharmacological Audit Trail hierarchy.

Greater reliance on information gained from evaluating probe compounds [35] as well as multiple probe or candidate compounds will become more of the standard clinical evaluation paradigm to address efficacy readouts and safety end points. This approach represents a more realistic drug development situation where customized plans for different molecules to answer specific efficacy or safety questions will result in a greater likelihood of clinical success.

9.3.3 Application of Early Adaptive Clinical Design Strategies

Many of the industry-wide initiatives cite the employment of adaptive design strategies as a key solution to addressing the "pipeline problem" for translating new biomedical discoveries into human medicine and disease treatment. The process of adaptive design involves learning, selecting, and confirming aspects of drug research and must proceed rapidly, efficiently, and seamlessly in the design process.

Adaptive clinical trial design is an initiative highly endorsed by both the Food and Drug Administration [45] and the European Medicines Agency (EMA) and is intended to give the clinical trial investigator many options to explore the safety and efficacy aspects of the drug therapy. The integral component of the framework is flexibility, with the adaptive designs allowing for preplanned modifications and changes to the trial based on various emerging data, biomarker data, and other end points. Modifications occur based on unblinded data and can include halting a trial early due to safety, futility, or efficacy at interim analysis without the need to file time-consuming amendments. There are several adaptive clinical trial paradigms available including a randomization design, a group sequential design, a sample size re-estimation design, a drop-the-loser design, an adaptive dose-finding design, a biomarker-adaptive design, an adaptive treatment-switching design, a hypothesis-adaptive design, an adaptive seamless Phase 2/3 trial design, and a multiple adaptive design [46]. The advantages of this process are the more efficient use of clinical trial participants, a quicker path to success or failure, and the saving of

valuable time and resources. Cited disadvantages of this approach include the need for complicated and complex adaptive clinical trial design protocols, which can be costly and time-consuming to prepare.

One of the tools that can be efficiently utilized in the adaptive clinical design process is microdosing. As discussed previously, microdosing trials [47] are typically utilized for obtaining human pharmacokinetic information on investigational new drugs quickly, so that key inputs may be communicated back to both the drug and clinical design teams. Historically, these studies provided little or no information on drug safety and efficacy; however, more recently, the application of microdosing schemes to positron emission tomography (PET) studies [48] has enabled the determination of both plasma and tissue drug concentrations. More enhanced information on tissue concentrations and human plasma/tissue distribution profiles are direct corollaries to efficacy and can be important parameters for subsequent pharmacokinetic-pharmacodynamic modeling and prediction. The results from these pseudo-efficacy studies can be used to adapt the next stages of the clinical trials or reiterate a clinical evaluation with additional drug candidates. More advanced applications of microdosing for non-radiolabeled drug systems have even been expanded to effectively increase the understanding of pharmacokinetic fates and biodistribution of drug metabolites, as well as simultaneously characterize several lead drug candidates and/or investigational tool probe compounds via highly efficient "cassette dosing" schemes [49, 50].

Microdosing should be considered as an important tool in the early drug development space as a method of rapidly relaying important information on drug parameters to both research and early clinical teams. This includes employing traditional microdosing studies into early adaptive clinical design plans for a drug asset or assets, where key information on the human pharmacokinetic fate of investigational drug assets is sought. Additionally the use of PET microdosing studies is to provide important early biomarker information on temporal tissue distribution to inform efficacy parameters.

As can be gleaned by the discussions of flexible adaptive clinical dosing and microdosing with various drug candidates, probes, and tracers, the cornerstone of a successful adaptive clinical design program must include flexible formulation design and drug delivery. Early formulation scientists will be required to support a variety of diverse formulations necessary to address all the aspects of adaptive clinical designs. For example, designs that include microdosing evaluations may need specific low-dose oral, intravenous, or combination dosage forms of one or several investigational drug assets, typically known as cassette doses. Due to the flexibility aspects, dosage form platforms need to accommodate wide dose ranges, and enabled dosage forms may be needed for adequate dose delivery. Extemporaneous preparation is one means to ensure wide-range dose flexibility [51] and can be utilized to prepare modified release dosage forms [52]. It is important to fully consider all dosage forms that can support exploring complex PK/PD and PK/safety relationships. This includes short- and long-duration modified release, combinations of immediate and controlled release dosage forms, as well as combinations of oral and subcutaneous or intravenous administration [53]. Eventually, rapid

small-scale, robust GMP manufacture using small-scale equipment or pod-based manufacturing [54] may be implemented to explore a variety of dosage forms for continually larger clinical populations.

Adaptive clinical trial formulation needs are typically therapeutic area specific. For example, the general clinical paradigm for oncology utilizes oral solid dosage formulations in patients for Phase 1 clinical trials, but may also include traditional intravenous infusion (chemotherapy)-type dosage form. Some adaptive oncology clinical trials are now designed to combine novel drug therapies with marketed standard of care cancer treatments [55] or may utilize molecular imaging biomarkers that may require significant, additional formulation and drug delivery efforts [56].

Alternatively, early clinical assessments for central nervous system (CNS) therapeutics may require the concurrent intravenous delivery of drug asset-related PET tracers and probe compounds distinct from the drug asset [57]. Clinical trials in metabolic diseases may require the delivery of various dosage forms (e.g., controlled release) to provide varied exposure profiles to assess key safety studies. In general, there is a strong correlation between early flexible formulation and the more popular trends of personalized medicine. In these cases better biomarkers and genetic screening may dictate dosage levels and safety margins. Successfully achieving efficacy and safety end points may lie in the ability to probe and learn about the drug assets and targets through flexible formulation paradigms available in the early clinical development space.

While the concept of adaptive design is most often focused on impacting clinical design and speedier patient outcomes, there are other aspects of adaptive design in the drug discovery and development processes. Most recently, adaptive design has also been invoked as a method in the design of active drug substances. In the cited example [58], a desired poly-pharmacological profile was achieved using an automated, adaptive design approach. Using this process, analogues were generated and prioritized against a set of objectives and demonstrated an impressive 75% success rate for in vivo target engagement. Overall, the adaptive design process is considered the new hallmark of drug discovery and development for new therapeutic innovations. Supported by the Critical Path Initiative recommendations, the derived benefits are expected to be shortened drug development timelines, more efficient use of resources and ultimately the faster, more efficient delivery of transformational medicines to patients.

9.3.4 Use of Fully Integrated Information Technology (IT) and Knowledge Management (KM) Systems as a Key Success Factor

There is a tremendous amount of data and information generated in the drug discovery and clinical evaluation process, and an integrated, robust knowledge management system is vital for the early collaborative research process. Starting from very early in the discovery stage, knowledge bases are needed to capture,

organize, and make available all the relevant data information gathered on the drug targets and drug assets as they become available.

The systems must be capable of rapid data acquisition and processing, as efficient and accessible knowledge management systems are essential for cross-functional team collaborations. These systems will also be useful in the proposed cross industry sharing of target, probe, population, and placebo data in nonproprietary fashion [35, 59]. As stated previously, establishing these integrated systems and platforms in the early development interface will help ensure that predictive models are accessing the most current data in real time. Additionally, if these models are established early, they can "learn" with each new informational input and become more powerful and predictive for specific drug programs [19, 42]. An integrated information platform facilitates the collaborative nature and holistic systems aspects that will facilitate clinical and translational success.

9.4 Summary

In summary, most solutions to the *pipeline problem* can be correlated to key actions that begin in the early development space, strengthening the interactions and links between basic laboratory researchers and clinical practitioners. These collaborative interactions must involve a bidirectional communication and coordination of key drug asset development studies, incorporating insights from a variety of interdisciplinary internal and external participants. Building comprehensive learning models may involve nontraditional early development activities including the early clinical evaluation of several key lead molecules (i.e., cassette-type microdosing) and the co-development of key, biomarker, and diagnostic systems for newly emerging disease targets. The increasing use of pilot methodology studies to more fully understand drug action and drug target engagement is expected to continue and facilitate deeper clinical understanding. More rapid progress of drugs to clinical trials and ultimately to the market is expected to occur from key early development activities. These include early biomarker and diagnostics identification and co-development, early predictive model development and integration, application of early adaptive design strategies utilizing flexible formulation approaches, and integration of information technology and knowledge management systems for efficient collection and sharing of vital drug asset and drug target information and learning.

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Chapter 10
Design of Clinical Formulations in Early Development

Catherine M. Ambler, Avinash G. Thombre, Madhushree Gokhale, and John S. Morrison

Abstract Thoughtfully designed early clinical formulations not only meet the needs of the study at hand and inform the development of the commercial product, but can influence the direction of the clinical program as well as provide further guidance to potential backups still in exploratory stages. This chapter focuses on the various types of early clinical formulations, why they are developed, and how the preclinical formulation space helps to guide initial clinical formulation selection. Impacts on clinical program development will be presented through case studies and examples in context of the types of clinical studies being supported: what is the goal of the clinical study, what questions need to be answered, and how will the information be used in subsequent development? Additional factors influencing formulation selection such as patient needs, the practicality of switching between dosage forms, and commercialization potential will be reviewed.

Keywords Extemporaneous preparation • Modified release (MR) • Solubilization • Clinical formulation • First in human (FIH) • Probability of technical success (PTS) • Accelerated development • Case studies

10.1 Introduction to Early Clinical Studies

The primary goals of a first-in-human (FIH) clinical study are to assess safety and tolerability of a new chemical entity. In general, FIH studies are not intended to evaluate therapeutic effect; however, the incorporation of biomarker monitoring

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where possible is not uncommon. First-in-human programs are generally conducted as small, in-house studies, where the subjects can be carefully monitored by trained medical professionals. The subjects are typically healthy volunteers at this stage, which allows safety to be assessed independent of other preexisting medical complications and in the absence of concomitant medication. However, in certain therapeutic areas such as oncology, where life-threatening disease, narrow therapeutic windows, and more severe side effect profiles may be expected due to the mechanism of disease treatment, FIH studies are typically conducted with patient populations. Placebo control groups are also utilized in early clinical trials, with matching formulations developed to maintain "blinding" during the study.

Though a number of study designs may exist to accomplish the goals of these initial Phase I clinical trials, they typically include some form of stepwise single- and multiple-dose escalation in order to establish initial human PK data, evaluate any toxicity/adverse events associated with the test compound, and establish a maximum tolerated dose. Factors to consider when selecting an appropriate design include known and/or predicted information on the behavior of the compound of interest, such as pharmacokinetic parameters, dose range to be evaluated, and safety data available, as well as related aspects such as anticipated dropout rates; statistical considerations in data interpretation; incorporation of additional study arms for evaluation of parameters such as food effect, dose frequency, or alternative formulation approaches; and the flexibility to adjust the clinical plan to real-time data as needed.

A high-level overview of the main study design strategies for early clinical trials is presented in "First in Human Studies: Points to Consider in Study Placement, Design and Conduct" [1]. In general, early clinical strategies likely fit into a parallel- or a crossover-type design. A parallel study design is a standard approach in FIH, in which dose escalation occurs stepwise over several cohorts of subjects with each cohort receiving one dose of the investigational compound. This design is illustrated in Fig. 10.1. Here, each cohort may consist of 8–12 subjects, two of which are randomized to placebo. In a single ascending dose (SAD) escalation, a single dose of one dose strength (i.e., Dose 1) is administered to Cohort 1, and the subjects are monitored for the determined period before completing their involvement with the study. The next cohort of individuals would then repeat the process

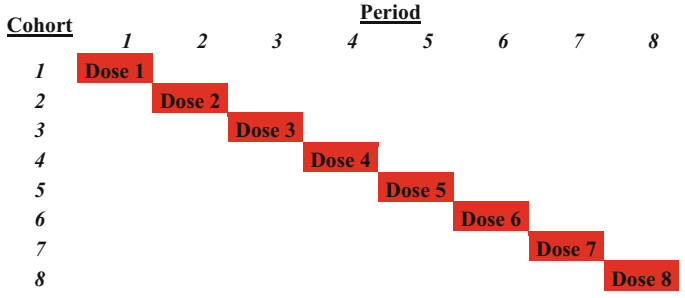

Fig. 10.1 Parallel study design for first-in-human single ascending dose escalation protocol

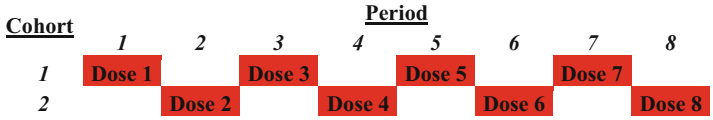

Fig. 10.2 Crossover study design for first-in-human single ascending dose escalation protocol

with the next sequential dose strength and so forth until reaching a predetermined endpoint in dose escalation or other stopping criteria.

Alternatively, a crossover study design as illustrated in Fig. 10.2 allows for study subjects to receive additional single doses of the test compound during the dose escalation process. This crossover design can be beneficial in allowing for direct comparison of differing dose levels, formulations, or administration frequencies within single subjects (i.e., the subject is their own control group), but may not be appropriate in instances, for example, where PK profiles require extended washout periods between dosing intervals. In this example, similar numbers of subjects may be enrolled in each cohort as compared to the parallel design, and both active and placebo doses are incorporated into the randomization strategy. However, as each cohort receives more than one dose level during the escalation process, fewer total subjects may be required.

In the case of either design, a similar strategy could also be applied to a Phase I multiple ascending dose (MAD) study, though each dosing period would be longer (e.g., 14 days of dosing at the given dose strength instead of 1 day as for SAD).

Other design types, or modifications of those above, are also available to meet the needs of a given study. Key to each Phase I study, however, is the ability to slowly increase the amount of compound administered to the subject while monitoring for safety and obtaining key information about the human pharmacokinetic profile of the investigational material.

Initial starting dose levels are selected based upon careful review and understanding of preclinical safety, pharmacokinetic, and pharmacodynamic data. In particular, the no observed adverse effect level (NOAEL) from the most relevant species in preclinical toxicology studies is weighed heavily in the dose selection process [2]. In cases where there is a precedence for the mechanism of action and known pharmacology, historical review of data may also be used to adjust the starting dose accordingly.

Prior to clinical evaluations, a prediction of the human pharmacokinetic (PK) profile is made based upon both in vitro assays and scaling of data obtained from in vivo testing in preclinical species. Though these predictions provide the starting assumptions of how the drug candidate will perform in humans and allow for estimations of dose requirements, there are limitations to these methods. Early human PK data is therefore highly valuable in refining these dose estimations, and adjustments to dosing strategies based upon earlier predictive tools may need to be made.

It is important to note that this increased understanding of human PK not only impacts the progression of the current clinical candidate, but potential backup

strategies as well as related therapeutic targets. Information can be fed back into early discovery to design around newly discovered deficits or improve predictive models to assist in more effective compound selection. For example, a longer than expected human half-life of a clinical compound that was projected to be cleared very quickly could lead to consideration of additional molecular substrate that was previously considered less than ideal from this perspective, allowing for efforts to focus on improvements of other aspects of the backup API profile. Alternatively, plasma exposures that are significantly different than expected for the clinical candidate could lead to increased understanding of transport mechanisms that are underrepresented in preclinical species, potentially leading to greater understanding of their role and better predictive tools or screening methods for subsequent chemical substrate.

10.2 First-In-Human (FIH) Phase I Clinical Formulation Design

There are many types of formulations available that will enable the Phase I clinical program. Most approaches can be divided into one of the two categories: speed to Phase I [3] or early positioning for final formulation [4]. Proponents of the first approach cite the high attrition rate observed in clinical trial progression, with statistics suggesting only around 13% of small-molecule clinical candidates progress from Phase I to NDA approval (based upon an evaluation of industry-progressed compounds in the 1993–2004 time frame) [5]. These approaches generally favor less resource burn in early clinical development. The second strategy aims at greater initial investment in a more robust formulation strategy, with more rapid progression to the commercial space for those candidates that survive. In either case, however, consideration of both the study design and goals, as well as the physical and chemical properties of the candidate of interest, must be considered in selecting the appropriate formulation design. For example, will the formulation need to support extremely low or high dose levels? What does the stability profile look like? Where is the study to be conducted, and what is the anticipated duration of dosing? Does the compound display high solubility, or are there limitations that require an enabling technology to deliver the dose range required? In addressing some of these questions, information obtained from dosing formulations used in the preclinical space can significantly inform the selection of clinical formulation options. Preclinical efficacy models may offer information on effectiveness of solution dosing or handling concerns with high viscosity suspensions, for example. Enabling formulations may have been required for adequate preclinical exposures, which may be pursued clinically for similar reasons. Likewise, challenges faced with high doses in toxicology studies may translate to similar challenges in high dose escalations in the clinic. Was a dose proportional increase in exposure achieved or did exposure plateau during escalation, and if so, why?

Many dosage form options exist for progressing early clinical studies. Formulation options which can cover broad ranges of dose strengths and allow for greater flexibility in dose adjustment offer significant benefit to early stages of clinical development. As mentioned above, preclinical predictions do not always translate to human PK performance, and the ability to quickly adjust to changing needs or explore additional dosing strategies (such as dose frequency/divided dosing, dose timing, with or without food, etc.) as the need arises is extremely helpful in this early space.

10.2.1 Simplified Manufactured Dosage Forms

Perhaps the most simplified formulation approach is an aqueous solution or suspension of the clinical candidate. Commonly referred to as a powder-in-bottle (PIB) or drug-in-bottle (DIB) formulation, the active pharmaceutical ingredient (API) is contained in a drinking vessel ready for constitution at the time of dosing. Little development work is required for this type of approach, aspects such as stability programs are streamlined due to the absence of additional excipient components in the formulation, and multiple contract organizations offer services for the rapid manufacture of the supplies. A similar approach can be taken by filling unformulated API powder into capsules (PIC) or drug in capsule (DIC), which can add convenience in dose preparation and administration. These formulations are most suited to compounds with favorable physical/chemical properties, therefore not requiring enabling formulation to achieve desired exposures.

10.2.2 Traditional Manufactured Solid Dosage Forms

Traditional formulated solid oral dosage forms such as immediate release tablets or capsules may fit the flexibility requirement described above through the manufacture of several dose-strength options. Administering multiple-dose units from a few strength options during a given dosing interval can cover a range of potential dose needs. For example, in a study described by Ku [4], three dose strengths (1, 10, and 100 mg) of a capsule formulation were manufactured to support a single or multiple ascending dose (SAD or MAD) study requiring a range of 1–800 mg doses. Tablet combinations for the single ascending dose study are shown in the table below (summarized from original text [4]) (Table 10.1).

This strategy generally requires longer initial lead times (to account for greater development, analytical characterization, stability data, manufacturing, and packaging needs) and estimation of dosing ranges earlier than other strategies. However, it can also position the program for more rapid progression into future clinical studies as solid dosage forms more easily support larger or longer-duration study needs, as well as those requiring out-patient use. These same formulations may be

Table 10.1 Dosing table illustrating tablet requirements for a single ascending dose study

	1 mg	10 mg	30 mg	100 mg fasted	100 mg fed	200 mg	400 mg	800 mg
1mg tablet	1							
10mg tablet		1	3					
100mg tablet				1	1	2	4	8

readily progressed through to Phase II studies and beyond, potentially without further development required. Additionally, time savings can be realized by the elimination of bioavailability studies required to switch between formulation types later in development [4].

10.2.3 Extemporaneously Prepared Dosage Forms

Extemporaneous preparation (EP) is a process wherein a formulation consisting of a drug or combination of drugs and/or excipients is prepared under the supervision of a licensed pharmacist. This creates a customized medication dosage form in accordance with the clinical protocol. Extemporaneous preparation provides an additional option for formulation support in early clinical trials. It removes the manufacturing and packaging components of a traditional supply chain process, as well as the extensive stability requirements associated with most pre-manufactured dosage forms. Increased speed, decreased cost, and lower bulk supply requirements are some of the advantages, as is a high degree of flexibility to meet changing dose requirements. Doses are prepared "just in time" to meet the specific dose strength required for an individual subject in a given escalation period or cohort of a study.

An EP-osmotic capsule is the extemporaneously prepared version of the traditional osmotic capsules [6], developed to enable rapid testing of an modified release (MR) dosage form in a Phase 1 setting. It can also be used to evaluate whether the drug present in an MR formulation requires solubilization. Since the drug candidate is released from the EP-osmotic capsule further down the GI tract, it can be used to evaluate colonic absorption potential. The capsule body and cap are manufactured under GMP conditions and stored as inventory so that they are readily available when needed. The drug layer is compounded and the capsule assembled at the clinical site (hence, called EP-osmotic capsules). At Pfizer, the capsules are relatively large (equivalent to a size #00 gelatin capsule) because they are designed to deliver any dose in the 1–200 mg range. Since the permeability of the capsule shell determines the rate of drug release and the capsule shell is premade, there is very

limited flexibility in the drug release rate. The Pfizer capsules provide either a short (80% released in about 6 h) or a long (80% released in about 14 h) duration.

For early clinical candidates, the EP process is also appropriate when an early comparison of immediate vs. modified release (IR vs. MR) delivery profiles is desired or when an MR formulation is most appropriate for the compound, but for business reasons, a traditional MR development is not endorsed. The EP process has been applied to MR formulations such as matrix tablets and osmotic capsules [7] to address this need as discussed in the case studies section.

The EP process can be applied to an extensive variety of formulations, encompassing simple solution or suspension options, enabled formulations, hand-filled capsules, and even tablet formulations. A few examples are provided in the case studies section.

10.3 Understanding Modified Release Formulations in Early Clinical Studies

10.3.1 General Considerations

Data from the FIH or other early clinical studies in healthy volunteers may highlight the need for an oral modified release (MR) formulation to modulate the pharmacokinetic profile of a drug candidate and enable its continued clinical progression. The translation of the medical need to the design attributes of the most appropriate MR dosage form needs to be considered carefully against the potential limitations of the MR dosage form. Ideally, in order to effectively develop an MR formulation, it is important to have an understanding (or at least a hypothesis) of the mechanism of action for the drug candidates and the PK–PD relationship, in order to assess the impact of the release rate on the exposure-response framework. Some broad guidelines are provided below, but a careful case-by-case analysis is required based on the particular PK characteristics and the pharmacology of the drug under consideration.

An extended release (ER) dosage form with relatively long delivery duration (12–16 h) has the potential to reduce dosing frequency and increase patient convenience/compliance. The formulation type can also increase the duration of effect by maintaining a certain Cmin while decreasing systemic side effects by lowering the Cmax/Cmin ratio. Alternatively, if the objective is a reduction in Cmax-related side effects, an ER dosage form providing a relatively short delivery duration (6–8 h) with or without a time lag may suffice.

A delayed release (DR) dosage form, such as enteric-coated tablets or multiparticulates, with an approximately 2 h time lag followed by relatively short delivery duration (2–4 h), has the potential to decrease local GI side effects by reducing the gastric and duodenal exposure to the drug candidate. Acid-labile molecules can also benefit from a DR dosage form. A longer time lag would be

required for a locally acting drug candidate with a site of action in the lower GI tract (i.e., the colon).

Depending on the pharmacokinetics and pharmacodynamics of the particular drug under consideration, complex MR delivery profiles may need to be considered. For instance, an IR + ER combination may be needed to rapidly achieve steady state. Alternatively, if only a brief ligand-receptor interaction is required several times a day or if tolerance produced by continuous exposure is a concern, an IR + DR combination or pulsatile delivery profile may be sufficient.

Two examples of commercially marketed MR dosage forms with complex drug release profiles are Ambien CR® (Sanofi-Aventis) indicated for the treatment of insomnia [8] and Concerta® (Janssen) indicated for the treatment of attention deficit hyperactivity disorder (ADHD) [9]. For Ambien CR®, the product profile goals were to treat patients having trouble with sleep onset or sleep maintenance while reducing the residual drug effects on cognitive or psychomotor functioning after awakening in the morning. This translated to an MR product with a rapid initial release to produce sleep onset, extended release beyond 3 h to support sleep maintenance, and no release after approximately 4 or 5 h to minimize residual effects. For Concerta®, the product profile goals were to reduce the dosing frequency from two or three times per day (BID or TID) regimen to improve compliance, as the target patient population was children requiring dosing during school hours. This translated into a drug release pattern that consisted of quick initial release for rapid onset of efficacy, increasing release during the day to maintain efficacy without developing tolerance, and gradual tapering in the late afternoon to early evening.

The following considerations are important in the decision to pursue an MR dosage form for a drug candidate. Additional considerations may be necessary on a case-by-case basis:

- Is the onset of action important? Due to slower absorption, the Tmax with an ER dosage form is generally longer.
- Is the drug poorly absorbed in the lower GI tract? For drugs with poor colonic absorption, the bioavailability of an ER formulation could be lower than the IR formulation (for the same dose).
- Does the drug have a high first-pass metabolism? The bioavailability of the drug candidate could be significantly reduced due to a slower release rate.
- Does the drug have significant P-gp efflux or gut wall metabolism? These factors can also negatively impact the bioavailability of the MR formulation relative to an IR formulation.
- For MR formulations containing more than double the IR dose, is the formulation technology robust? Dose dumping is a concern for drug candidates with a narrow therapeutic index.
- Are the adverse events related to Cmax or local GI irritation? In these cases an MR formulation is a potentially resolution. However, for drug candidates with adverse events (AEs) due to toxicity associated with a certain threshold plasma concentration, an MR formulation may not resolve the issue.

10.3.2 Ideal Drug Candidate for Modified Release Formulations

The physicochemical, biopharmaceutical, and pharmacokinetic properties that make for an ideal candidate for MR are highlighted in Table 10.2 with the desirable range of the attributes highlighted.

Additional data that can help assess the feasibility of an MR dosage form include (1) solubility, pKa, and pH-solubility profile; (2) stability including pH stability and potential for oxidative degradation (since many MR dosage form excipients can undergo oxidative degradation and initiate drug candidate degradation); and (3) permeability, including Papp (e.g., in RRCK cells) and P-gp/efflux liability (e.g., Papp B → A/Papp A → B in MDR1-transfected MDCK cells).

This information can be used at the lead development and candidate selection (LD/CS) stage to establish a suitable candidate for development if it is known that MR will be needed. This information is also useful in selecting backup molecules for a drug candidate already in early clinical development.

10.3.3 Strategic Considerations in Modified Release Deployment

Frequently, project teams must decide the appropriate stage of compound development for MR technology deployment. This is a strategic question, and the answer will depend on the particular candidate under consideration as well as the particular organization making that decision. Figure 10.3 presents one general framework for analyzing the situation depending on the stage of development, which may influence the decision to deploy MR.

A framework to analyze whether or not to deploy MR technology is presented in Fig. 10.4. "Confidence in MR" refers to how likely is that an MR formulation will meet the medical/clinical need for which it is being considered (i.e., how strong is the rationale for an MR dosage form). This is often dependent on how well the drug target, pharmacology, and PK-PD relationship are understood or precedented.

The term "probability of technical success" (PTS) refers to the availability of appropriate MR technology. If the physicochemical, biopharmaceutical, and PK properties of the drug candidate are ideal and well developed, mature platform technologies, such as hydrophilic matrix tablet, osmotic technology, and multiparticulates, can be deployed to achieve the desired release profile, PTS is considered to be high. At the other extreme, if new technologies need to be developed to achieve the target profile, the PTS is considered to be low.

If there is low confidence in MR, investments should be made to increase the confidence, and if the PTS is low, investments should be considered to increase PTS.

In Tables 10.3 and 10.4 several real-world examples are presented in which the framework described above was applied to make decisions regarding pursuit of the development of MR formulations with proprietary Pfizer drug candidates.

Table 10.2 Physicochemical, biopharmaceutical, and pharmacokinetic properties of an ideal candidate for MR (Adapted slightly from Ref. 10)

	Factor	Criteria	Comments
Physicochemical factors	Dose	< 1 mg	Greater development complexity (potential drug content uniformity issue)
		> > 250–300 mg	May need more than one tablet to accommodate the drug load
		10–250 mg	Average degree of difficulty
	Dose/solubility ratio (highest dose/lowest solubility in the pH range 1–7.5 for bases and 5–7.5 for acids)	< 1 ml	Several technology options exist for CR development
		1–100 ml	Average degree of difficulty
		100–1000 ml	MR development will be challenging but feasible
		>1000 ml	Requires solubilization and MR development will be difficult
		>10,000 ml	MR development practically impossible
	Stability	Generally stable as a solid/solution and with common MR excipients	Predict average degree of difficulty
		Compound shows or is predicted to have significant degradation	Predict higher degree of difficulty
Biopharmaceutical factors	Absorption mechanism	Transcellular passive diffusion	Average degree of difficulty
		Other mechanisms including efflux	Performance may be difficult to predict
	Regional permeability (colonic absorption)	Poor absorption, Papp, RRCK $<10^{-6}$ cm/s	MR formulations with prolonged delivery duration may not be feasible. Typically will not be bioequivalent to IR
		Moderate absorption, Papp, RRCK 10^{-6}–10^{-5} cm/s	MR development challenging but feasible. May or may not be bioequivalent to IR
		Good absorption, Papp, RRCK $>10^{-5}$ cm/s	MR development should be feasible. Likely to be bioequivalent to IR
PK factors	Half-life	<1–2 h	Half-life too short for MR development
		2–10 h	Acceptable half-life
		> > 10 h	Compound may not need MR for reducing dosing frequency
	Metabolism and efflux	High presystemic or first-pass metabolism	Relative BA of MR formulation may be low
		Compound is P-gp or CYP3A4 substrate	MR performance difficult to predict (depends on dose and Km, Vmax)

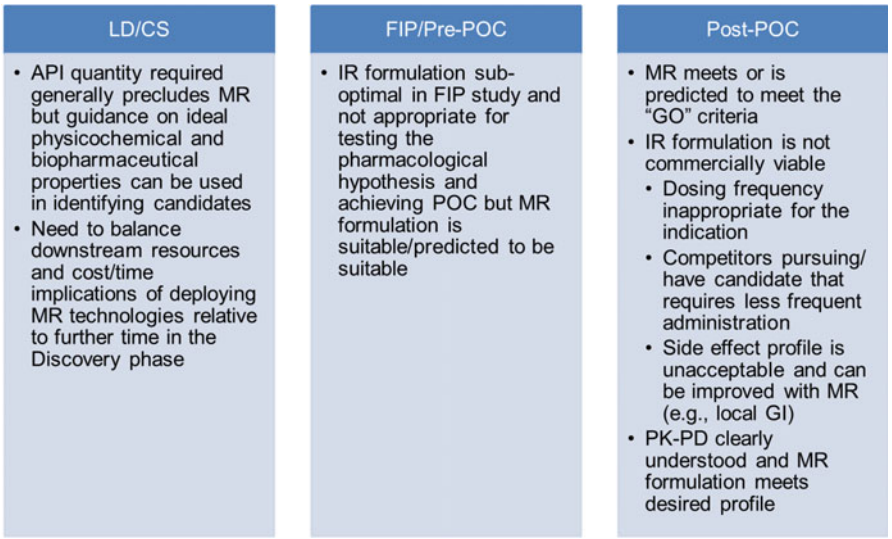

Fig. 10.3 Strategic considerations in deciding when to deploy MR technology

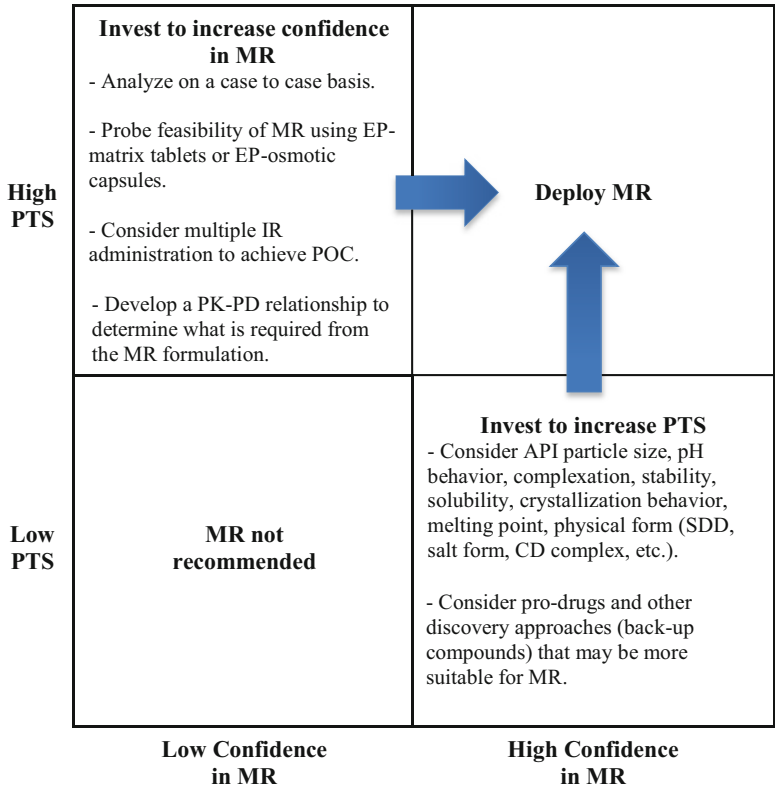

Fig. 10.4 Strategic considerations for MR deployment (probability of technical success vs. confidence in MR technology)

Table 10.3 Case studies and examples of applying the framework for MR formulations in early- and late-stage clinical development

	Drug A	Drug B	Drug C
MR rationale	Improve efficacy by increasing Cmin w/o increasing Cmax	Reduce AE	Reduce AE related to high peak/trough
Dose (mg)	10 mg	5 mg	200 mg
D/S (mL)	15 mL	45 mL	880 mL for API 500 mL for SDD
Stability	No known issue	No known issue	No known issue
Absorption mechanism	Transcellular	Transcellular	Transcellular
Colonic absorption	Unknown	Unknown	Unknown
Effective half-life	~4 h	~8 h	~3 h
Metabolism/ efflux	MDCK AB ratio: 14.7	Unlikely P-gp substrate	Highly effluxed ER = 42
Confidence in MR	Low	Low	Low
PTS for MR	High	High	Moderate
Recommendation	Short and long EP-osmotic capsule	Short and long EP-osmotic capsule	Short and long EP matrix tablet

Table 10.4 Additional case studies and examples of applying the framework Modified Release (MR) formulations deployment framework for MR formulations in earlyand late-stage clinical development

	Drug X	Drug Y	Drug Z
MR rationale	Maintain C > Ceff QD dosing a MUST	QD dosing	QD dosing to be competitive
Dose (mg)	30 and 120 mg	82.5/165/330 mg	20 mg
D/S (mL)	85 and 350 mL	<10 mL	100 mL
Stability	No issue expected	Known incompatibilities	Oxidation (=no PEG/PEO)
Absorption mechanism	Transcellular	High permeability	Moderate permeability
Colonic absorption	Unknown	Reduced due to active transport in upper GI	Unknown
Effective half-life	~5 h	~6 h	~3 h
Metabolism/ efflux	CYP3A4	Negligible metabolism	CYP3A4/P-gp efflux
Confidence in MR	High	High	High
PTS for MR	High	High	High
Recommendation	Short EP-osmotic capsule because limited API) followed by SCT	GR matrix tablet	Short and long EP-osmotic capsule to define optimum MR followed by ECS

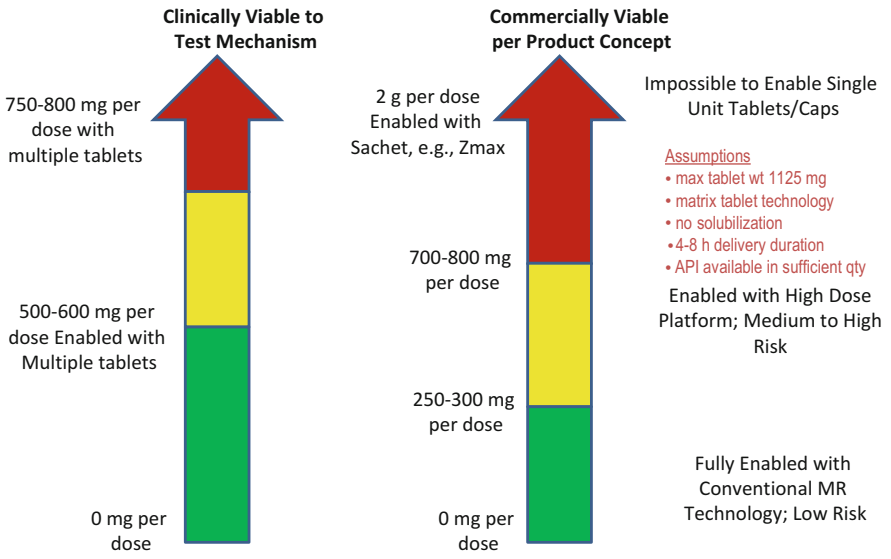

Fig. 10.5 Enabling versus commercially viable doses in modified release formulations

10.3.4 Translation of Modified Release Options from Early to Later Development

Frequently, in order to rapidly initiate a POC study, particularly for first-in-class compounds, multiple tablets or capsules are dosed. Such "fit-for-purpose" formulations allow for rapidly answering the POC feasibility rather than delay the collection of this crucial information for formulation optimization. The most important factor to consider in the translation of MR formulations from early clinical development to late-stage development and commercialization is the dose. Typically, the quality target product profile (QTPP) requires a single dosage unit (tablet or capsule) to be administered. It can be impossible to develop a single unit tablet or capsule that is swallowable, if the dose is greater than 750 or 800 mg. In such cases, the dosage form/formulation platform needs to change. For example, a powder for oral suspension was used to deliver a 2 g dose in the case of azithromycin extended release [11, 12]. Figure 10.5 graphically depicts the doses that are clinically viable vs commercially viable.

10.4 First-In-Human Case Studies

The following case studies are provided to illustrate some formulation examples with contemporary proprietary drug candidates. These examples do not include structures, therapeutic targets, or extensive physicochemical and biological data.

10.4.1 Study A: Powder in Capsule

Compound 1 was a free-flowing, nonhygroscopic crystalline material, with high solubility and high permeability. Some degradation in aqueous media occurred over time, limiting the effective use period of a solution or suspension dosage form. The anticipated clinical dose covered a range of 1 mg to 200 mg in a Phase I clinical study. Extemporaneously prepared powder in capsule (EP-PIC) is selected as the formulation for the FIH program, which allowed for rapid advancement to the clinic. This formulation option avoided potential solution stability concerns, required minimal dose preparation efforts and no manufacturing activities, and maintained flexibility to adjust doses in real time during the course of the clinical evaluation. As compared to other solid dosage forms, the EP-PIC also did not require additional excipient compatibility studies, as it was comprised of only API in a capsule shell.

10.4.2 Study B: Enabled Formulation

Compound 2 was a crystalline-free acid, with high lipophilicity and low solubility. A dose escalation strategy was anticipated to cover a broad dose range, between 1 and 1000 mg, though significant difficulty in predicting clinical PK was anticipated for this molecule. Therefore, a high degree of flexibility in dose selection and the ability to adjust dose level during the clinical study were desired. An enabling formulation such as a spray-dried dispersion (SDD) may be utilized to overcome solubility and resulting bioavailability limitations. An EP suspension of the SDD formulation allowed for greater flexibility in dose adjustments in response to real-time clinical data. This maintained the ability to reach high dose strengths as required during escalation, which would be difficult to deliver in traditional solid dosage form options.

While EP offers many benefits to an FIH program, it is a strategy that may not be so suitable for longer-term progression. Preparation of individual unit doses can be highly tailored for use in small studies, but resource requirements can limit the applicability in studies with larger cohort sizes or long durations. EP may also not be suited to compounds with significant stability concerns, such as limited solution stability, or situations which may necessitate additional storage or packaging requirements, such as hygroscopicity limitations or photostability challenges. However, it can be used to quickly reach certain key decisions in a program, to explore variables that may be of key importance to later development, or to answer questions that are needed to inform backup programs still in discovery, for example, influence of particle size on bioavailability, taste profiles and masking options, or influences of solubility vs. dissolution.

10.4.3 Study C: Particle Size Evaluation

In this study, an immediate release formulation was being progressed through an FIH program with Compound 3, a free base with low solubility and moderate permeability. An evaluation of the effect of particle size on the bioavailability of the compound was necessary to decide on a product profile for later clinical and ultimately commercial solid dosage forms. Extemporaneously prepared suspensions were produced at the clinical site using small quantities of API milled to different particle size specifications and dosed as part of a crossover cohort inserted in a single-dose escalation protocol. This approach allowed for minimal resource utilization while providing an understanding of the particle size effect for the drug candidate and permitted doses to be selected and inserted into the clinical program based upon real-time data. Results of this study directly informed solid dosage form development efforts, as well as backup discovery efforts.

10.4.4 Study D: EP-Osmotic Capsule (Adapted from Ref. 7)

An IR formulation of a candidate for the treatment of type 2 diabetes showed dose-dependent gastrointestinal (GI) side effects. There was a possibility that these effects could be mechanism related; hence, the confidence in MR resolving the issue was considered to be low. However, MR formulations are known to reduce GI side effects. The dose and solubility properties of the compound were such that the probability of technical success (PTS) was considered to be high. Therefore, a colonic absorption study using EP-osmotic capsules was undertaken with two different release durations to determine MR feasibility with minimal investment in API and dosage form development. In the PK study, the Cmax was blunted as expected, but the AUC was lower compared to an IR control (and this AUC lowering was even worse with the long-duration formulation). On the basis of the data, we concluded that an MR formulation with a short delivery duration had the best chance of mitigating the GI side effects.

10.4.5 Study E: Modified Release (Adapted from Ref. 7)

An IR formulation of an anti-infective in development did not meet the target pharmacokinetics (PK) criteria to maintain a sufficient threshold plasma concentration (Cmin) or steady state greater than the minimal efficacious concentration (MEC) with a dose of less than 1 g. PK simulations indicated that MR formulations could overcome these issues. However, there was insufficient API for traditional MR development and manufacture of clinical supplies. Therefore, evaluating the feasibility of a MR formulation was performed with two matrix tablet formulations extemporaneously prepared at the clinical PK study site. The results showed that

both MR formulations met the desired progression criteria, as predicted by the simulations. Unfortunately, the development of this compound was terminated owing to an adverse toxicological finding in preclinical studies.

10.5 Formulation Design Following First-In-Human Studies

10.5.1 General Considerations

Moving into the next phase of clinical development introduces greater focus on efficacy, and with this, the endpoints and parameters of the studies change. For example, a move from healthy volunteers to patient populations. The inclusion of patient populations in a clinical trial brings additional complexity to the study design and execution. Design aspects such as larger cohort sizes and more extended dosing intervals, longer recruitment periods, multiple study sites, and out-patient study designs must be considered not only for the program as a whole but also when selecting an appropriate dosage form to support the clinical evaluation. Logistical aspects of the supply chain play a greater role, as do other facets such as used period and expiry dating, ease of self-administration, etc.

Some formulation options which are readily applicable to FIH study usage become less desirable for later stage of clinical trial development. For example, while an extemporaneously prepared solution in bottle may be a perfect fit for a 1 day in-house dosing during an FIH dose escalation study, it becomes much less practical for patients to take home and store a month's supply. Not only does it become more cumbersome for the patient, it places a much greater demand on the resources of the pharmacy supporting the study. The benefits of speed and flexibility for dose adjustment are no longer the focus, whereas the need for larger quantities, longer expiry periods, and broader distributions are desired.

Additionally, the unique needs of the patient population play a role. Is the patient able to swallow a tablet or capsule? Patients with dysphagia, for example, may require alternative dosing options. Perhaps the patient requires administration of medication through a nasogastric tube. Pediatric or geriatric populations may also find more traditional dosage forms, a challenge from this perspective, and other options may be more practical. If more specialized dosage forms are anticipated, understanding the available formulation space in discovery and early development can benefit the later progression of the drug candidate. This allows for more development time to optimize the dosage form to meet the patient needs. By the same token, this is an area where later-stage development can also influence early discovery programs. Gaining knowledge about the target product profile of compounds in a given therapeutic area or for specific patient populations can be highly useful in designing new chemical substrates, where specific properties can be built into the molecules to enable greater compatibility with the target drug product.

Whether trial endpoints to identify a positive outcome are achieved in a short time period versus a prolonged evaluation also requires consideration. For example, reduction in postoperative pain may be assessed more easily over a relatively short study time frame and is likely to be studied within a controlled clinical or hospital setting. Alternatively reduction in inflammation associated with an autoimmune disorder may require many months or years to evaluate. In the former, a fast onset formulation may be ideal, some dosage form manipulation could be acceptable given the setting of the patient use, and feedback on dosage form performance is likely to be quick. In the latter example, however, more complex release profiles may be desired. Patients will be self-administering over a longer duration so the dosage form must be convenient and straightforward. Consequently, opportunities for optimization may be limited due to the prolonged interval of performance-indicating data collection.

Related questions of dosing duration, frequency, and compliance may need to be addressed as well. Based upon the human PK parameters determined in FIH, for example, is once daily dosing feasible or will BID dosing be required to achieve anticipated efficacious plasma concentrations? Can the dosing interval be supported for the duration of the trial, and is it commercially feasible?

Well-thought-out and carefully designed formulations at each stage of a drug candidate's progression will answer many of these questions, not only informing the ultimate development of commercializable products, but positioning the drug candidate for more efficient progression through clinical development as well as helping to guide discovery efforts toward new molecule design and selection based upon that knowledge.

The desired or critical PK parameters vary depending on indication and mechanism of action. In some cases, for immediate onset of action, rapid attainment of therapeutic concentrations is required, whereas for some other cases where target exposure is needed to be maintained over a longer period of time after repeated dosing, the steady-state concentration (Css) and lowest concentration at the end of dosing (Ctrough/Cmin) are the most important PK parameters.

The robustness of the target product profile defined prior to FIH depends on a number of factors. For instance, how much is known about the pharmacological class of molecules and translatability from preclinical PK (absorption/clearance), PD, and disease models? As exposure data from FIH becomes available, the target product profile, formulation strategy, and dose are further refined.

The choice of formulation and drug delivery technology can significantly impact the drug release, absorption, pharmacokinetic (PK) profile, and ultimately pharmacodynamic response. Thus, formulation plays a crucial role in development efforts. Alignment between discovery, preclinical efforts, and development is critical, as the routine use of in vitro screening tools and in silico PK modeling provides potential biopharmaceutical risk assessments early in the process. Clinical studies and formulation strategies are designed to address these risks via tailored formulations.

Some common pharmaceutics-related problems observed during first-in-human single ascending dose studies include variable or low exposures, Cmax-related

adverse effects, food or pH effects, and PK profiles dramatically different from preclinical projections. An a priori assessment of these potential outcomes and backup strategies with flexible clinical protocols are beneficial, but not always feasible. A translational approach to development with a quick feedback loop into the clinic is an attractive opportunity to address these concerns. Some organizations manufacture drug candidate formulations immediately prior to dosing at a colocated GMP production and clinical testing facility. Using this approach, the drug product can be manufactured within days without the need for extensive scale-up and stability assessments. The clinical data from one study period can be quickly reviewed and used to select the next formulation option to be delivered. This flexibility of CMC aspects and real-time feedback from the clinic enables faster selection of an optimal formulation to advance into commercial development.

Salts or crystal engineering approaches to design cocrystals, micronization to reduce particle size, use of amorphous material or lipid-based self-emulsifying systems, and complexation with cyclodextrins are approaches to overcome solubility limitations and improve in vivo exposures.

As first-in-human data becomes available and the dose range is refined, the formulation development efforts may move toward either an enabled formulation or consider a conventional dosage form depending on the direction of dose adjustment.

Computational simulations using physiology-based pharmacokinetic modeling software (e.g., GastroPlus, Simulations Plus, Inc., Lancaster, CA) are routinely used to assess impact of interacting formulation and physiology on drug candidate exposure profiles. These models are revised and refined iteratively with additional human PK data, allowing for better predictions and formulation optimization. Kesisoglou et al. present an example where in silico modeling PK absorption modeling approaches were used to fit data from the FIH studies and generate PK parameters [13]. The model was then used to predict effect of particle size. The authors presented a case study in which dissolution-limited absorption was assessed based on early human data, incorporating dissolution rates from final drug product rather than just primary particle size data. This in vitro dissolution data for API with different particle size distributions (PSDs) was used in the model to identify an optimal PSD for the desired in vivo exposures.

Amorphous solid dispersions with polymeric carriers and/or surfactants are prepared by spray drying, hot-melt extrusion, and lyophilization or through the use of supercritical fluids. These amorphous solids have a higher energy than crystalline material leading to solubility improvement which can be several orders of magnitude greater and hence can increase in vivo exposures significantly. Several reports in literature highlight the improved exposures and PK seen by the use of amorphous solid dispersions compared to crystalline materials [14–18].

Self-emulsifying drug delivery systems (SEDDS) are another enabling technology to improve oral absorption of lipophilic drugs [19]. Lipid-based formulations (LBFs) may be composed of oils, cosurfactants, and cosolvents that solubilize a drug candidate. These systems can rapidly form oil-in-water (w/o) fine emulsions when dispersed in aqueous phase under mild agitation, and depending on particle

size of droplets formed, they can be either classified as microemulsifying or nanoemulsifying. Since the drug is in a solubilized form, these lipidic systems bypass dissolution and can result in supersaturated levels of free drug in the lumen providing bioavailability enhancement and can overcome a pH or food effect. These systems can also potentially overcome low and variable PK issues. Additional types of lipid formulations are also possible. Chakraborty et al. [20] provides an in depth review of lipid-based formulations and related in vitro and in vivo considerations.

Another enabling approach is nanocrystal formation in which the particle size of crystalline material is reduced to the nanometer range with wet-milling with beads, high-pressure homogenization, or controlled precipitation. Physical stability of these suspensions is typically improved by the use of hydrophilic polymers or surfactants. The use of nanocrystal technologies has shown enhanced bioavailability ranging between 1.7–60-fold and 2–30-fold for Cmax and AUC compared to micrometer range of crystalline solids for various molecules [21–23]. Commercially though very few nanocrystal oral formulations using NanoCrystal® (Elan Drug Technologies) and IDD-P® (SkyePharma) are available.

To reduce timelines between various clinical studies and enable quick changes to formulations, probe arms using alternate formulation strategies can be built into the FIH studies. For example, in cases where enabling technologies such as spray-dried dispersions are typically included as the primary first-in-human approach, a probe arm using crystalline API may be included to provide an early assessment. This provides the opportunity for a quick formulation development "pivot" if doses significantly change beyond FIH.

Additional aspects that can be explored as an extension of first-in-human studies or to further probe absorption liabilities for a given drug include food effect evaluation, absorption site studies, or comparison across various formulations which typically is done as a relative BA study in a crossover controlled limited subject study design. These studies feed into formulation strategies for beyond Phase I to address any PK-related issue that can be fixed by formulation.

A preliminary food effect study is also evaluated as part of Phase 1 studies typically at the expected efficacious doses. This enables an early read of potential food effect as well as enables incorporation of instructions for food related to dosing in future clinical protocols. If a food effect is identified, typically solubilized formulations or amorphous dispersions can all be potential strategies for mitigating food effect.

Some drug candidates display regional-specific absorption. If the absorption occurs primarily in the proximal intestine rather than the entire GI, any differences in upper GI concentration will impact absorption and ultimately the exposure profile. In such cases, site of absorption studies are conducted to guide further drug product development. These can be conducted in a noninvasive way using timed delivery capsules with a radiolabel included in the drug reservoir for locating the capsule via gamma scintigraphy. When the capsule reaches the desired location in the gastrointestinal tract, the capsule drug reservoir contents are expelled. PK profiles from delivery to various sections of the GI are obtained and compared to the

original reference study to assess for regional absorption. This information is incorporated into absorption models to optimize and guide formulation development. This is especially useful for drug candidates with very short half-lives for which extended release dosage forms are considered as a potential option.

10.5.2 Other Specific Considerations

As mentioned previously, Phase 1 formulations are typically focused on enabling a quick path to the clinic and may not be viable commercial formulations. More robust, commercially relevant formulations must be developed for the drug candidate to proceed, and bridging studies are often required. Some examples include a change in API form, formulation technology, or supplier of the active raw material. In the latter case, if the material has a different impurity profile, additional toxicology studies may be needed.

The pharmaceutical development section of the eCTD outlines these changes to formulation and any data supporting its comparability. Sometimes preclinical studies are also done ahead of clinical bridging studies to show that proposed changes are either still consistent with the Phase 1 PK or provide any desired altered PK if that is the driver for the formulation change.

Bridging across formulations can also be performed as an extension of the Phase I study, with the new formulation introduced at a given dose along with the current formulation to assess PK. However, more frequently bridging studies across formulations are performed as part of well-controlled relative bioavailability (BA) studies. Relative BA studies are typically designed as crossover two sequence studies of test and reference formulation. Relative BA studies are typically conducted as an internal risk assessment. Bridging or adjusting the dose based on the improved formulation may not require the strict regulatory criteria of statistical power for typical bioequivalence (BE) studies.

The main purpose of BA studies is to support and facilitate formulation development and optimization efforts. Food effect assessments are typically performed with this study format, especially for solubilized or lipidic formulations. Food can alter BA by various means, including delayed gastric emptying, stimulated bile flow, altered gastrointestinal (GI) pH, increased splanchnic blood flow, altered luminal metabolism, and physical or chemical interaction with dosage form. In general, meals that are high in total calories and fat content are more likely to affect GI physiology, thereby resulting in a larger effect on the BA. Modified release formulations are tested for both food effect and dose dumping which is especially important for pediatric formulations [24]. Typically, these studies are conducted in normal healthy volunteers to minimize any other considerations such as disease state or co-medications.

Pediatric formulations are a key area of development and rather complex and time and cost intensive given the differences in age/weight, physiology differences in adults versus children, route of administration, excipients, importance of taste

masking, volume, and size of dosage form. To avoid delays in clinical studies in children, often simple enabling formulations like powder mixes, granulates, or pellets in bottles are used to start early pediatric assessment in non-pivotal studies. Since these formulations are not optimized for commercial viability, further formulation development specifically designed for pediatrics is required. Various bridging options can be considered for pediatric development depending on overall strategy development incorporated different formulations, age groups, and physiology groups.

Palatability is another key consideration assessed for pediatric development. Most often due to ethical consideration, extensive biopharmaceutical data across formulations is not available in pediatric population, and by default adult relative BA studies in healthy volunteers (except in certain cases like oncology where disease adult patients are relevant) are used for supporting pediatric development studies as mentioned above.

Other considerations for pediatric formulations include palatability and the potential for excipient interaction with the drug candidate or pediatric patient physiology [25]. The latter is important as transport systems (e.g., P-gp transport inhibitors), first-pass metabolism (e.g., CYP inhibitors), and GI pH (e.g., pH modifiers) may not be fully mature in pediatric population. Initial relative BA studies comparing adults and children with the standard formulation must be completed prior to any additional PK manipulating dosing option studies.

Occasionally, formulation bridging can occur without conducting human PK studies if the change being proposed is minor and in vitro evaluations are deemed sufficient. The SUPAC document provided by the FDA has guidance around the nature of changes for IR or MR formulations and criteria for justifying a waiver for any clinical bridging studies. For example, a high-solubility, high-permeability BCS Class 1 drug product undergoing a minimal formulation changes may have a bridging study waived if a simple dissolution test demonstrated equivalence to the original formulation. Alternatively, in vivo animal model bridging study may be required to verify that the efficacy or safety of the product is consistent with what has been produced throughout the development program. Bridging study may also be waived for pediatric formulations based on in vitro dissolution data and adult PK data so long as linear PK is anticipated. Also, for drugs that are in the same dosage form or the same manufacturing process and composition as adult formulation and solution formulations and where excipients are not expected to impact the pharmacokinetics, bridging studies may be waived [26, 27].

10.6 Summary

Early clinical formulation design must simultaneously balance many factors, providing appropriate exposure profiles to evaluate the safety, efficacy, and ultimately commercializability of a promising drug candidate. Prior to first-in-human (FIH) studies, the design must be sufficiently flexible to accommodate the drug candidate physicochemical and biopharmaceutical properties as well as the projected but unknown pharmacokinetic profile, dose amount, and frequency.

Dose manufacturing options typically pursued at this stage may be a simple powder in bottle (PIB) or powder in capsule (PIC), as well as the more traditional formulated capsules or tablets. Specialized modified release options may also be pursued for drug candidates with gastrointestinal stability issues, specific site of action or site of absorption concerns, or requiring alternative exposure profiles. Extemporaneously prepared formulations for on-site compounding (OSC) allow for greater speed and flexibility in the clinical plan and can even include preparation of osmotic capsules.

Following the initial FIH studies, alternative concerns such as gastric pH, coadministration with food, impact of particle size, dose escalation, and specialized patient populations such a pediatrics are often investigated. These factors may require even more specialized formulations, including amorphous solid dispersions (ASDs) or lipid-based formulations (LBFs) to achieve the desired exposure profiles.

Ultimately, clinical formulation design plays a key role in enabling better drug design. Gaining and transferring early knowledge of the compatibility, capability, and limitations of the formulation as well as the drug candidate safety and efficacy readouts back into the discovery space enable research scientists to optimize drug candidate structural properties and maximize the likelihood of achieving an approved medicine.

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Chapter 11
Translational Research: Preclinical to Healthy Volunteer to Patient

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Abstract The development of medicines involves not only the translation of information collected in preclinical in vitro and in vivo experiments to clinical studies in healthy volunteers, also known as T1 translation, but additionally the optimization of the new medication within the environment of medical care, the translation from healthy volunteer to patient, also known as T2 translation. How do the pharmacokinetic parameters vary with different doses? What is the best dose? Can the new medication be combined with other therapies? This chapter begins with a discussion of the overall strategy and individual steps to determine a drug candidate's pharmacology, absorption, distribution, metabolism, and elimination (ADME), and drug-drug interactions in clinical studies, as well as subsequent evaluation of preclinical predictions. Integration of concentration-time data to develop population pharmacokinetic models and the use of biomarkers in clinical studies are discussed. Biomarkers can be particularly helpful in diseases where obvious clinical pathology may lag behind changes in laboratory values or other more easily measured surrogates. Case studies are also presented illustrating applications of translational research in the development of possible therapeutic agents for the treatment of osteoporosis and Alzheimer's disease.

Keywords Clinical pharmacology • Biomarkers • T1 translation • T2 translation • ADME • Drug-drug interactions

11.1 Introduction to Clinical Pharmacology Studies

Translational research has been defined as the interpretation of discoveries made in nonclinical experiments into clinical interventions for the diagnosis, treatment, prognosis, or prevention of disease with a direct benefit to human health [1]. This process begins with the basic premise that observations made in one experimental

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condition may be predictive of outcomes of similar experiments performed under different circumstances. For example, the pharmacokinetic parameters of an antibiotic observed in mice are expected to be predictive of the pharmacokinetics of the same antibiotic in dogs, and both preclinical estimations of parameters are expected to be predictive of the parameters observed in humans [2]. Successful and efficient drug development makes use of all available information so that new medications are approved in a timely manner with reasonable use of resources. The goal of drug development is to provide new medications to treat unmet medical needs in a wide variety of patients using convenient, safe, and effective dosing, within an environment of meals, work, other medications, and non-pharmacological treatments. Information from unsuccessful drug development programs is also studied to gain a better understanding of the reasons for failure and what changes in the development program would be more likely to result in an effective and safe medicine. Two roadblocks to translation of new knowledge into clinical practice were identified by a group of researchers at the Institute of Medicine who reviewed a large number of translational research studies [3]. The first roadblock occurs between basic biomedical research and clinical science. This is referred to as T1 translation and much of this book is devoted to this stage. The second roadblock occurs between clinical science and knowledge versus improved health and is referred to as T2 translation. This chapter also explores the various studies that are conducted during the T2 stage of translational research, which expands upon early clinical data to the information needed to integrate a new medication into routine clinical practice.

During the conduct of drug candidate studies in human subjects, data are collected that expand treatment knowledge of disease and under ideal circumstances result in a new approved medication. The low number of ultimately successful drug development projects is well known; as most promising high school athletes fail to play in professional leagues, most new chemical entities entering phase 1 of clinical development will not be approved and launched. However, thoughtful analysis of clinical studies with potential medications that fail to achieve their goals can still contribute to improving the general care of patients. This information can be used to develop new methods and new understandings that will in turn influence the development of subsequent better compounds.

11.1.1 Preliminary Clinical Development Plan and First-In-Human Study Design

An outline of the clinical plan is drafted at the earliest stage of drug development, soon after there is some evidence of physicochemical stability, suitable pharmacokinetics, pharmacological activity, and tolerability. The clinical plan assures that development project planning provides sufficient time and resource support. Regulatory guidances are available for some indications and define the necessary evidence required to show efficacy. A precursor to the clinical development plan

is the target product profile [4]. The target product profile describes the minimum acceptable and ideal attributes of the new medicine: indication, target population (including geographical location), efficacy, safety, drug-drug interaction profile, formulation, dosage, stability, and cost. The target profile can also be described through a mock-up of an optimal prescribing information summary.

Long before the first subject is enrolled in the initial clinical study, the development team drafts idealized prescriber information for the proposed product. This includes the dosing regimen and directions needed for dose adjustment due to administration with food or other medications, as well as differences in age, size, gender, ethnic or racial background, and other sources of differences in response. Considerations are made with respect to the current standard of care for the disease and what factors contribute to dissatisfaction by prescribers and patients. Then the team expands the idealized prescribing information, outlining the clinical trials that are needed to support the desired statements in the various sections. Next, each discipline, including biostatistics, clinical, clinical pharmacology, medical, preclinical drug metabolism, pharmacokinetics, and safety, among others, assesses each clinical trial to determine what specific information is required. The necessary pharmacokinetics, pharmacodynamics, safety, and efficacy data are reviewed to reasonably design each clinical trial and specify what the source of the information will be. The end result is a clinical development plan, which may evolve as studies are conducted and new information is acquired and incorporated. Efficient clinical development requires contingencies and mitigation strategies if unexpected results arise.

Successful completion of the clinical plan will provide the information needed to demonstrate the safety and effectiveness of the new molecular entity (NME) in patients requiring treatment. It is recognized that there will be gaps in this knowledge, as it is impossible to perform an exhaustive evaluation of all potential demographic characteristics of patients, severity of disease, and combinations of medications that may be coadministered. These knowledge gaps are first bridged through systematic investigation of the most likely causes of pharmacokinetic variability that determines the drug exposure patients will experience. Models are also developed to describe exposure and response relationships related to efficacy and safety. Any remaining gaps in knowledge may be addressed in post-approval commitments in the form of additional studies, registries, risk management plans, and safety surveillance. The clinical development plan is broken into phases [5]:

- Phase 1—information is gained about the pharmacokinetics and safety of the NME across a broad range of doses.
- Phase 2—preliminary evidence for clinical responses of both efficacy and safety is collected in patients who are representative of the larger population with the disease of interest, across a range of doses lower than those used in phase 1.
- Phase 3—confirmation of efficacy and safety is collected in an even more narrow range of doses, but usually across a more heterogeneous collection of patients with the disease of interest.

The first-in-human (FIH) trial marks an important step in drug development. Immediately prior to the start of the study, a regulatory package known as an investigational new drug (IND) application is submitted. This document includes in vitro and preclinical assessment of pharmacological activity, pharmacokinetics, safety, and toxicokinetics, as well as the proposed FIH protocol describing the subjects, dosing, and observations to be made. A key component of the IND is the protocol with the proposed first dose and subsequent dosing escalation plan in the FIH study. The preclinical studies of efficacy and toxicity are carefully reviewed to identify the no observed adverse effect level (NOAEL) and the minimal anticipated biological effect level (MABEL). Simulations of the anticipated human exposure are translated from the preclinical observations, developed using allometric scaling, or physiologically based pharmacokinetic (PBPK) models. With the growing number of monoclonal antibodies and other biological therapeutics in development, target-mediated drug disposition (TMDD) is also an important tool in predicting exposure [6]. The planned dosing escalation is chosen to envelop the anticipated clinically effective dose as well as maintain a sufficient safety margin below the dose anticipated to be associated with unacceptable side effects [7].

FIH studies sequentially enroll small groups of healthy volunteers or patients into cohorts, numbering 6–8 individuals, where all subjects in the same cohort receive the same, single dose of active drug or placebo in a blinded, randomized fashion. Subsequent groups of subjects are enrolled upon assessment of safety. An example of this study type, also referred to as single ascending dose (SAD) studies, is discussed by Muralidharan et al. [8]. In cases where there is specific concern about the potential safety of the investigational drug product, each individual subject will be dosed in sequence, rather than dosing all individuals in the group at the same time. The initial dose administered is usually much lower than that anticipated for clinical efficacy. Dose escalation continues until the likely therapeutic range of exposure is achieved or until there is an unacceptable incidence or type of adverse reaction. The goals of the FIH study are primarily safety: determining the maximum dose with an acceptable safety and tolerability profile as well as any observed dose-limiting adverse events. Single-dose pharmacokinetic parameters are also investigated, providing a first assessment of pharmacokinetic and possible concentration-effect information.

Since each individual receives only a single dose and the FIH studies generally involve small numbers, intersubject variability can confound the results. There are generally only five or six different dose levels, with approximately six individuals receiving each dose and two or three additional subjects receiving a placebo in each group. These data can be combined and used to develop a model to validate preclinical simulations as well as to simulate different designs for subsequent multiple ascending dose (MAD) studies as well as other single-dose study designs of doses and conditions not tested. FIH studies may be expanded to include other cohorts if early information is needed for subsequent development. For instance, a cohort of subjects may receive the drug after consuming a meal instead of in the fasted state, or a cohort of Asian subjects may be enrolled to facilitate early inclusion of Asian patients in a subsequent global trial. Collecting and evaluating

electrocardiograph (ECG) data may permit an early assessment of potential cardiovascular safety signals [9].

Another important use of FIH study data is to evaluate the in silico pharmacokinetic predictions made prior to the availability of clinical data. The reasons for inaccurate predictions can be then investigated and used to improve future predictions. The concentrations observed in the subjects are compared to preclinical exposures to confirm whether human pharmacokinetics will achieve concentrations sufficient for efficacy but below concentrations associated with unacceptable toxicity. Biomarkers may also be assessed as well as evidence of efficacy and toxicity to begin establishing a concentration-effect relationship that will guide subsequent study design and ultimately patient dosing. The range of concentrations evaluated in these FIH studies is usually the largest of any in the clinical portion of drug development.

11.1.2 Research Goals and Study Design of Phase 1 Clinical Pharmacology Studies

The FIH and MAD studies are primarily directed toward T1 translation research, seeking confirmation of safety and pharmacokinetic parameter estimates from preclinical data across a range of doses used to subsequently guide phase 2 and phase 3 studies. In contrast, other phase 1 studies have T2 translational research goals and are conducted to gain information that will optimize exposure in patients with different intrinsic and extrinsic factors. Such studies to evaluate altered pharmacokinetics include food effect studies, drug-drug interaction studies, the effect of renal or hepatic impairment, the effect of gender, the effect of age (either advanced or pediatric), metabolic studies, and the effect of dosage form design or manufacture on pharmacokinetics. The effect of race, such as Asian or black or white, or ethnicity, such as Hispanic, may be studied in phase 1 clinical trials, but also may be investigated using population pharmacokinetic analyses of combined studies. Examples of the questions that may be asked are shown in Table 11.1.

Despite the many different goals, essentially all phase 1 clinical pharmacology studies contain the same elements (subjects, dosing, and observations) and ultimately are analyzed similarly. Enrolled phase 1 subjects are generally healthy volunteers, or if they have some clinical pathology such as renal impairment, their condition is relatively stable, so that participation in a short clinical study does not pose a serious health threat. The test medication is administered as either single doses or multiple doses, but always within a clinically relevant range. The FIH/SAD and MAD studies have the broadest range of doses and should represent all subsequent exposures observed in the clinical program. Doses used to assess the effect of coadministration with food, other medications, or the impact of end-organ impairment should fall within the ranges tested in the FIH and MAD studies. These doses must also be adjusted to account for predicted exposure changes to retain the

Table 11.1 Example of T2 translational research studies with tigecycline

Research question	Study
Does administration of tigecycline with food change the pharmacokinetics?	Muralidharan et al. [8]
Does prolonging the length of the infusion decrease the incidence of nausea or vomiting caused by tigecycline?	Muralidharan et al. [8]
How is tigecycline metabolized and excreted?	Hoffmann et al. [10]
Do the recommended doses of tigecycline change oropharyngeal and intestinal microflora?	Nord et al. [11]
What are the pharmacokinetics of tigecycline in Chinese subjects?	Jiang et al. [12]
Are the concentrations of tigecycline in the lung adequate to treat pneumonia?	Conte et al. [13]
Does renal impairment change the pharmacokinetics of tigecycline?	Korth-Bradley et al. [14]
Does hepatic impairment change the pharmacokinetics of tigecycline?	Korth-Bradley et al. [15]
What are the concentrations of tigecycline in tissues?	Rodvold et al. [16]
Does tigecycline coadministration change the pharmacokinetics of digoxin?	Zimmerman et al. [17]
Is there a difference in tigecycline pharmacokinetics between men and women or between young and old adults?	Muralidharan et al. [18]
Does tigecycline prolong corrected QT intervals in healthy subjects?	Korth-Bradley et al. [19]

safety margin and protect the subjects participating in the clinical trial. Other than the FIH, MAD, and some drug-drug interaction studies, most phase 1 studies are designed as 2-period crossover studies of single-dose administration under standard versus test conditions. The observations made throughout phase 1 trials include safety assessments of vital signs, laboratory chemistry, hematology, and urinalysis; informative biomarkers that may signal efficacy or safety concerns; blood and urine drug concentration analyses; as well as, in the case of biologics, antidrug antibodies or neutralizing antibodies. Pharmacokinetic and pharmacodynamic parameters are calculated, and the comparisons between the test and standard conditions are summarized using inferential statistics. Data collected for all studies are frequently amalgamated using population pharmacokinetic methods to extract a robust description of the pharmacokinetic parameters, their variability, and important causes of intersubject variability. To permit analyses across studies, data about the dosage form used must be carefully recorded, including lot and batch number, bioanalytical method, and dissolution testing.

Study protocols are developed by a small group of individuals, including clinical, clinical pharmacology, statistical, and operations expertise. The FIH and MAD studies are conducted consecutively, although there may be some overlap as once the safety of a particular dose is completed in the FIH, the MAD may begin at a daily dose not exceeding the dose originally administered in the FIH. For example, if a dose of 100 mg was safely administered to subjects in the FIH, doses of 25 mg twice daily may be initiated in the MAD study without waiting for the completion of all cohorts in the FIH. Once the FIH and MAD studies are completed and the

collected data interpreted, all of the other planned phase 1 studies may be initiated in parallel. However, given the expense and purpose of these studies, they may be staggered such that new data become available just in time to inform the next study. Studies intended to optimize treatment in phase 3 are conducted prior to those required for final registration. Phase 1 studies in vulnerable populations such as pediatric subjects or lactating women are not usually conducted without a compelling need, such as information being required to initiate a phase 3 clinical trial involving the same group.

11.1.3 Data Analysis and Interpretation

The end product of a clinical study is a study report summarizing the resulting observations. Pharmacokinetic parameters are summarized as are the safety findings and any other observations found. The results of these studies are then used to make decisions previously identified as important in the clinical plan. For instance, were the effective or toxic exposures observed in preclinical models observed in the subjects studied? Were dose-limiting side effects observed? Were the single-dose observations predictive of the observations after multiple-dose administration? Do the pharmacokinetic parameters change significantly when the medication is administered with food or other medications compared to when administered alone? The responses to these questions are used to design the phase 2 and phase 3 clinical trials.

11.2 Drug-Drug Interactions

Few patients, especially those with chronic conditions take a single medication. A survey of almost 7000 Irish citizens aged 50 years and older, who lived in the community, rather than residing in a nursing facility or long-term hospital, reported that 17% took more than five medicines each day [20]. With aging, the frequency of polypharmacy and the potential for drug-drug interactions (DDI) increases. Almost 60% of a group of Belgian seniors, aged 80–100 years, who lived in the community, took more than five medications, and 9% took more than ten each day [21].

Understanding potential drug interactions between an NME and other medications that may be used concurrently is an integral part of the NME safety assessment. If an NME is shown to increase or decrease the exposure of concomitantly administered medications, unanticipated safety (toxicity) or reduced efficacy could occur, respectively. Conversely, the NME exposure may be altered as a result of other medications. In the past, conducting drug-drug interaction studies was empiric and based upon either the likelihood of medications being coadministered or to establish a potential advantage over other medications if a lack of interaction could be shown. For example, the prescribing information for the nonsteroidal anti-inflammatory drug (NSAID) etodolac, approved in 1991, lists ten potential drug

interactions, three of which are negative, showing no impact of either medication on the pharmacokinetics of the other. There is little detail in the prescribing information about the specific routes of metabolism, but concern is raised about potential for interactions due to the extensive protein binding of etodolac. In contrast, the prescribing information for the NSAID celecoxib, approved in 1998, lists information for many of the same potential drug interactions listed for etodolac, but also indicates metabolism primarily occurs by CYP2C9 oxidation.

With better understanding of the role of Biopharmaceutics Drug Disposition Classification System (BDDCS) [22] and metabolic pathways with respect to potential drug-drug interactions, fewer empiric drug interaction studies are performed. Additionally, the drug-drug interaction studies that are conducted provide further information about specific metabolism and transporters. The regulatory guidances on the investigation of drug interactions provide detailed directions to systematically approach the potential for drug interactions [23, 24]. While the study of a particular combination of interacting medications may have specific applicability to patients taking them, the results of drug-drug interaction studies also have more general contributions to clinical practice. For instance, drug-drug interactions aid in establishing the particular pathways by which NMEs are metabolized and eliminated by CYP450 enzymes or transporters, respectively. Metformin, for example, is a medication commonly used as the first pharmacotherapy for patients with type 2 diabetes. It is also recommended as a probe substrate for OCT2 transporters, despite not being associated with drug-drug interactions of current clinical importance [24].

The availability of PBPK models developed using results from nonclinical studies combined with human pharmacokinetic data from the FIH studies offers the first simulation of the metabolic pathways for an NME [25]. These data are also useful for prioritizing drug-drug interaction studies.

11.3 Pharmacokinetics in the Patient

Most phase 2 and phase 3 studies will include assessment of the exposure obtained in the patients who participate in clinical trials. Some phase 2 and 3 studies will have pharmacokinetic substudies that collect a complete pharmacokinetic profile in a subset of patients participating, while other studies will collect a smaller number of samples from some or all of the patients and perform a population pharmacokinetic analysis [26]. In patient populations that are small in number, such as in the study of rare diseases, or in patients who cannot be easily studied, such population pharmacokinetic studies may also provide information that is normally assessed in a phase 1 study, such as impact of race/ethnicity or a concomitant medication [27] on pharmacokinetics. Exposure-response may also be explored using the concentration data along with observations of efficacy and/or adverse events. Alternatively, the concentration data collected in phase 3 studies may simply be summarized or compared to what has been previously described in phase 1 studies in healthy volunteers.

11.4 Biomarkers in Clinical Development

The Biomarkers Definitions Working Group, which is comprised of members from FDA, NIH, academia, and industry, has defined a biomarker as "[a] characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [28]. The working group also defined the following end points for biomarkers:

- *Clinical end point*: A specific characteristic or variable of the disease that reflects how a patient feels or functions or how long a patient survives.
- *Surrogate end point*: A biomarker intended to substitute for a clinical end point. A clinical investigator uses epidemiological, therapeutic, pathophysiological, or other scientific evidence to select a surrogate end point that is expected to predict clinical benefit, harm, or lack of benefit or harm.

Biomarkers are used in drug development to increase the probability of the clinical efficacy signal [29]. Depending on the stage of development, biomarkers can be used for different purposes. In very early stages of development, they help identify targets that correlate with clinical benefit. In the later stages of development, they help identify subpopulations most likely to respond to the drug candidate [30]. Some biomarker terms commonly used in the literature are:

- Diagnostic biomarkers, which provide the means to define a population with a specific disease
- Prognostic biomarkers, which correlate with health outcomes
- Predictive biomarkers, which define populations that might respond more favorably to a particular intervention from an efficacy or safety perspective

Although biomarkers are typically associated with efficacy measurements, biomarkers of safety and tolerability are also important [31]. One such example is the QTc interval prolongation which has been accepted by regulatory authorities as a safety marker for the potential of a drug to cause torsades [32].

The following subsections provide some examples of biomarkers in drug development from a few disease areas.

11.4.1 Biomarkers of Bone Health

Osteoporosis is a major health issue in postmenopausal women as it is associated with bone fractures. The prevalence of osteoporosis in developed countries is estimated to be 40% in women in their sixties and 70% in women in their eighties [33]. Bone turnover markers have been widely used in clinical trials for developing drugs to treat osteoporosis. Markers of bone turnover can be stratified regarding their origination from the bone mineral unit (BMU) (Fig. 11.1).

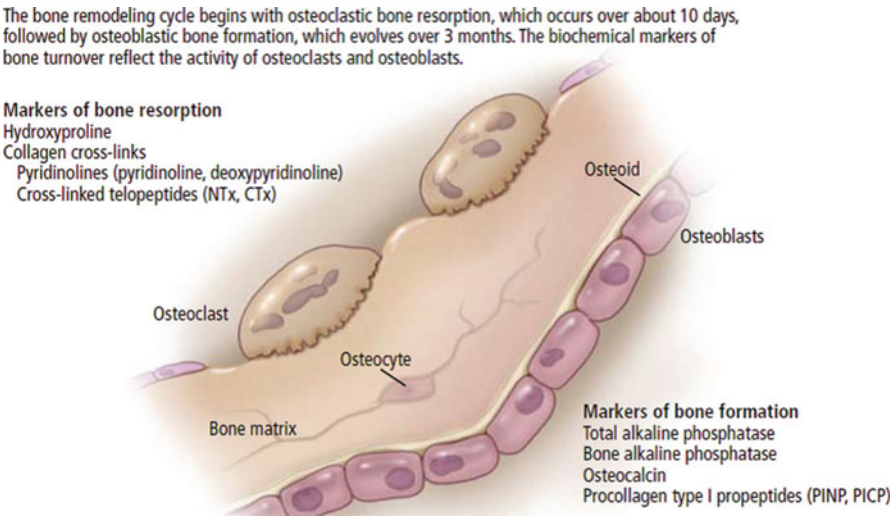

Fig. 11.1 The bone remodeling cycle. Medical Illustrator: Ross Papalardo. Reprinted with permission from Singer FR, Eyre DR. Using biochemical markers of bone turnover in clinical practice. Cleve Clin J Med. 2008; 75:739–750. Copyright © 2008 Cleveland Clinic Foundation. All rights reserved.

Bone resorption markers measure collagen type I degradation products released during osteoclastic resorption of bone. The most common markers of bone resorption measure peptide fragments deriving from collagen type I, such as CTX-I, NTx, ICTP, and pyridinolines [34]. Bone formation markers measure enzymatic activity of osteoblasts, bone proteins, and fragments of procollagens released during bone formation. The common bone formation biomarkers most often evaluated are bone-specific alkaline phosphatase, osteocalcin, or PICP/PINP [34]. Phase 2 and phase 3 trials of antiresorptive treatments have shown changes in bone turnover markers in as little as 3 months after drug administration and a negative correlation between most treatments and markers [35]. Several studies have demonstrated the correlation between plasma levels of bone formation versus bone resorption markers and risk of fracture [36–39]. Additionally, with antiresorptive treatments, the correlation between 6-month change in uCTX and 4-year change in spine and hip bone mineral density (BMD) was $r = -0.41$ and $r = -0.42$, respectively ($P < 0.001$) [40]. Bone biomarkers therefore provide valuable information in choosing the correct treatment and regimen by helping determine the efficacy of treatment early on.

Fosdagrocorat is a first-in-class, selective, high-affinity, dissociated agonist of glucocorticoid receptor (DAGR), in development to retain anti-inflammatory efficacy of glucocorticoids while reducing the unwanted side effects which include bone loss [41]. It is currently also under investigation for the treatment of rheumatoid arthritis. In phase 2 proof of concept trial, bone turnover markers procollagen type 1 N-terminal peptide (P1NP; bone formation) and urinary N-telopeptide/urinary

creatinine ratio (uNTx/uCr; bone resorption) were included as primary end points in addition to the clinical primary efficacy end point of ACR20 responses in a study to provide information on bone loss. Prednisone dosed at 5 and 10 mg daily were used for comparison of the bone loss effects. The results showed that suppression of bone formation biomarkers with fosdagrocorat 1, 5, and 10 mg was non-inferior to prednisone 5 mg daily [42]. The primary end point results support the underlying transrepression/transactivation ratio with fosdagrocorat different from that observed with prednisone in this clinical setting and may provide an improved benefit-risk ratio as it appears that fosdagrocorat causes less bone loss than prednisone.

11.4.2 Biomarkers in Inflammatory Diseases

11.4.2.1 Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by persistent inflammation and joint damage with a variable course of symptom improvement and worsening and different pathogenic mechanisms leading to common signs and symptoms [43]. Rheumatoid factor (RF), an antibody not found in the serum of healthy people, is one of the most widely used biomarkers in RA diagnostics [44] and is elevated in 60–80% of established and 50–60% of early RA cases. Anti-citrullinated protein antibodies (ACPAs) have recently emerged as highly sensitive and specific serological markers of RA, providing a superior alternative to the RF test in the laboratory diagnostics of RA. ACPAs production can precede the onset of RA symptoms by years and ACPA-positive individuals with undifferentiated arthritis have shown higher risk of developing RA [45]. Since ACPAs are associated with pronounced radiographic progression, they also have an important prognostic role [46]. Another class of novel antibodies in RA patients are the anticarbamylated protein (anti-CarP) antibodies [47]. These antibodies were detected in about 45% of RA patients and also in approximately 30% of ACPA-negative patients [47]. A novel algorithm, named a multi-biomarker disease activity (MBDA) score, has been developed based upon concentration measurements of 12 serum biomarkers (SAA, IL-6, TNF-RI, VEGF-A, MMP-1, YKL-40, MMP-3, EGF, VCAM-1, leptin, resistin, and CRP) to assess disease activity [48]. The MBDA score was significantly associated with the conventional disease activity score, which is a composite index of the number of swollen and painful joints [49]. Changes in MBDA scores were able to demonstrate clinically relevant reductions in disease activity scores, suggesting that this test has the potential to more quantitatively evaluate disease activity. Developing such biomarkers can help the clinical management of RA patients, both with regard to the prognosis and follow-up of patients during treatment.

11.4.2.2 Psoriatic Arthritis

Psoriatic arthritis (PsA) is an inflammatory arthritis condition associated with psoriasis. Approximately one-fourth of patients with psoriasis of the skin also develop PsA [50]. Identifying biomarkers, including genetic, blood, tissue, and transcriptional markers, would help in assessing the pathogenesis, diagnosis, and therapy of PsA. Increased serum concentrations of inflammatory markers like CRP, P-selectin, and haptoglobin and proinflammatory cytokines such as TNF, IL-6, IL-8, and IL-12 have been reported in studies with psoriatic patients [51]. Alenius et al. [52] have shown that serum IL-6 levels are elevated in PsA patients compared with psoriasis-only patients as well as that serum IL-6 correlated well with joint counts, ESR, CRP, and serum IL-2Rα. A combination of biomarkers that include Hs CRP, osteoprotegerin, matrix metalloproteinase 3, and CPII:C2C ratio has been suggested by Chandran and Gladman [53] to differentiate between PsA and psoriasis-only patients. Patients with PsA have also been shown to have higher concentrations of Dkk-1 and M-CSF levels compared to healthy controls [54]. It is anticipated that utilizing biomarkers such as these will aid in the diagnosis and treatment of PsA.

11.4.2.3 Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) involves chronic inflammation of all or part of the digestive tract. IBD primarily includes ulcerative colitis and Crohn's disease. The diagnosis of ulcerative colitis (UC) and Crohn's disease (CD) is largely based on endoscopic and histologic assessment of the inflamed tissue. Noninvasive, economical tests that can accurately rule in or rule out IBD as well as differentiate CD from UC would provide a valuable clinical resource [55]. Concentrations of fecal calprotectin, lactoferrin, and CRP have each been correlated with histologic and endoscopic disease activity in patients with UC and CD [56, 57]. Roseth et al. [58] demonstrated that patients with CD or UC who had remission following medical therapy demonstrated large reductions in levels of fecal calprotectin (to below 50 μg/g). Biomarkers have also been developed to identify patients that are likely to experience disease recurrence after treatment. Several studies have shown that in patients with quiescent disease, increased concentrations of fecal calprotectin predict disease relapse within 12 months, particularly in patients with UC [59, 60]. PR3-ANCA was proposed by Mahler et al. [61] as a novel biomarker. When tested by highly sensitive assays, PR3-ANCA was preferentially detected only in patients with UC and not in CD and therefore has clinical diagnostic and prognostic significance for IBD patients.

11.5 Case Study: Development of Bazedoxifene for the Prevention and/or Treatment of Postmenopausal Osteoporosis

The bazedoxifene case study is presented as an example of a traditional development plan that proceeded in a conventional manner. The target indication was well described and effective treatments available. Osteoporosis is a skeletal disorder characterized by compromised bone strength and increased risk of fracture [62]. A significant proportion of individuals with osteoporosis are postmenopausal women. After menopause, it is believed that the associated decline in estrogen production leads to an increased rate of bone turnover, with the net bone loss contributing to reduced bone strength and an increased risk of vertebral and other fractures [63]. One of the major determinants of bone strength and osteoporotic fracture risk is bone mineral density (BMD), as assessed by dual-photon absorptiometry (DPA) or dual-energy X-ray absorptiometry (DXA). BMD is reported as a T-score (≥ -1.0 is normal, between -1.0 and -2.5 is osteopenia, ≤ -2.5 is osteoporosis). Additionally, biochemical markers of bone turnover (e.g., serum type 1 collagen C-telopeptide [CTx]) are increased in metabolic bone disease that involves accelerated bone loss. Currently available pharmacologic agents for postmenopausal osteoporosis either maintain or increase BMD and decrease the rate of bone turnover.

Nonsteroidal selective estrogen receptor modulators (SERMs) are tissue selective agents, which act as estrogen receptor agonists in some tissues and as estrogen receptor antagonists in others [64]. Estrogens and SERMs are implicated in a variety of functions in the body, most notably the reproductive system, skeletal remodeling, and vasomotor functions, and they exhibit their mechanism of action by binding two types of estrogen receptors (ERs): ERα and ERβ. An ideal SERM would have beneficial agonistic effects on bone as well as the cardiovascular system to prevent bone loss and cardiovascular events without inducing hot flashes. The SERMs would also have antiestrogenic effects on the breast and endometrium to reduce the risk of breast and endometrial cancers. Bazedoxifene acetate is a SERM that is approved and marketed as Conbriza® or Viviant™ in a number of European and Asian countries for the prevention and/or treatment of osteoporosis in postmenopausal women. It was developed based on preclinical screening criteria, including favorable skeletal effects without adverse stimulation of endometrial or breast tissue.

In addition to showing an effect on BMD, regulatory guidances for developing drugs to treat or prevent osteoporosis require clinical demonstration of reduced fractures in postmenopausal women, which unfortunately is not directly measurable in a nonclinical setting [65]. For purposes of clarity and simplicity in discussing efficacy, this case study will focus primarily on BMD and bone turnover markers and no other efficacy measures such as vasomotor symptoms.

11.5.1 *In Vivo Pharmacology: Effects of Bazedoxifene on Bone Repair in Monkeys and Rats*

In a preclinical study with aged cynomolgus monkeys, bazedoxifene was evaluated for safety and efficacy toward preventing ovariectomy (OVX)—induced bone loss [66]. Animals (18 per group) underwent OVX and were administered bazedoxifene (0.2, 0.5, 1, 5, or 25 mg/kg/day) or vehicle, by daily oral gavage for 18 months. Animals in the second control group were sham operated and vehicle administered, also by daily oral gavage for 18 months.

Bone turnover markers were assessed at 6, 12, and 18 months, along with bone densitometry using DXA and peripheral quantitative computed tomography. Animals were sacrificed after 18 months, and uterine and pituitary weights were evaluated. Additionally, histomorphometric and biomechanical measurements were performed. OVX monkeys receiving vehicle showed increased bone turnover associated with osteopenia and slight decreases (not statistically significant) in biomechanical strength parameters at the lumbar spine and femoral neck. Bazedoxifene partially preserved bone mass by preventing the OVX-induced increases in bone turnover. Although the response was similar among all bazedoxifene-treated groups, the strongest efficacy was observed at 25 mg/kg/day. Treatment with bazedoxifene did not adversely affect measures of bone strength and was well tolerated; there was no evidence of uterotrophic activity, mammary tissue was unaffected, and there were no adverse effects on plasma lipids. The results of this study indicated that treatment of ovariectomized cynomolgus monkeys with bazedoxifene at least partially prevented changes in bone remodeling that correlated with increases in bone mineral density while maintaining bone strength and a favorable safety profile.

In a 6-week OVX rat model study [67], bazedoxifene was effective in maintaining bone mass at doses as low as 0.1 mg/kg/day, reaching maximal significant efficacy at a dose of 0.3 mg/kg/day. This dose maintained vertebral compressive strength (a surrogate for a reduced incidence of fracture) equivalent or better than the sham-operated animals. The histological quality of bone (assessed at the proximal tibia) was maintained and correlated well with the increases in BMD and compressive force data. In particular, bazedoxifene treatment prevented the loss in trabecular bone and prevented the increase in turnover markers induced by OVX, without affecting dynamic parameters of bone formation compared with sham-operated animals.

11.5.2 *Preclinical Pharmacokinetics*

Following administration of 0.2 mg/kg IV bazedoxifene to OVX female rats, the large volume of distribution (16.8 mL/kg) indicated wide tissue distribution. Plasma clearance (3.9 L/h/kg) was high, and the elimination half-life ($t_{1/2}$) was

short (3.8 h) [68]. Bazedoxifene was orally absorbed at a moderate rate in rats, reaching a mean maximum peak concentration of 4.7 ng/mL, at 2 h from a 1 mg/kg dose. The absolute bioavailability in rats was approximately 16%, based on the $AUC_{0-\infty}$ values obtained after the oral and IV dosing,

Following intravenous or oral administration of 1 mg/kg of radiolabeled [^{14}C] bazedoxifene to female rats, the mean elimination plasma half-life assessed by total radioactivity was approximately 29 h, showing the persistence of radiolabel on metabolites, compared with the previous assessment of 3.8 h, above. The plasma concentration versus time profiles following both intravenous and oral administration showed secondary peaks. This suggested either enterohepatic circulation or absorption from both small and large intestines, possibly mediating a relatively long elimination half-life and the slow total elimination of bazedoxifene-associated radioactivity.

Several aspects of the disposition of bazedoxifene in rats appear to be similar to those observed in postmenopausal women following oral administration. The absolute bioavailability after oral administration was low in both species: approximately 6% in women after a 10 mg dose (~0.14 mg/kg) and 16% in rats [69]. The pharmacokinetic profiles of bazedoxifene in women and in rats were indicative of enterohepatic recirculation, which may contribute to a long $t_{1/2}$ (approximately 30 h in women). Bazedoxifene was extensively metabolized in male and female rats, as well as in postmenopausal women through the glucuronidation pathway to yield bazedoxifene-5-glucuronide as the major circulating metabolite in both species. Little P450-mediated metabolism was evident in rats or humans. This observation was also supported by in vitro incubations of bazedoxifene in rat liver microsomes, which showed bazedoxifene-5-glucuronide was formed at a higher rate than bazedoxifene-4′-glucuronide. However, in humans bazedoxifene-4′-glucuronide was the predominant metabolite in hepatic microsomes [70].

11.5.3 Translation to Clinical Evidence of Efficacy

Two large, prospective, global phase 3 trials of bazedoxifene for osteoporosis prevention and treatment have been completed [71, 72]. Both studies were randomized, double-blind trials and included a placebo as well as a positive control (raloxifene).

In the 2-year study by Miller et al. [71], a total of 1583 healthy postmenopausal women were enrolled. These patients had either bone mineral density T-scores at the lumbar spine or femoral neck between −1.0 and −2.5 or clinical risk factors for osteoporosis and were randomly assigned to one of five groups: bazedoxifene 10, 20, or 40 mg/day, placebo, or raloxifene 60 mg/day. All the women received daily elemental calcium (600 mg) supplementation. Compared with placebo, all doses of bazedoxifene and raloxifene prevented bone loss at all four skeletal sites evaluated. The mean percent change differences in lumbar spine bone density from baseline at 24 months relative to placebo were 1.08% ± 0.28%, 1.41% ± 0.28%,

and 1.49% ± 0.28% for BZA 10, 20, and 40 mg, respectively ($P < 0.001$ for all comparisons). All the bazedoxifene treatment groups had significantly greater bone mineral density over the total hip in comparison with the placebo group at 6, 12, 18, and 24 months. Significant reductions in serum levels of osteocalcin and cross-linked CTx from baseline and relative to placebo were observed for all doses of bazedoxifene treatment by 3 months and were sustained until the end of the study ($P < 0.001$).

In a 3-year study by Silverman et al. [72], 7492 healthy postmenopausal women aged 55–85 years with osteoporosis were enrolled. These patients were defined by low bone mineral density or radiographically confirmed vertebral fractures and were randomized to treatment with bazedoxifene 20 or 40 mg/day, raloxifene 60 mg/day, or placebo. All the subjects received daily oral calcium (≤1200 mg) and vitamin D (400–800 IU) supplementation. The primary end point was the incidence of new vertebral fractures after 36 months, and secondary end points included nonvertebral fractures, bone mineral density, and bone turnover markers.

Treatment with bazedoxifene 20 and 40 mg and raloxifene 60 mg significantly increased lumbar spine and total hip bone mineral density, as well as reduced serum levels of osteocalcin and CTx compared with placebo. Among 6847 subjects in the intention-to-treat population, which includes every subject who is randomized and ignores noncompliance, protocol deviations, or withdrawal, the incidence of new vertebral fractures was significantly lower ($P < 0.05$) for bazedoxifene 20 mg (2.3%), bazedoxifene 40 mg (2.5%), and raloxifene 60 mg (2.3%) compared with placebo (4.1%). Relative to placebo, bazedoxifene 20 mg and 40 mg and raloxifene 60 mg reduced the risk of new vertebral fractures by 42%, 37%, and 42%, respectively. There were no significant differences in the incidence of new vertebral fractures between the bazedoxifene and raloxifene treatment groups. The incidence of nonvertebral fractures with bazedoxifene 20 mg (5.7%) and 40 mg (5.6%) and raloxifene 60 mg (5.9%) did not differ significantly from placebo (6.3%). However, in a post hoc analysis of a subgroup of women at higher fracture risk (femoral neck T-score ≤ -3.0 and/or at least one moderate or severe vertebral fracture or multiple mild vertebral fractures; $n = 1772$), bazedoxifene 20 mg showed 50% and 44% reductions in nonvertebral fracture risk relative to placebo ($P = 0.02$) and raloxifene 60 mg ($P = 0.05$), respectively. A similar reduction in nonvertebral fracture incidence was observed with bazedoxifene 40 mg, but the difference was not statistically significant.

Subjects who completed the 3-year study were eligible to enroll in a preplanned 2-year extension study [73]. Of the 4991 women who completed the initial 3-year study, 4216 chose to enroll in the extension study. In the extension study, subjects receiving bazedoxifene 40 mg were transitioned to bazedoxifene 20 mg after 4 years (40/20 mg), while those receiving bazedoxifene 20 mg continued. At 5 years, the incidence of new vertebral fractures in the intent-to-treat population was significantly lower with bazedoxifene 20 mg (4.5%) and 40/20 mg (3.9%) versus placebo (6.8%; $P < 0.05$), with relative risk reductions of 35% and 40%, respectively. Nonvertebral fracture incidence was similar among groups. In a subgroup of higher-risk women ($n = 1324$; femoral neck T-score ≤ -3.0 and/or

≥1 moderate or severe or ≥2 mild vertebral fracture[s]), bazedoxifene 20 mg reduced nonvertebral fracture risk versus placebo (37%; $P = 0.06$); combined data for bazedoxifene 20 and 40/20 mg reached statistical significance (34% reduction; $P < 0.05$). As shown in preclinical studies, as well as 2- and 3-year clinical studies, the effect of bazedoxifene continued for 5 years of treatment, significantly increasing BMD and reducing bone turnover versus placebo ($P < 0.05$) and was generally safe and well tolerated.

11.5.4 Translation of Preclinical to Clinical Evidence of Safety

Consistent with the nonclinical findings, women receiving bazedoxifene in the phase 3 trials had incidence rates of both common and serious adverse events similar to those for subjects receiving raloxifene or placebo. Additionally, there were no observed cardiac conduction safety signals. Measurement of QTc duration from ECGs collected from subjects in phase 1 thorough QT study at both therapeutic (20 mg) and higher (120 mg) doses did not reveal prolongation of the interval in subjects receiving bazedoxifene.

In the 2-year phase 3 study [71], serum concentrations of total cholesterol and low-density lipoprotein cholesterol were decreased with bazedoxifene and raloxifene treatments compared with placebo, whereas serum levels of high-density lipoprotein cholesterol were elevated after treatment with both SERMs. Although significant increases from baseline in the median concentrations of triglycerides were observed among women receiving bazedoxifene 20 mg and 40 mg and placebo, there were no significant differences between the groups.

In the 3-year phase 3 trial [72], bazedoxifene 20 mg and 40 mg doses were well tolerated. The incidences of adverse events, serious adverse events, discontinuations because of adverse events, and deaths in the bazedoxifene groups were generally similar to those in the placebo group. The most common adverse events were abdominal pain, accidental injury, arthralgia, back pain, flu syndrome, headache, hypertension, infection, and pain. The incidences of vasodilatation (hot flushes) and leg cramps were similar among the bazedoxifene and raloxifene treatment groups, but both were significantly higher than those in the placebo group. Most cases of vasodilatation were mild to moderate in severity and did not pose a safety concern sufficient to warrant discontinuation of therapy.

The overall frequencies of cardiovascular events and stroke were low and evenly distributed among the groups. The incidence of venous thromboembolic events (deep vein thrombosis and/or pulmonary embolism) was higher in the active treatment groups than in the placebo group, primarily because of an increased incidence of deep vein thrombosis, although the overall incidence of venous thromboembolic events in the active treatment groups was very low (<1%).

There was no significant difference in the incidence of venous thromboembolic events among the bazedoxifene and raloxifene treatment groups.

Breast and endometrial safety is an important issue in the clinical development of new SERMs. Special attention was paid to evaluation of the reproductive tract and breast safety in the phase 3 studies of bazedoxifene. In the 2-year phase 3 trial [71], bazedoxifene was not associated with a significant change from baseline in mean endometrial thickness compared with placebo. There was no confirmed diagnosis of endometrial hyperplasia or malignancy in the bazedoxifene and raloxifene groups. Furthermore, there were no significant differences among the groups in the change from baseline in ovarian volume, number or size of ovarian cysts, or incidence of ovarian cancer. Reports of breast pain and breast cancer were low and evenly distributed among the groups.

In the 3-year phase 3 trial [72], there were no significant changes in the mean endometrial thickness from baseline or in the incidence of endometrial hyperplasia and endometrial carcinoma among the groups. There were no clinically important changes from baseline in number or size of ovarian cysts among the groups. No significant difference was reported in incidence of breast cancer among the groups. There was a significantly lower incidence of fibrocystic breast disease for bazedoxifene compared with raloxifene. Mammogram analyses after 24 months of therapy revealed that the mean percent changes in breast density from baseline were low and did not differ significantly among the groups. In the 2-year extension study [73], bazedoxifene showed no evidence of endometrial or breast tissue stimulation over 5 years of therapy, consistent with findings at 3 years. Fewer cases of endometrial carcinoma were reported in the bazedoxifene group compared with the placebo group.

11.5.5 Summary

As may be seen by review of the previous case study, although there was no use made of PBPK modeling, population pharmacokinetic analyses, or exposure-response analysis to provide a quantitative underpinning of the development program, safety and efficacy were demonstrated in the clinical program.

11.6 Case Study: Development of Bapineuzumab for the Treatment of Alzheimer's Disease

Alzheimer's disease (AD) is the most common form of dementia, characterized by a gradual decline in cognitive functioning and memory [74]. Approximately 5.4 million Americans are affected by this illness. Thirteen percent of persons 65 years of age and older have AD, with 1275 new cases per 100,000 patients (65 years of

age and older) reported annually [75]. Advanced age is found to be the greatest risk factor for AD, although baseline mild cognitive impairment, previous head trauma, family history, and genetics also play a role [74]. The genetic factor, apolipoprotein E-ε4 (APOε4) genotype in particular, has been linked to late-onset AD. Inheriting a single ε4 allele increases the risk of late-onset AD by a factor of 4, whereas inheriting two alleles increases the risk by a factor of 19 [75–77].

The etiologic mechanism of AD is unknown; however, several postulated theories involve the formation and accumulation of amyloid beta (Aβ) peptide plaques in the cerebral vasculature. These peptides coincide with and appear to influence many of the abnormalities that result in the cognitive decline observed in patients with AD. Amyloid beta peptides are neurotoxic and drive the aggregation of cytotoxic tau proteins, also known as neurofibrillary tangles. Impaired cholinergic transmission (resulting from amyloid beta peptides) further fuels tau protein formation. Mitochondrial functioning also becomes impaired as a result of amyloid beta peptides and yields reactive oxidative species that mediate vascular inflammation and injury. The degree of amyloid beta plaque accumulation correlates with severity of disease [74].

Ever since the discovery that Aβ is the major constituent of amyloid plaques in AD and that familial AD results from mutations in the gene for amyloid precursor protein (APP) or in genes responsible for processing APP to Aβ [78], there has been a push to develop anti-amyloid therapeutics. Driven in part by the success of antibody therapies to target and destroy tumor antigens in neoplastic disease and by the absence of competition from less costly small molecules, immunotherapy against Aβ emerged as the industry's best hope for the first marketable disease-modifying agent for AD. Strategies to develop disease-modifying therapies for AD have been explored with the goal of reducing the formation of cerebral amyloid beta plaques via passive immunity or immunization to cause development of anti-amyloid beta antibodies.

In this section, we will discuss the example of bapineuzumab (AAB-001, Pfizer Inc./Janssen Pharmaceutical), a recombinant humanized monoclonal IgG1 antibody against the β-amyloid (Aβ) N-terminus(Aβ1–5), based on the murine antibody 3D6 and intended to promote Aβ clearance from the brain (molecular weight, 148,764 Da). Translational research for this compound was challenging as it is a biologic, rather than a small molecule, and its mode of action precludes its administration to healthy volunteers.

11.6.1 In Vivo Pharmacology

Bapineuzumab, a monoclonal antibody, is produced by a recombinant Chinese hamster ovary (CHO) cell culture process. It contains 1334 amino acids and is composed of two heavy chains and two light chains, which are disulfide bonded to form a tetramer. Each heavy chain is fully glycosylated and contains N-linked oligosaccharides of the type commonly observed in mammalian IgG antibodies.

Bapineuzumab selectively binds human Aβ peptide and was thought to provide passive immunity, reducing the formation of Aβ plaques and slowing disease progression.

Studies have been conducted to show that subcutaneous administration of murine monoclonal and polyclonal antibodies directed to the N-terminal region of Aβa1–42 are capable of reducing amyloid burden in PDAPP mice [79, 80]. Schenk reported that immunization of the young animals essentially prevented the development of Aβ plaque formation, neuritic dystrophy, and astrogliosis. Treatment of the older animals also markedly reduced the extent and progression of these AD-like neuropathologies. Janus et al. [79] demonstrated that immunization by administration of the monoclonal antibodies 3D6 and 10D5, which recognize Aβ amino acids 1–5 and 3–7, respectively, bound to plaque and significantly reduced amyloid burden, as did a polyclonal antibody preparation to Aβ1–42.1. These results raised the possibility that immunization with amyloid-β antibodies may be effective in preventing and treating Alzheimer's disease.

Morgan et al. [81] showed that in a transgenic model for AD in which mice develop learning deficits as amyloid accumulates, vaccination with Aβ protects transgenic mice from the learning and age-related memory deficits that normally occur in this model for AD. During testing for potential deleterious effects of the vaccine, all mice performed superbly on the radial arm water-maze test of working memory. Later, at an age when untreated transgenic mice show memory deficits, the Aβ-vaccinated transgenic mice showed cognitive performance superior to that of the control transgenic mice and, ultimately, performed as well as non-transgenic mice. The Aβ-vaccinated mice also had a partial reduction in amyloid burden at the end of the study. From these results, the authors concluded that this therapeutic approach may prevent and, possibly, treat AD. Because human IgG1 is most similar to mouse IgG2a, bapineuzumab was engineered as a human IgG1 isotype to retain the properties attributed to antibody isotype.

11.6.2 Preclinical/Clinical Comparison of Pharmacokinetics

Pharmacokinetic behavior of bapineuzumab was evaluated in mice, Sprague-Dawley rats, New Zealand White rabbits, and cynomolgus monkeys. PK data in all animal species tested showed a biphasic decline after IV administration, dose-dependent increases in bapineuzumab area under the curve (AUC) and peak plasma concentration (Cmax), a long elimination half-life ($t_{1/2}$), and limited distribution (primarily to the vascular compartment), characteristics that are typical of monoclonal antibodies. Simulations based on the minimal effective serum concentration needed for efficacy in the PDAPP mouse and the PK parameters in animal species indicated that a human dose in the range of 1–5 mg/kg would maintain the desired serum levels at or above 3.7 μg/mL for approximately 1 month.

In clinical studies of humans, bapineuzumab was administered intravenously and reached peak concentrations at the end of the 1-h infusions. Both single-dose

and multiple-dose pharmacokinetic studies were performed. After single doses administered to patients with Alzheimer's disease [82], a threefold increase in dose from 0.5 to 1.5 mg/kg resulted in approximately sixfold greater exposure, while the additional increase from 1.5 mg/kg to 5 mg/kg resulted in an increase of only 2.6-fold higher. The volume (Vss) was small (69.7 ± 10.1 mL/kg), clearance low (0.190 ± 0.040 mL/h/kg), and half-life long (24.0 ± 4.0 days) after a single 0.5 mg/kg dose.

A population pharmacokinetic analysis of the samples collected as part of the phase 3 studies, where patients received doses every 13 weeks for a total of six doses of 0.5, 1, or 2 mg/kg, infused over 1 h, confirmed the pharmacokinetic characteristics observed in the single-dose phase 1 study [83]. Although there was an observed impact of body weight on both volume and clearance, there was no evidence that APOε4 carrier status affected the clearance of bapineuzumab, and the target concentration appeared to be maintained.

There was no investigation of the potential for drug-drug interactions because monoclonal antibodies such as bapineuzumab are generally considered to be catabolized and eliminated by processes involved in the turnover and degradation of endogenous immunoglobulins [84]. Such monoclonal antibodies are degraded to constituent amino acids, which can then be reincorporated into newly synthesized proteins or utilized as an energy source. They are not metabolized via cytochrome P (CYP) 450 systems, but are degraded to individual amino acids, no reactive metabolites are generated. Consequently, traditional metabolism, elimination, or drug-drug interaction studies, as would typically be conducted for small-molecule drugs, are not considered necessary or useful for biologics such as bapineuzumab [24].

11.6.3 Preclinical/Clinical Evidence of Efficacy

Assessment of cognition is difficult in preclinical models. Healthy volunteers can be assessed and serve as positive control subjects. Validated efficacy assessment scales for AD include the Mini-Mental State Examination (MMSE), Alzheimer's Disease Assessment Scale—Cognitive Subscale (ADAS-Cog), and Disability Assessment for Dementia (DAD). These tools are designed to evaluate several areas of function, including cognition, functional capacity, behavior, general physical health, and quality of life. MMSE and ADAS-Cog assess cognitive areas whereas DAD is a functional test.

Most assessment tools are designed to be completed either by the patient (in the early stages of disease), the caregiver, or the patient's primary healthcare provider. Most often, a combination of tests is needed to complete an evaluation of the patient's overall condition. Assessments given by the primary caregiver often evaluate not only the patient's condition but also the caregiver's own well-being, which can be an important factor in deciding whether a particular treatment strategy has proven beneficial.

For patients with Alzheimer's disease or a related dementia, there is no single test that can simultaneously assess all areas of functioning. The aim of such tests is to better understand the actual efficacy of treatments and to develop a comprehensive, practical assessment that can be administered quickly by a clinician.

The first phase 2 study [85] enrolled 234 patients, randomly assigned to receive placebo or bapineuzumab in four dose cohorts (0.15, 0.5, 1, or 2 mg/kg). Patients received six infusions, 13 weeks apart, with final assessments at week 78. The prespecified primary efficacy analysis assumed linear decline and compared treatment differences within dose cohorts on the ADAS-Cog and DAD scales. No significant differences were found in the primary efficacy analysis. Exploratory analyses combined dose cohorts and did not assume a specific pattern of decline. These exploratory analyses showed potential treatment differences ($P < 0.05$, unadjusted for multiple comparisons) on cognitive and functional end points in study "completers" and APOε4 noncarriers. Reversible vasogenic edema, detected on brain magnetic resonance imaging (MRI) in 12/124 (9.7%) bapineuzumab-treated patients, was more frequent in higher-dose groups and APOε4 carriers. Six vasogenic edema patients were asymptomatic; six experienced transient symptoms. Overall, primary efficacy outcomes in this phase 2 trial were not significant. However, the potential treatment differences in the exploratory analyses were encouraging and supported further investigation of bapineuzumab in phase 3 with special attention to APOε4 carrier status.

A second phase 2 multicenter, placebo-controlled, double-blind, ascending-dose study [86] was conducted in 28 patients 50–80 years of age who received doses of 0.5, 1, or 2 mg/kg or placebo. As in the previous study, patients received up to six infusions 13 weeks apart and underwent positron emission tomography (PET) scans at baseline and at weeks 20, 45, and 78 in order to determine the retention ratio of Pittsburgh compound B (^{11}C-PiB), which is thought to reflect the clearance of amyloid beta peptides, in predefined cortical areas of the brain. Even though bapineuzumab patients experienced reduced cortical ^{11}C-PiB retention from baseline, increased retention was observed in the placebo group. The clinical impact of this phase 2 study was unclear, and further investigation was deemed necessary for possible translation into clinical benefit.

Treatment response in patients with the APOEε4 genotype versus patients without the APOEε4 genotype was assessed in two phase 3, multicenter, randomized, double-blind, placebo-controlled studies [87]. The first study, enrolling 1331 patients without APOEε4, compared active treatment, 0.5 and 1 mg/kg of bapineuzumab, each administered as six infusions, 13 weeks apart, to placebo. The second study enrolled 1121 patients with the APOε4 genotype and compared responses after six infusions of bapineuzumab (0.5 mg/kg) given 13 weeks apart to placebo. The co-primary end points for each trial were the changes in ADAS-Cog and DAD scores from baseline. Secondary end points included brain amyloid burden on PiB-PET, cerebrospinal fluid (CSF) phospho-tau, and MRI brain volume. Neither study found any statistically significant differences in the co-primary efficacy end points of ADAS-Cog nor DAD scores versus placebo in APOε4 carriers and noncarriers. However, a secondary end point analysis revealed a

reduction in amyloid plaques on PET imaging in APOε4 carriers in mild AD patients. Similar results were also reported by a pooled analysis of the two trials in both co-primary and secondary end points. In addition, significant decreases in CSF phospho-tau concentrations in APOEε4 carriers receiving 1 mg/kg, were observed in a subgroup analysis of patients with mild and moderate AD. Pooled analyses also revealed a decline in MRI brain volume. However, based on the lack of clinical efficacy in ADAS-Cog and DAD scores in these two studies, phase 3 trials investigating the long-term efficacy and tolerability of bapineuzumab over a 4-year period were halted.

11.6.4 Evidence of Safety

Adverse events that were reported as being greater than 5% (and twofold higher than placebo) in the phase 2 study [85] included back pain, anxiety, vasogenic edema (VE), paranoia, vomiting, hypertension, weight loss, skin laceration, gait disturbance, and muscle spasm. Vasogenic edema was the only event noted to be dose related; it was detected on MRI in 12 patients (9.7%), but it was not detected in any patients receiving placebo. Ten of the 12 cases of VE were detected in APOε4 carriers, and incidence rates increased as bapineuzumab doses were escalated. Half of these patients were symptomatic, with most patients commonly reporting headache, confusion, vomiting, and gait disturbances. Symptoms resolved in the majority of patients after bapineuzumab was discontinued.

In the second phase 2 study [86], adverse events were reported in 19 of 20 bapineuzumab-treated patients and in all eight placebo-treated patients. The events most commonly reported (in 10% or more of bapineuzumab patients) were headache, fatigue, nasopharyngitis, diarrhea, urinary tract infections, falls, abrasions, and muscle spasms. Most adverse events were generally mild to moderate and transient; however, serious events were reported in four bapineuzumab-treated patients and in three placebo-treated patients, with no relation to dose. Two patients in the 2 mg/kg bapineuzumab group experienced cerebral VE, as identified on MRI scans, and were found to be APOε4 carriers. Both patients were asymptomatic and developed VE after the first bapineuzumab dose. The edema resolved after treatment was discontinued.

Both phase 3 studies reported a higher rate of VE in both the APOε4 carriers and the noncarriers after treatment. Treatment-emergent vasogenic edema was observed in 15.1% of the APOε4 carriers receiving bapineuzumab versus 0.2% of patients receiving placebo. Of the noncarriers, 4.2%, 9.4%, and 0.2% of patients in the 0.5, 1 mg/kg, and placebo groups, respectively, were affected. Of the APOε4 carriers and noncarriers found to have VE, 2.4% and 1.5% reported symptoms. Other adverse events such as syncope, dehydration, and pneumonia occurred at similar rates between patients receiving bapineuzumab and placebo in both APOε4 carriers and noncarriers.

As noted above, bapineuzumab was found to cause vasogenic edema (VE) of the brain in some subjects. This term describes a radiographic finding detected as high signal intensity on fluid-attenuated inversion recovery (FLAIR) MRI sequences. Recognition of what was labeled VE in early trials of bapineuzumab led to the institution of MRI assessments at prespecified time points. However, the term VE was problematic since it was used to include FLAIR hyperintensities seen both in the brain parenchyma (consistent with cerebral edema) and in cerebral sulci (indicative of extraparenchymal effusion).

The MRI signal abnormalities observed in VE are believed to be related to movement of amyloid from the parenchyma into the perivascular space as well as removal of vascular amyloid. It is hypothesized that these shifts in amyloid result in extravasation of fluid, which in turn manifest as several amyloid-related imaging abnormalities (ARIA), including edema (ARIA-E) and hemosiderin deposition (ARIA-H) , either within the brain parenchyma or within cortical sulci.

The smaller findings of ARIA-H are microhemorrhages and are seen with increased incidence in patients with AD. Small hemosiderin deposits occur spontaneously in up to 19% of normal elderly people 3 and up to 32% of AD patients [88].

ARIA-E and ARIA-H can occur spontaneously or in the course of treatment with amyloid-lowering agents [89]. Most cases of VE have not been associated with clinical observations. However, some cases of VE have been associated with clinical observations such as altered mental status, seizures, gait difficulties, visual disturbances, elevated blood pressure, vomiting, headache, fatigue, dizziness, syncope, weakness on one side of the body, and irritability. Mild increases in white blood cells and elevated protein levels have been reported in the cerebrospinal fluid (CSF) of some subjects with VE who have undergone lumbar puncture. VE generally is observed after the first or second infusion, although it has been observed after later infusions in some subjects. In individual subjects, MRI findings may vary over time, but VE usually improves gradually. In some subjects, neurological deficits have persisted after MRI resolution of the VE. Although symptoms have improved in most subjects, not all subjects have recovered, and it is possible that complications associated with VE could lead to chronic disability and/or death.

Although the underlying pathophysiological mechanisms for ARIA are unknown, it has been proposed that a reduced vascular integrity caused by an aggressive lowering of both central and vascular Aβ might be involved [90]. Additionally, these ARIA phenomena are not associated with evident restricted diffusion, tissue necrosis, or other evidence of cytotoxic edema. It is assumed that they represent transient breakage of the blood-brain barrier (BBB).

Findings from using the PDAPP mouse model imaged with gadolinium-enhanced dynamic MRI resulted in detection of numerous BBB disruptions upon anti-Aβ immunotherapy. However, PDAPP control and wild-type mice showed no evidence of leakage; these disruptions can occur within a week of treatment initiation and resolve quickly, within 7 days, despite persistence of antibodies, and these experiments support the hypothesis that treatment with bapineuzumab leads to leakage from amyloid-laden vessels consistent with ARIA in PDAPP mice.

The authors concluded that this methodology may be useful to explore the underlying biological mechanism of BBB leakage and assess the safety profile (ARIA) of other anti-Aβ compounds.

11.6.5 Biomarkers

AD is a progressive neurodegenerative disease characterized neuropathologically by cerebral neuronal loss, deposits of extracellular amyloid beta (Aβ) plaques, and intraneuronal neurofibrillary tangles with accompanying decreases in CSF of Aβ and increases in CSF tau proteins such as total tau and phosphorylated tau. The effect of bapineuzumab on CSF biomarkers reflecting Aβ homeostasis, neuronal degeneration, and tau-related pathology, in the patients enrolled in the previously described phase 2 studies was reported by Blennow et al. [91]. Within the bapineuzumab group, at the end of the study, a decrease was found both for CSF T-tau (−72.3 pg/mL) and P-tau (−9.9 pg/mL) compared with baseline. When comparing the treatment and placebo groups, this difference was statistically significant for P-tau ($P = 0.03$), while a similar trend for a decrease was found for T-tau ($P = 0.09$). No clear-cut differences were observed for CSF Aβ. The authors concluded that CSF biomarkers may be useful to monitor the effects of novel disease-modifying anti-Aβ drugs in clinical trials.

As mentioned previously, carbon-11-labeled Pittsburgh compound B (^{11}C-PiB) PET is a marker of cortical fibrillar Aβ load in vivo. In the first phase 2 study described above, patients had ^{11}C-PiB-PET scans at baseline and at weeks 20, 45, and 78. The primary outcome was the difference between the pooled bapineuzumab group and the pooled placebo group in mean change from screening to week 78 in ^{11}C-PiB cortical to cerebellar retention ratio averaged across six cortical regions of interest. It was concluded by the authors that ^{11}C-PiB-PET seemed to be useful in assessing the effects of potential AD treatments on cortical fibrillar Aβ load in vivo.

11.6.6 Exposure-Response Analyses

Exposure-response (ER) analyses evaluate relationships between drug concentrations or exposure (AUC) and relevant response or pharmacodynamics end points, including the study of the impact of covariates, defined as variables that may be possibly predictive of the response.

Samtani et al. [92] established a population-based pharmacodynamic disease progression model using pooled data from the phase 3 studies described above. This model estimated longitudinal changes in disease progression, measured by ADAS-Cog/11 scale, after bapineuzumab treatment, and identified covariates contributing to the variability in disease progression rate and baseline disease status. The analysis showed no effect of bapineuzumab on ADAS-Cog/11 progression rate,

consistent with the lack of clinical efficacy observed in both studies. Sex, APOEε4 carrier status, and years since disease onset significantly influenced baseline ADAS-Cog/11 scores, while age, years since disease onset, and APOε4 carrier status had significant effects on disease progression rate. The influence of bapineuzumab exposure on disease progression was not significant.

An ER analysis using a disease progression model for the DAD end points was conducted using data from the two phase 3 studies described previously [93]. It evaluated longitudinal changes in DAD scores, baseline factors affecting disease progression, and bapineuzumab effect on disease progression. Similar to ADAS-Cog, the estimated treatment effect of bapineuzumab was not significant, consistent with lack of clinical efficacy observed in the primary analysis. The model suggested however that progression of DAD tended to decrease with increase in bapineuzumab exposure. The ER relationship was similar regardless of the APOε4 status but more pronounced in patients with mild AD. Baseline disease status, age, memantine (a concomitant medication indicated for Alzheimer's disease) use, and years since onset had significant effects on baseline DAD scores.

11.6.7 Summary

The extensive analysis of the data collected in the clinical studies conducted to investigate the effectiveness of passive immunization in the treatment of Alzheimer's disease was unfortunately unable to identify patients for whom treatment would be successful, despite early demonstration of promise of limiting disease progression. The clinical trials were informative with respect to assessing efficacy, tolerability, biomarkers, and imaging techniques. Exploratory analyses showed hints at cognitive benefit in mild patients, and an alternative baseline for the Mini-Mental State Examination suggested a benefit [94]. Perhaps treatment would be effective if administered earlier, and perhaps it is important to better understand the impact of APOε4 carrier status [95].

11.7 Conclusions

T2 translation research refers to those later-stage clinical studies that are aimed at translating knowledge from preclinical and early-stage clinical observations of healthy volunteers into patients and ultimately toward treatment plans. As has been described in this chapter, the repeated use of careful experimental design, followed by appropriate analysis, modeling, and simulation to describe pharmacokinetic parameters in humans, can help in the efficient and robust development of medications. Significant learnings can also be gained from drug candidates and studies that were not successful. While careful observations of safety signals and evidence of efficacy must be made in patients treated in clinical trials, future

translational activities are greatly aided by attempts to link clinical observations to data from previous preclinical models (T1) as well as those in healthy volunteers participating in the FIH trials (T2).

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Chapter 12
Regulatory Aspects at the Drug Discovery Development Interface

Lynn Gold and Ken Phelps

Abstract While discovery scientists are interested in proof-of-concept data, regulators seek key safety data prior to advancing new drug candidates into first-in-human (FIH) clinical trials. Safety as well as therapeutic activity data continues to be collected and evaluated at each subsequent clinical trial stage of a program to gain proof of concept. This requires clear research and development targets to help guide the development pathway to provide phase-appropriate investigational new drug (IND) submissions.

In early discovery a customized development target product profile (dTPP) describing the key attributes of the proposed product is recommended. This dTPP defines the important aspects of the discovery target that guide the development program, and as it evolves, it becomes aligned with what regulatory reviewers have outlined as the key information for ultimate approval of the proposed product. These details provide scaffolding for a development pathway with appropriate regulatory targets and a foundation for the initial IND submission. The IND is the detailed description of the sponsor's safety data, clinical study design, and drug product.

The goal of early studies in humans is the generation of sufficient data to transition to the next critical milestone and eventually to a safe, therapeutically beneficial, and therefore marketable drug product. Ultimately, this necessitates developing a product that keeps all stakeholders in mind: the innovator, the patient or end user, and regulators. Essential to achieving that goal is a strong foundation that is described in the IND.

Keywords Investigational new drug • Discovery • Safety • First-in-human • FDA • Clinical hold • Drug substance • Drug product • Nonclinical • Clinical

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12.1 Introduction

The focus of drug discovery is on evaluating and optimizing compounds that can provide a therapeutic effect against a specific disease state. In addition to efficacy, drug candidates must also demonstrate appropriate safety and pharmacokinetic profiles. Extensive datasets are generated in preclinical species to provide confidence that the compound is worthy of advancement into the resource-intensive clinical space. However, none of this work receives regulatory scrutiny until the compound is ready to be advanced into clinical trials.

For a promising drug candidate to become a commercially viable drug product, it must receive approval from the specific regional regulatory agency in which the product is to be marketed. Approval requires testing under controlled settings in clinical trials designed to evaluate safety and efficacy. This applies to both synthetically prepared new chemical entities (NCEs) and new biological entities (NBE) produced from recombinant cell lines. While the regulatory concepts for both therapeutic modalities are similar, the specific requirements are distinctly different. This chapter focuses on NCEs.

There are different regulatory agencies for different countries or regions in which the first-in-human (FIH) study may be performed. Examples include the US Food and Drug Administration (US FDA); the European Medicines Agency (EMA); Health Canada; the Ministry of Health, Labour and Welfare (MHLW) in Japan; and Brazil's National Health Surveillance Agency (Anvisa). All regulatory agencies review preclinical efficacy, safety/toxicology, and pharmacokinetic data as well as the proposed clinical plan.

First-in-human (FIH) testing requires a great amount of scrutiny, as there are many unknowns at this stage. In the USA, the FDA requires the submission of an investigational new drug (IND) application to advance to FIH studies. The IND is discussed further below.

12.2 US FDA Regulatory Expectations and Guidelines for a Phase 1 FIH Clinical Study

A new drug candidate must meet regulatory requirements prior to human testing. If the new drug candidate is a new chemical entity, defined in 21 CFR §314.108(a) as a drug that contains no active moiety that has been approved by the FDA in any other application submitted under Section 505(b) of the Federal Food, Drug, and Cosmetic Act (see Example 1), then an IND will be required to allow the first-in-human (FIH) clinical study to proceed. The FDA also classifies these drugs as new molecular entities (NME). The key is the definition of active moiety per 21 CFR §314.108 (a); an active moiety is the molecule or ion, excluding those appended portions of the molecule that cause the drug to be an ester, salt (including a salt with hydrogen or coordination bonds), or other non-covalent derivatives (such as a

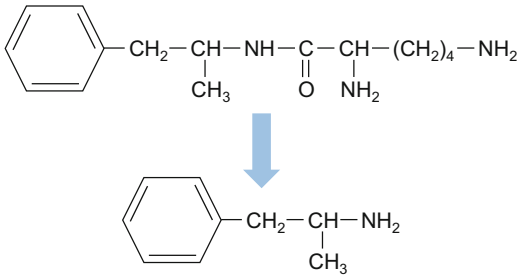

Fig. 12.1 Lisdexamfetamine to dextroamphetamine (Vyvance® NDA 21977)

complex, chelate, or clathrate) of the molecule that is responsible for the physiological or pharmacological action of the drug substance.

Example 1
An instructive example distinguishing a new chemical entity is the drug product Vyvance® (NDA 021977 February 23, 2007), containing the drug substance lisdexamfetamine dimesylate. The active moiety of this compound, dextroamphetamine, was previously approved in a new drug application (NDA) for Dexedrine® (NDA 017080, August 2, 1976). However lisdexamfetamine is a lysine prodrug of dextroamphetamine, requiring metabolic cleavage of the lysine amide bond to release dextroamphetamine, as shown in Fig. 12.1. As such, lisdexamfetamine is a new chemical entity and has been classified as a new molecular entity by the FDA.

Terminology can differ among the regulatory agencies. The EU has published a position paper on this topic, along with a description of when exclusivity can be obtained for a new active substance (March 2015). Article 10.2.b of Directive 2001/83/EC states, "The different salts, esters, isomers, mixtures of isomers, complexes or derivatives of an active substance shall be considered to be the same substance unless they differ significantly with regard to safety and/or efficacy."

Regulatory agencies have developed guidance documents to aid researchers in advancing NCEs to FIH studies. Guidance documents can be found here: the FDA website for guidances, the International Conference on Harmonisation (ICH) website for harmonized guidances, and the European Medicines Agency (EMA) website for human regulatory guidance documents. Further information can also be obtained from the pharmacopeia in the region in which the FIH studies are planned.

The FDA's guidance documents are based on information gathered from the FDA's vast experience with a wide variety of development programs across the pharmaceutical industry, which no one individual drug sponsor organization possesses. Discovery organizations can benefit from these guidance documents, which are continually drafted, published, and updated by the FDA to enable the optimal

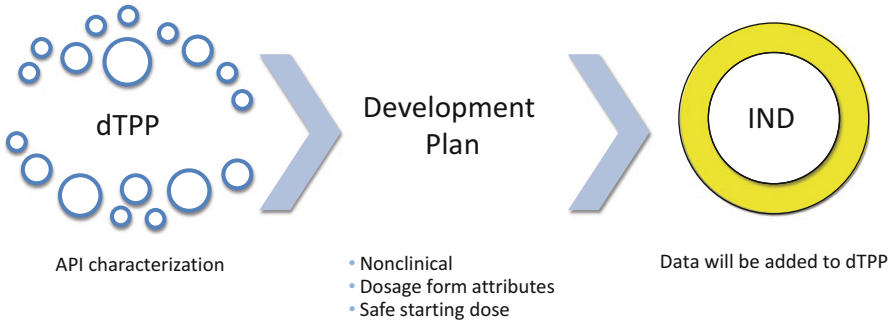

Fig. 12.2 The development target product profile (dTPP) drives the development plan based on the gaps needed to support the IND filing goal for the proposed new drug product

and most efficient translation of molecules to medicine. Prior to clinical testing, an investigational new drug (IND) application must be submitted. This in turn requires establishing a development plan based on the development target product profile (dTPP) to achieve the IND target, as shown in Fig. 12.2.

12.3 The Drug Development Plan

A development plan establishes a pathway to an IND. This chapter primarily focuses on the US filing requirements and may also reference the United States Pharmacopeia and National Formulary (USP/NF) for any available methods and monographs describing active pharmaceutical ingredients (APIs), analytical methods, dosage forms, container closures, and excipients. Other pharmacopeias may be referenced in specific cases depending on the supporting data and circumstances. An IND may be prepared that contains completely novel information and therefore does not reference anything in the USP/NF, except possibly the general chapters. The value of the USP/NF is as a benchmark for aspects of development programs.

All sponsors with development programs at the interface between the early-stage development and the FIH studies should brainstorm the various alternative development pathways available. Key nonclinical studies provide a mechanism for establishing safety guidelines prior to the FIH study initiation. Consideration should be given to all aspects that are known and potentially unknown about the drug product under development. Think about the impact on safety regarding the use of the proposed new drug product (Example 2).

Example 2
At the time of this work, a tragic example of the potential consequences of unknowns in a phase 1 (FIH) study of the investigational drug BIA 10–2467 (an inhibitor of fatty acid amide hydrolase or FAAH) involving healthy volunteers is currently being investigated to understand the cause(s). The FDA is participating in a thorough evaluation of the early safety data generated to support the Bial-Portela & Ca. SA of Sao Mamede do Coronado, Portugal, study of the investigational drug BIA 10–2467, conducted in France, resulting in a halted clinical study with the death of one healthy volunteer and neurological injury to four others (FDA January 31, 2016). The lessons learned from this program will inform the expectations for development programs for early safety studies for new chemical entities (NCEs) in the future. Close attention to the evolution of these learnings is warranted. The FDA will also collect and review safety information pertinent to FAAH inhibitors under investigation in the USA.

12.4 Development Target Product Profile

A team of research and development scientist needs a common target for their product development, which is represented by the bulls eye in Fig. 12.3. The target product profile (TPP) summarizes the product to be developed; desired features, studies, and activities necessary to demonstrate safety and efficacy; and distinguishing features that provide a competitive advantage. An early version of the TPP is a development (d)TPP, represented by the blue ring of the target in

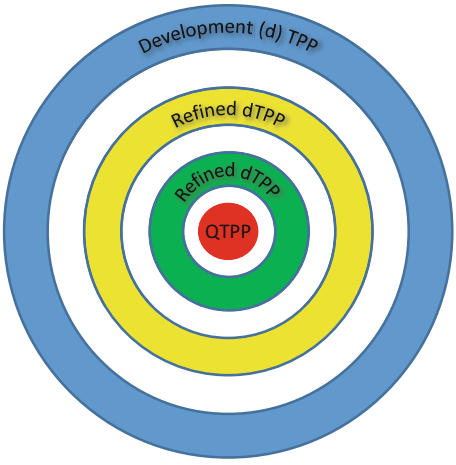

Fig. 12.3 Target product profile bulls eye

Fig. 12.3. The early development target requires refinement as additional knowledge is gained. As the discovery process evolves, the dTPP also evolves, through versions that become closer and closer to the quality (Q)TPP, which represents a safe and efficacious drug product (depicted by the bullseye in Fig. 12.3). A TPP helps communicate the development intentions for a new program to all stakeholders, which at an early stage are representatives of various scientific disciplines on a project team and possibly regulatory reviewers. The FDA documents *Guidance for Industry and Review Staff Target Product Profile—A Strategic Development Process Tool* (March 2007) and *Guidance for Industry*: *Q8 (R2) Pharmaceutical Development* (November 2009) both describe the use of TPPs.

A TPP is customized to the sponsor's development program, with the concept of the labeling for the commercial drug product in mind. As the TPP evolves, it may eventually become the package insert. Often, a sponsor will work backward from the desired labeling drug attributes and cautions to determine optimum development choices. This approach supports the design of efficacy, safety, and toxicology studies as well as critical quality attributes for the proposed drug product.

At a minimum key attributes initially included are indication and usage, dosage and administration, and dosage forms and strengths under consideration. Eventually the TPP is expanded to include dosage forms and strengths that represent the commercial target, contraindications, adverse reactions, clinical pharmacology, nonclinical toxicology and clinical studies, warnings and precautions, drug interactions, use in specific populations, drug abuse and dependence, over dosage, description, how supplied, storage and handling, and patient counseling information.

The first steps in the preparation of a dTPP are to define the known attributes of the new moiety and to consider what the patient and regulatory needs are as dictated by the phase of development the product is in. This begins with a dTPP that outlines the known characteristics of the drug substance, including physical and chemical attributes, some of which are inherent and others based on the current route of synthesis. Characteristics such as impurity profile and particle morphology can be critical especially if the route of administration is a solid oral dosage form. In contrast, if the proposed product is an oral solution, the impurity profile is important, but the particle morphology is less critical. The initial development priority to achieve the IND is safety. This takes the form of a well-characterized impurity profile in the early nonclinical studies for a potential oral dosage form and sterility if the dosage form is intended to be parenteral.

It is also important to consider which attributes might be altered during the development program and the impact of these alterations. For example, an early development program may begin with a parenteral solution formulation to gain critical human pharmacokinetic data such as volume of distribution and clearance. Subsequently it may advance to an oral dosage form such as drug in bottle (DIB) or drug in capsule (DIC) for further development. As a result, bridging studies are typically required to demonstrate the similarities and differences in the systemic exposure from the different delivery paradigms. Additionally, bridging of the purity

and potency for the different dosage forms and formulations is critical to support the exposure levels established in the various early development studies. Nonclinical studies using relevant and comparable dosage forms also provide guidance for clinical studies toward establishing dosage formulation specifications.

Example 3
Example development target product profile (dTPP) for an oral solution evolving into an example quality target product profile (QTPP) for a solid oral dosage form

Item	dTPP	QTPP
Description	Solution for injection	White film-coated oval tablet embossed with "logo" on one side and plain on the other
Active pharmaceutical ingredient (API)	Chemical name	Chemical name
Chirality	Racemic	S-Enantiomer
Polymorphic form	Unknown	Amorphous form
Impurities	NMT 5%	Not more than 2%
Dosage form	Parenteral solution	Solid oral dosage form
Strength	100 mg/10 mL	50 mg, 100 mg, and 200 mg
Dosing instructions	One infusion of 10 mL per day	Take one tablet twice a day for 2 weeks
Assay	90.0–110.0%	92.0–110.0%
Impurities/related substances	Total impurities NMT 5% Unknown impurities NMT 0.1%	Total impurities NMT 2% Unknown impurities NMT 0.1% Specified impurities NMT 1%
Dissolution	Not applicable	85% of the drug is dissolved in 15 min
Uniformity of content	According to USP <905>	According to USP <905>
Packaging	10 ml Vial	14 tablets per blister Two blisters per carton
Particulate matter	Per USP<788>	Not applicable
Microbial evaluation	Not applicable	USP<61> and <62>
Bioburden	USP<85>	Not applicable
Sterility	USP<71>; terminal sterilization	Not applicable
Storage conditions	Store at room temperature per USP definition	Store at room temperature per USP definition
Retest data/expiration date	12 months, room temperature	36 months, room temperature

In addition, the quality of the API and the excipients in a new drug product for the early development studies should be considered and documented. Some excipients can impact the release of the drug or the gastric retention

(continued)

of the drug and others may not. For example, peppermint flavor is known to reduce gastric motility. Careful consideration of all attributes will allow for a more complete interpretation of the experimental data that are obtained.

Target product profiles are living documents intended to provide an overall goal for the safety and efficacy of a new investigational drug. They should provide an accurate snapshot of attributes at a specific stage in development and are modified as new learnings are provided from the ongoing research program and throughout the life of the product.

12.5 The Investigational New Drug (IND) Application

Once an internal drug candidate has been selected for development-based target activity, efficacy, physicochemical properties, pharmacokinetics, safety, tolerability, and/or toxicity profile, the next milestone is the first-in-human (FIH) clinical trials. For FIH studies targeting the US market, an investigational new drug (IND) submission is necessary to demonstrate the safety of the compound. It is valuable and instructive to conduct a pre-IND meeting with the FDA.

12.5.1 Pre-Investigational New Drug (PIND) Submission Meeting

Prior to preparing an IND submission, a gap analysis is recommended to establish a risk assessment for the program. The identified risks provide key questions that can be vetted with the FDA review division in a pre-IND (PIND) meeting (May 2009). This is also an excellent opportunity to engage the FDA review division and clarify the nonclinical and clinical requirements for the investigational new drug (IND) application.

Profiles which represent high risk include:

- New disease areas with no current FDA guidance documents.
- New targets which pose safety and efficacy concerns.
- New drug classes which invoke pharmacokinetic, pharmacodynamic dosing and duration of action concerns.
- -New dosage forms which may invoke pharmacokinetic, pharmacodynamic dosing and duration of action concerns.
- New manufacturing processes which pose yield, formulation, and cost of goods questions.
- Uncertain intellectual property which casts doubt on the potential return on investment.

If the proposed drug is to be used in a new therapeutic area, this will require significant background and justification. However, a drug targeting a well-established therapeutic area with a straightforward animal species model can be justified based on the current available literature.

A PIND meeting request includes a list of questions to be reviewed and addressed by the appropriate FDA division. This may result in a face-to-face meeting, a teleconference, and written responses only (WRO), the meeting request may be denied, or a different approach may be suggested depending on the perceived risk profile of the drug candidate and the therapeutic area. When a meeting is granted, a PIND meeting package must be provided containing background information regarding agency questions as well as the draft dTPP. Written responses to the sponsor's questions are provided prior to the meeting, and meeting minutes are typically provided by the FDA following the meeting. The PIND meeting minutes contain information from the FDA for the sponsor to consider prior to the investigational new drug (IND) submission.

12.5.2 IND Introduction

The simplest and currently recommended approach to the construction of the IND submission is to follow the electronic common technical document (eCTD) format (GFI May 2015). Commercial INDs may still be filed as paper applications up to May 15, 2018. From that date forward, commercial INDs will be required to be in eCTD format for FDA review.

In addition to the IND relevant CFR section references, the current FDA guidance documents can be found on the FDA website. A list of some guidance documents, relevant to an IND to support a FIH study, is provided in the supplemental information at the end of this section. These guidance documents provide a framework for the structure and content of an IND at the various stages of development. The overall structure of an eCTD-formatted IND is well defined; however, each IND submission is a unique work product.

12.5.3 IND Preparation

The current version of the Comprehensive Table of Contents Headings and Hierarchy (Version 2.3, 2014) describes the appropriate module and section for all information in an IND and NDA. The IND in eCTD format will have five modules (also all covered in the paper submission). A list, description of the modules, and a reference to the appropriate guidance document are provided in Table 12.1. The reference column lists the International Conference on Harmonisation (ICH) Harmonised Tripartite Guideline describing content and structure of the various modules of the CTD. It is also noted that the FDA

Table 12.1 Description of modules in eCTD

Module	Description	Comment	Reference[a]
1	Administration and prescribing information	Required form 1571 Prior final FDA correspondence (final pre-IND meeting minutes) Cover letter Environmental analysis Clinical label Investigator brochure	The eCTD backbone files Specification for Module 1[b]
2	Overall summaries for Modules 2, 3, 4, and 5	Section 2.3: summary of DS and DP from Module 3 Section 2.4: summary of nonclinical study reports Section 2.5: summary of clinical protocol	M4Q[c] M4S[c] M4E[c]
3	Quality-drug substance and drug product	• Drug substance characterization, synthesis, critical quality attributes, and stability • Contract sites supporting documentation for the API • Quantitative drug product formulation • Contract sites supporting documentation for the drug product • Drug product formulation history table including what was dosed in any nonclinical studies and the batches to be used in the proposed clinic trial (pharmaceutical development summary) • Assessment of excipients at the maximum expected dose for the clinical study versus the values in the IDD • Summary of status of methods and certificates of analysis • Overview of process and any critical in-process testing as appropriate • Ongoing stability data to support the length of the clinical study (1 to 3 months accelerated and long-term storage data)	M4Q(R1)[c]
4	Nonclinical study reports	Nonclinical study reports supporting the established starting dose with a safety margin	M4S[c]
5	Clinical study reports	The FIH clinical protocol Form 1572 Investigator CVs	M4E[c]

[a]FDA guidance documents found at www.fda.gov and the United States Pharmacopeia and National Formulary (USP/NF) serve as resources for the content of these sections
[b]Module 1 is regional information; this reference is the FDA document for the US requirements
[c]International Conference on Harmonisation (ICH) Harmonised Tripartite Guideline

Guidance for Industry (GFI) documents should be consulted as well as the current volume of the United States Pharmacopeia and National Formulary (USP/NF) for methods and best practices for various drug substances, excipients, and drug products. The USP/NF does not provide a monograph describing the testing for a new drug substance but includes descriptions of the standard methods that may be used to characterize a new drug substance. The USP/NF also has information pertaining to various dosage forms and the important testing criteria to be considered during development.

12.5.3.1 Module 1

The information requirements for Module 1 are regional requirements (not harmonized) and are not included in the ICH Harmonised Tripartite Guideline. A reference to the FDA specifications for Module 1 is provided in Table 12.1. Section 1.2 should include a cover letter to the appropriate FDA review division, including the sponsor's point of contact for the review division. The letter should also refer to any key discussions that have occurred with the review division prior to this submission. Any points that are critical to the reviewer's understanding of the contents of the IND should be described in the letter or separately in a reviewer's guide. Administrative and prescribing information is also provided in Module 1.

All available letters of authorization (LOA) for the FDA to reference active Drug Master Files (DMF) in the IND are included in Section 1.4.1. Drug Master Files are dossiers that have been prepared by suppliers of components that a sponsor is using in a particular drug product. A DMF is a tool for a supplier to protect any proprietary information that is crucial to their business. Most suppliers have active DMFs to support their products. These files contain proprietary information defining the starting materials, process, safety, test methods, specifications, container closure, stability, and other tests supporting the safe use of the subject of the DMF. Often the drug substance section of the application will reference a supplier's DMF; however, this is not mandatory. Examples of DMFs that should always be included are those that support the container closure material, unless the container is a new item. Excipients are often supported by a DMF, but the supplier may require a supply agreement prior to committing to support the submission with an LOA for the initial IND. In cases in which the supportive data for the component are readily available for incorporation into the IND, a DMF would not be required. This however must be assessed on a case-by-case basis.

An environmental analysis or claim for exclusion is required in Section 1.12.14. Draft labeling for the active and placebo (if the study design describes one) investigational drug product should be included in Section 1.14.1.2, and the investigator brochure as described in the FDA GFI: Good Clinical Practices for Investigator's Brochure is included in Section 1.14.4.1. A general investigational plan for the sponsor's initial IND is provided in Section 1.20.

12.5.3.2 Module 2

Module 2 contains summary sections; Section 2.2 is the overall summary introduction. Section 2.3 is discussed with Module 3, sections 2.4 and 2.6 are discussed with Module 4 and Sections 2.5 and 2.7 are discussed with Module 5.

12.5.3.3 Module 3

A full Module 3 (Quality-Chemistry Manufacturing and Controls (CMC)) consists of the drug substance and the drug product documentation.

The drug substance Section 3.2.S includes details about the source of the drug substance, the starting materials, receiving specifications, synthesis, in-process controls, purification, release test methods, release specifications, stability test methods, stability specifications, and shelf life for the active pharmaceutical ingredient (API). At the FIH stage of development, the analytical support for the drug substance is required but does not need to be fully validated; as the program advances to efficacy studies (phase 3), the API methods must be validated and provided in the IND.

The drug product Section 3.2.P typically includes the proposed quantitative drug product formulation to be used in the clinic, safety of the excipients, impurities in the drug product, discussion of the novelty of the dosage form, container closure components, analytical methods, and stability of the packaged final dosage form. The listing of the maximum potency for all components in the drug product found in the FDA Inactive Ingredient Database for the intended dosage form is typically included to support the safety of the final dosage form. At the FIH stage of development, the drug product analytical support is required but does not need to be fully validated, as described above for the drug substance. As the program advances to efficacy studies, the drug product methods must be validated and provided in the IND.

Section 2.3 is a summary of all of the CMC information on the drug substance and drug product and is typically limited to 40 pages or less. In the early development, an entire Module 3 may be represented in Section 2.3 within the 40-page limit, and in such cases in the initial IND submission, Section 2.3 often replaces the entire Module 3. In contrast, when a sponsor has a significant pharmaceutical development "story" for the initial IND, a full Module 3 (e.g., greater than 40 pages) is provided and Section 2.3 only includes a high level summary.

12.5.3.4 Module 4

Module 4 contains the nonclinical study reports supporting the IND submission. These include single-dose toxicity studies and repeat dose toxicity studies in appropriate species, which allow the estimation of the appropriate safe starting

dose. The translation of animal exposure into human exposure with the human equivalent dose estimation and justification is necessary, and the FDA Guidance for Industry: *Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers*, July 2005, provides additional information for performing this translation with drug products intended for systemic exposure. The exact content is based on the indication, target population, dosing regimen, and length of clinical study proposed and the amount of supporting information currently available to support the opening protocol dosing.

Section 2.4 is a summary of nonclinical data supporting the IND with the safety, primary and secondary pharmacology, pharmacokinetics, pharmacodynamics, and toxicology of each of the components as well as the proposed drug product for use in clinical trials as described in Module 4. In the early stages of drug development, the draft versions of the nonclinical study may be included in the IND, with the agreement of the FDA prior to the submission and the understanding that the submission will be updated when the final reports are completed. PIND meetings provide an opportunity for preliminary comments from the agency about the available nonclinical study data.

More detailed summaries and tabular listings of the nonclinical data are provided in Section 2.6.

12.5.3.5 Module 5

Module 5 contains the clinical study protocol supporting the IND submission. Typically this module includes the opening FIH protocol for the IND and any supporting literature references.

A summary of the clinical overview is provided in Section 2.5, describing the planned clinical study to support the IND submission. Included in this section is an overview of the product development rationale, biopharmaceutics clinical pharmacology, efficacy, safety, and a risk benefit summary for the proposed drug product for use in the clinical trials as described in Module 5.

Additional detailed summaries and tabular listings of the clinical data that are provided in Section 2.7 will not be applicable at this stage of development.

12.5.4 IND Submission

In parallel with the submission of an IND, the clinical study protocol is reviewed by the Institutional Review Board (IRB). An IRB is a group designated to review biomedical research involving human subjects to assure the protection of the rights and welfare of those subjects. IRBs may approve or disapprove the proposed research as well as request modifications to the clinical study protocol prior to allowing the study to be conducted.

The agency has a 30-day review period from the acknowledged receipt of the submission to allow the proposed clinical program to proceed or to place the program on clinical hold. The sponsor may receive questions from the agency during this review period; they will only allow brief periods of time for the answers to be provided. Delay in responding to these requests from the agency can result in clinical hold responses. A response from the agency that states a program is on clinical hold means that the clinical program to evaluate the investigational drug product in humans may not proceed until the stated items in the letter are resolved to the agency's satisfaction. There can be many reasons for clinical hold such as not enough details associated with the drug product characterization, an impurity that the agency knows to be at a toxic level, a request for additional toxicology data prior to dosing humans, or concern that the starting dose or dose escalation is not appropriate to name a few.

The IND goes into effect 30 days after the agency receives it unless the agency notifies the sponsor that the investigations described in the IND are subject to clinical hold per 21 CFR § 312.42. This notification could be provided by telephone, by other means of rapid communication, or in writing. This will be followed as soon as possible and no more than 30 days after imposition of the clinical hold, with a written explanation of the basis for the hold. The sponsor can request in writing that the clinical hold be removed once a complete response to all the clinical hold issues identified in the clinical hold order has been provided to the agency. The FDA will have 30 calendar days after receipt of the request to respond to the sponsor in writing.

If the agency does not notify the sponsor of any clinical hold issues within 30 days after acknowledged receipt of the submission, the sponsor may proceed with the clinical study activities (such as shipping of clinical supplies to the clinical site, or screening of subjects.). Subjects may receive doses of clinical trial material once the IND is in effect provided that the IRB has reviewed and approved the clinical study protocol.

12.5.5 IND Maintenance

Subsequent submissions to the IND should be numbered in sequence. These submissions will include protocol amendments, information amendments, and safety reporting. Information amendments can include new formulations, updated stability data, updates to manufacturing information, nonclinical study reports, and new clinical protocols. Annually, within 60 days of the anniversary date when the IND went into effect, a Data Safety Update Report should be submitted. E2F Development Safety Update Report: Guidance for Industry (GFI), August 2011, provides details on the preparation and submission of this report.

As the development program progresses, updates or revisions to the development target product profile are made with the appropriate nonclinical or clinical study data. The subsequent milestones are an End-of-Phase 2 meeting with the review division at the agency and/or the Pre-NDA meeting (GFI May 2009).

12.6 Summary

Early discovery is often thought to be far removed from regulatory submissions and human studies. However development programs require well-defined development target product profiles, which bridge the gap between discovery and regulatory submissions. Development programs can benefit from effective communication at appropriate milestones with the FDA in terms of guiding the project team on the journey toward an accepted IND submission.

References and Supplemental Information

1. FDA works with regulatory partners to understand French-based Biotrial phase 1 clinical study. http://www.fda.gov/Drugs/DrugSafety/ucm482740.htm?source=govdelivery&utm medium = email&utm_source = govdelivery, United States Food and Drug Administration. Accessed 31 Jan 2016.
2. Reflection paper on the chemical structure and properties criteria to be considered for the evaluation of New Active Substance (NAS) status of chemical substances, European Medicines Agency. draft. 26 March 2015.

Code of Federal Regulations: The FDA's portion of the Code of Federal Regulations (CFR) interprets The Federal Food, Drug and Cosmetic Act and related statutes. The following sections of the CFR are relevant to IND applications. Section 21 CFR § 312 and 21 CFR § 314 both contain information regarding IND applications. Orphan drugs regulations are provided in 21 CFR § 316. Good lab practices for nonclinical laboratory (animal) studies are provided in 21 CFR § 58. The protection of human subjects regulations are provided in 21 CFR § 50. Institutional Review Board regulations are provided in 21 CFR § 56. Drug labeling regulations for INDs are provided in 21 CFR § 201 and financial disclosures by clinical investigator regulations are provided in 21 CFR § 54.

Website links for various International Regulatory Agencies:
FDA: http://www.fda.gov/Drugs/default.htm
ICH: http://www.ich.org/home.html
EMA: http://www.ema.europa.eu/ema/
Health Canada: http://www.hc-sc.gc.ca/index-eng.php
MHLW: http://www.mhlw.go.jp/english/policy/health-medical/pharmaceuticals/
Anvisa: http://portal.anvisa.gov.br/wps/portal/anvisa-ingles

List of Guidance Documents that may be relevant for IND preparation can be found on the FDA website (www.fda.gov/regulatoryinformation/guidances/), some are listed below;

(a) CGMP for Phase 1 Investigational Drugs; Guidance for Industry, July 2008.
(b) Exploratory IND Studies; Guidance for Industry, Investigators, and Reviewers, January 2006.
(c) Content and Format of Investigational New Drug Applications (INDs) for Phase 1 Studies of Drugs, Including Well-Characterized, Therapeutic, Biotechnology-derived Products; Guidance for Industry, November 1995.

(d) Content and Format of Investigational New Drug Applications (INDs) for Phase 1 Studies of Drugs, Including Well-Characterized, Therapeutic, Biotechnology-derived Products, Questions and Answers; Guidance for Industry, October 2000.
(e) Safety Reporting Requirements for INDS and BA/BE Studies; Guidance for Industry and Investigators, December 2012.
(f) Bioavailability and Bioequivalence Studies for Orally Administered Drug Products-General Considerations, Draft Guidance; Guidance for Industry, July 2002.
(g) Bioavailability and Bioequivalence Studies Submitted in NDAs or INDs–General Considerations, Draft Guidance; Guidance for Industry, March 2014.
(h) IND Exemptions for Studies of Lawfully Marketed Drug or Biological Products for the Treatment of Cancer, Revision 1; Guidance for Industry, January 2004.
(i) Guideline for Drug Master Files; Guidance for Industry, September 1989.
(j) Required Specifications for FDA's IND, NDA and ANDA Drug Master File Binders.
(k) Immunotoxicology Evaluation of Investigational New Drugs; Guidance for Industry, October 2002.

Part V
Evolution of the Drug Discovery/ Development Paradigm

Chapter 13
Alternate Routes of Administration

Neil Mathias and Srini Sridharan

Abstract Patients, caregivers, payers, drug developers, and the continually evolving standard of care all play a critical role in shaping the drug product and formulation requirements to better meet unmet medical needs of patients. A significant area of growth in recent years has been in the non-oral route of administration (alternate route and injectable route of administration, RoA). The use of drug products for alternate route of administration or the use of drug-device combination products offers an opportunity to enable a product in situations where there are significant oral challenges, such as extensive gastrointestinal metabolism, low oral bioavailability, suboptimal oral PK, local gastrointestinal toxicity, or other adverse reactions. Additionally, drug-device combination products (both injectable and non-injectable for alternate route products) present an opportunity to consider an enhanced product that improves patient compliance and increases treatment options to manage diseases.

In this chapter, alternate routes of administration such as intranasal, inhalation, buccal/sublingual, and transdermal approaches for delivery of drug candidates to systemic molecular targets are discussed. The rationale for each route of administration, including their strengths and limitations, drug molecule developability criteria, and recommended preclinical testing experiments to enable such products, is reviewed.

There has been a steady trend over the past decade in which self-administration has become more and more prevalent among patients. As a result, devices are being developed that incorporate more patient requirements, such as portability, intuitiveness, ease of use, and other human factor considerations. In addition, with the growth of mobile health applications, devices are becoming more connected with mobile devices, enabling better patient compliance with treatment regimens and advancement in standards of care. Product trends and recent advances are outlined in this chapter together with strategies to consider for clinical testing.

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Keywords Non-oral delivery • Route of administration (RoA) • Self-administration • Drug-device combination • Non-invasive • Invasive injectable

Abbreviations

B/SL	Buccal/sublingual
BCS	Biopharmaceutical classification system
CVC	Central venous catheters
DPI	Dry-powder inhaler
ID	Intradermal
IM	Intramuscular
IN	Intranasal
INH	Inhalation
IV	Intravenous
MDI	Metered-dose inhaler
MN	Microneedles
NME	New molecular entity
PD	Pharmacodynamic
PFSs	Prefilled syringes
PICCs	Peripherally inserted central catheters
PK	Pharmacokinetic
RoA	Route of administration
SC	Subcutaneous
TD	Transdermal

13.1 Opportunities for Non-oral Routes of Administration (RoAs)

The successful discovery and development of new medicine hinges on the medicine's ability to treat a disease meet patient requirements, physician expectations, and regulatory requirements. Through this process, drug discoverers/developers are continually challenged to produce new molecular entities (NMEs) and advance them through long and expensive clinical trials. The high rates of attrition are typically attributed to lack of adequate safety, lack of measurable efficacy, undesirable pharmacokinetics, or insurmountably poor biopharmaceutical properties (solubility, solid- and solution-state stability, low bioavailability) [1, 2]. This has led scientists in biopharmaceutical and specialty drug delivery fields to seek new ways to deliver drugs and explore innovative strategies beyond the oral route of administration, thereby creating new product opportunities and treatment paradigms [3].

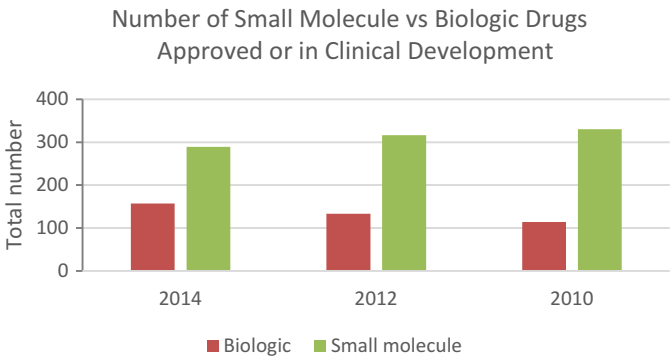

Fig. 13.1 Comparison of small molecule and biologics that are approved or in active clinical development programs

To examine the trends in product options, a snapshot review of approved and clinical-phase products between 2010 and 2015 was conducted using an online product tracking database, PharmaCircle [4]. Some interesting trends emerged:

1. The ratio of small molecule to macromolecules (peptides, biologics, RNA/DNA therapeutics, etc.) has been steadily decreasing over the years (Fig. 13.1), a reflection of significant advances in overcoming delivery issues associated with the new molecular types.
2. Seven of the top ten best-selling medicines in 2014–2016 are biologic medicines.
3. There is a greater willingness to develop NMEs for difficult to treat disease areas, such as targeting intracellular or nuclear targets, targeting specific organs, or using device technologies to achieve a desired target product profile [5].
4. The distribution of different routes for approved products is injectable products 24%, non-oral, non-injectable products 28%, and oral products roughly 48%.

13.1.1 Challenges to Oral Delivery

At the drug discovery-development interface, unexpected preclinical findings from oral route delivery can preclude advancement of a clinical drug candidate. Local toxicity in the gastrointestinal (GI) tract, physical/chemical instability in the GI tract, poor intestinal absorption, dose-limiting efflux, slow onset of action (delayed absorption), extensive gut and hepatic first-pass metabolism, suboptimal or highly variable PK profile, and high-dosing frequency/compliance issues are common scenarios that impede drug candidate progressions through development. A proven and successful strategy to circumvent these problems is to deliver drugs systemically via an alternate route of administration such as intranasal (IN),

buccal/sublingual (B/SL), pulmonary (P), or transdermal (TD) or injection via a drug-device combination product directly into the body [6]. This creates new product opportunities with distinct benefits over the oral route and has the potential to rescue a drug candidate originally intended for oral administration.

13.1.2 The Needs of the Patient, Caregiver, and Payer in Drug Product Design

Each route of administration offers unique and distinct drug product/dosage form opportunities that can be tailored to the disease state, designed to meet or improve on the existing standard of care drug products and adapted to leverage the physicochemical properties of the drug candidate. Several examples of approved drugs exist that highlight such unique synergies: intranasal triptans (sumatriptan, zolmitriptan) offer rapid-onset relief of migraine-related pain in minutes that is not achieved with an oral tablet; intraoral (B/SL) and TD opioid products eliminate GI-related adverse reactions of the oral products; and insulin- and GLP-1-based injectable products reduce injection frequency from daily to weekly or longer. On the other hand, there are scenarios where a non-oral alternate route of administration may not be viable, for instance, high dose may not be feasible for certain routes have dose amount or volume limits, or unfavorable physicochemical properties for a drug candidate to deliver the dose, or drug-induced irritancy reaction at the site of administration, or unfavorable product costs and payer economics for specialized drug products. Therefore, it is prudent to consider the selection of the appropriate route of administration and drug product design elements to be founded on not just biopharmaceutical and drug delivery principles, but also patient/caregiver preferences and the macroeconomic environment to maximize the chances of a drug candidate becoming a successful drug product.

13.1.3 Non-Oral Product Opportunities

This chapter describes the delivery considerations and factors in developing strategies to pursue non-oral alternate routes of administration (RoAs) for systemic delivery. The RoAs are divided into two categories:

1. Noninvasive, non-oral routes such as intranasal (IN), buccal/sublingual (SL), inhalation (INH), and transdermal (TD)
2. Invasive, injectable routes such as intravenous (IV), subcutaneous (SC), intradermal (ID), and intramuscular (IM) injections

In the following sections, the benefits and limitations of each RoA (Table 13.1), the delivery criteria unique to that route (Table 13.2), and generally preferred

Table 13.1 Advantages and limitations of alternate routes of administration

Route	Pros	Cons
Intranasal	– Highly permeable epithelia – Well-perfused mucosa – Convenient dosing with spray device – Commercially available nasal spray devices – Amenable to small molecules and peptides	– Limited dose and volume – Rapid mucociliary clearance (short duration for absorption) – Taste/sensory liability – Irritation potential in the sensitive nasal mucosa
Buccal/ sublingual	– Easy access, convenient dosing – Good patient compliance – More robust mucosa that is less sensitive to irritation – Several dosage form options (rapidly disintegrating tablet, wafer film, lozenge, spray, patch, tablet, etc.) – Amenable to small molecules and small potent peptides	– Limited dose and volume – Poor retention of dose in oral cavity – Clearance by saliva – Potential taste/sensory liability – Selective permeability – Potential local toxicity of adhered dose
Pulmonary/ Inhalation	– Large surface area for absorption – Thin epithelial barrier with moderate permeability characteristics – Very well perfused – Amenable to small molecules and macromolecules	– Limited dose and volume – Challenge to achieve reproducible deposition in deep lung – Particle clearance by mucociliary blanket and macrophages – Risk of safety signals or affected lung function with chronic dosing – Potential taste/sensory liability – Complicated drug-device product development
Transdermal	– Easy access, convenient to dose – Good patient compliance – Skin least sensitive barrier – Large surface area for treatment – Amenable to small molecules. Peptides require active TD delivery (discussed in TD section)	– Low rate and extent of dose delivery (requires good potency) – Toughest barrier to penetrate – Toxicity/irritation at site of application due to API or occlusion from a patch-based product or device

physicochemical properties for a drug candidate (Table 13.3) are described. One of the main challenges in biopharmaceutical research is to demonstrate delivery feasibility or developability potential of an RoA by correlating data from experimental models (in vitro, in vivo, and in silico) that are to some extent predictive of performance in humans. For each route, the preclinical methods available to assess delivery feasibility and safety at the site of dosing, as well as ways to assess the viability of specific drug product dosage form options, are highlighted. It is not the intent of this chapter to detail all facets of each route, but rather emphasize those that are generally more amenable and provide a holistic view of product development options at the discovery-development interface.

Table 13.2 General considerations for non-injectable, non-oral RoAs

	Route			
Consideration	IN	B/SL	INH	TD
General patient preference	Good	Good	Fair	Good
Therapeutic considerations (PK/PD)	Rapid onset	Rapid onset, pre-gastric or in testinal absorption	Rapid onset	Slow, sustained plasma levels
Delivery options	Solution (spray)	Solution or solid dosage form (buccal tablet, patch, or lozenge)	Inhalation product (powder, solution, or suspension)	Patch or topical presentation (ointment/spray)
Typical device	Single or multidose metered spray device	Patch system, metered spray device, or solid dosage form	Nebulizer, metered-dose inhaler, or dry-powder inhaler	Patch system (active or passive)
Permeability	Highly permeable	Moderate relative to nasal	Moderate relative to nasal	Poor permeability
Surface area	~150 cm^2	~215 cm^2	>75 m^2	~2 m^2 [a]
Selectivity for absorption	Small hydrophilic or hydrophobic molecules and peptides	Small molecules with greater lipophilicity	Small molecules and macromolecules	Small lipophilic molecules (passive), small charged molecules (active)
Vascular perfusion	Well perfused, drains into the vena cava	Well perfused, drains into the internal jugular	Complete perfusion, drains into pulmonary vein	Slow perfusion, drains into the peripheral capillaries
Aq. solubility requirements	Good aq. solubility	Good aq. solubility	Moderate solubility	Low aq. solubility
Mucosal environment	Lining fluid pH 5.5–7.4	Saliva 5.8–7.4, volume ~ 0.9 ml, 0.1 mm thick film	Lung surfactant containing lining fluid, pH 6.9	Hydrophobic stratum corneum, pH 5.5
Aq. pore-size radii	7 nm	–	5 nm	0.5–3.4 nm

Patient preference rating is based on criteria such as ease of dosing, convenience of dosing device/dosage form, patient compliance, complexity of usage instructions, and patient control of delivery [9–13]

[a]Injectable patch devices like patch pumps can be applied to the abdomen, back, upper arms (triceps), thighs, and buttocks

Table 13.3 Summary of criteria and generalizations for each alternate route of administration

Criteria	IN	B/SL	INH	TD (passive)
Max dose	20 mg	20–30 mg	~20 mg	10–20 mg
Volume (liquid formulation)	50–150 μl	<500 μl	<200 μl	<300 μl
BCS class	BCS I, III	BCS I–II	BCS I–III	BCS I–II
Preferred physicochemical properties:				
• MW	<1000	<500	<10,000	<500
• Log P[a]	1–4	2–4	−1 to 2	>2–5
• pK_a	4–9	4–9	4–9	Unionized
• pH range	4–7	3–8	3–7	4–7
Typical device/ dosage form	Nasal sprayer	Mouth spray, patch, film, solid dosage form, etc.	Inhaler	Patch, topical sprayer, cream

[a]Exceptions to these generalizations exist (e.g. nitroglycerine, nicotine)

13.2 Selecting Alternate Routes of Administration

The first step is to understand the drug target location and whether it can be accessed by local or systemic administration. Injectable routes typically deliver drug candidates invasively into the systemic circulation, whereas non-invasive RoAs can be considered for both systemic and local delivery. RoA for local delivery introduces the drug directly at the disease site, for example, intravitreal injection into the eye for macula retinal diseases (ranibizumab) or inhaled anti-asthmatic drugs dosed to the diseased respiratory airways. For systemic delivery, the choice of route is based on a variety of factors, such as properties of the molecule, desired pharmacokinetic and pharmacodynamic profile, and point of therapy (administered by a healthcare professional (HCP) in a hospital or doctor's office vs. self-administration at the patient's convenience). This chapter focuses on non-oral RoA for systemic delivery only and excludes local targeting.

To assess a drug candidate's developability potential for an alternate RoA, a good understanding is required of:

1. Therapeutic area considerations: location of the molecular target; need for chronic, sub-chronic, or acute treatment; desired PK profile (immediate release, pulsatile, or extended release); sensitivity to the diseased state (e.g., inhalation delivery when lung capacity is compromised); and standard of therapy.
2. Patient population considerations: at home or hospital/doctor's office dose administration; compliance to improve treatment outcomes (e.g., reduced frequency of dosing); specialized population preferences, e.g., pediatric or geriatric requirements; and disease co-morbidities or hindrances (e.g., reduced dexterity for arthritis patients).
3. Knowledge of physicochemical characteristics: molecular weight, ionization state, aqueous solubility, partition coefficient (log P), absorption kinetics, and solid-/solution-state stability.

4. Biopharmaceutical performance assessed through in vitro, in vivo and in silico assessment that informs the absorption potential, PK profile, and overall viability of a particular RoA.
5. Deliverability assessment: knowledge of the human dose requirements and the typical dose range anticipated. Generally, large doses are better suited to some injectable routes and the oral route. Most non-oral RoAs have limitations on the total volume or amount that can be dosed, which influences the overall target product profile that outlines the formulation and dosage form requirements.

For ease of discussion and comparison between RoAs, they have been categorized into two: noninvasive (non-injectable) and invasive (injectable) routes.

13.2.1 Non-injectable Routes of Administration (RoAs)

Each of the noninvasive RoAs (IN, B/SL, INH, and TD) and their general considerations are listed in Table 13.2. Patient and disease considerations, dosing regimen, and competitive landscape play a prominent role in selecting a viable RoA. Acute therapies are generally more amenable to some routes of delivery, while chronic therapies require a long treatment duration with significant regularity which tend to preclude some RoAs (IN, INH, and B/SL). Due to the nature of the mucosal barriers, IN, B/SL, and INH delivery favors rapid systemic absorption leading to a spike in plasma levels that must be reasonably well tolerated. These routes apply to both acute and chronic therapies such as pain management, migraine, cardiovascular disease, glucose control, smoking cessation, etc., where immediate relief is desired and a favorable PK-PD relationship exists. On the other hand, for TD products, a drug depot is formed at the site of dosing which leads to slow, sustained PK profiles and a low Cmax-to-Cmin ratio. Therefore, TD delivery is more suited to sub-chronic or chronically administered therapies such as chronic pain, chronic CNS disorders (depression, Parkinson's, dementia, and attention deficit-hyperactivity disorder), and hormonal therapies (contraception, hormone replacement, menopause, and osteoporosis).

The greatest limitation to broad application of noninvasive, non-oral RoAs is the maximum deliverable dose of 20–30 mg. For an NME entering clinical development, estimating a therapeutic dose for that route can be a challenge. As with any first in human (FIH) studies, interspecies allometric scaling is used to predict human PK parameters (clearance, volume of distribution, and plasma half-life) and estimate a human dose range that is based on the Cmax, AUC, and bioavailability from that specific RoA. More sophisticated variants of these methods have also been well published in the literature as well as in an FDA guidance document [7, 8]. Additionally, knowledge of the target therapeutic plasma levels from preclinical efficacy studies helps define the target PK profile for the indicated route.

Table 13.3 outlines limitations for IN, B/SL, INH, and TD routes based on dosing volume, mass, or size. Dose escalation via alternate RoAs is relatively

limited with single administration in most cases. Multiple administrations (spray actuations) or multiple dosing units could offer some dose flexibility. Nevertheless, in contrast to oral or injectable delivery, the boundaries are more restrictive, governed by clearance mechanisms that drain drug from the dosing site or increased risk of irritation in the local mucosa from a high drug load.

13.2.1.1 Intranasal (IN) Delivery

IN delivery is an attractive option when first considering a non-oral route of administration. It is non-invasive, relatively painless, and easy to administer; it does not require sterile processing and can be dosed in commercially available devices [14]. The leaky nature of the nasal epithelial barrier relative to other mucosae [15] and reduced metabolic capacity (relative to the gut/liver on a per gram tissue) can lead to rapid systemic absorption for both small molecules and peptides [14, 16]. Depending on the physicochemical properties of the drug candidate, it may be absorbed through paracellular or transcellular mechanisms or both. In general, in accordance with the pH-partition theory, small, unionized, lipophilic drugs tend to show the greatest potential for transmucosal transference [16, 17]. Molecules deviating from this ideal are more reliant on the less efficient paracellular pathway; however, limited surface area for diffusion at the perijunctional spaces and aqueous pores limits the rate of drug transport [18]. In any event, drug delivered to the nasal cavity has a relatively short residence time before mucociliary clearance drains the drug down the nasopharyngeal tract, with a half-life ranging from 20 to 30 min [19]. Doses must be delivered in a relatively small volume (~100 μl), requiring drug candidates with high solubility in a non-irritating aqueous vehicle.

To date, the maximum dose delivered in a single spray actuation from an approved nasal product (Imitrex®) is 20 mg. Dose escalation is possible by increasing the delivered maximum volume to 150 μl or via multiple actuations per nostril. Large volumes increase the probability of drug solution loss by nasopharyngeal drainage and swallowing [20]. Several techniques can be explored to enable solubility-limited drug candidates: standard preformulation-based salt-screening studies, formulating with non-irritating amounts of glycol- or glycerol-based cosolvents, complexation with solubilizing cyclodextrins, or pursuing a hydrophilic prodrug approach [14, 21]. Most nasal products for systemic delivery are solution-based formulations. Particulate suspensions (including nanosuspensions), gels, ointments, and powders can also be delivered, although the short residence time in the nasal cavity and the added dissolution and/or diffusion from a matrix can significantly limit the efficient delivery of meaningful amounts to the systemic circulation. Therefore, BCS I (highly soluble and highly permeable) compounds are the most amenable to IN delivery. However, BCS II and III drug candidates may be also suitable candidates if they possess suitable physicochemical characteristics: MW $<$1000 g/mol, log P between 2 and 4, and a pK_a maximizing the % unionized at the pH of nasal lining fluid (pH 5.5–7.4, Table 13.3) [16, 17, 22].

Off-the-shelf spray devices that deliver solution or suspension to the nasal mucosa are available for proof of delivery in clinical trials. The most commonly used devices are metered-dose spray pumps that deliver on average 100 μl (50–200 μl) as either a single dose or multiple doses from a reservoir device [23]. The latter for formulations with preservatives that limit microbiological growth over the formulation's shelf life. Preservative-free devices eliminate the use of preservatives in the formulation who's long-term use on nasal mucosa integrity has been somewhat controversial. Other novel device types are available with specialized spray pattern deposition for example olfactory targeting in nose-to-brain delivery, pressurized metered-dose sprayers, or nasal powder inhalers [23].

IN delivery can also be considered as a route to target CNS delivery, via nose-to-brain route [24]. Drug dosed to the olfactory region of the nasal cavity can be transported via ensheathed channels along the olfactory or trigeminal nerve to the cerebrospinal fluid and/or the adjacent regions in the brain, or become systemically absorbed and transported across the blood-brain barrier (BBB) into the brain parenchyma. The direct nose-to-brain route has been tested in preclinical species, but consistent and quantitative demonstration of brain or CSF delivery from nasally administered drug in humans has been limited [25]. Animal models with proportionally larger olfactory regions (obligate nose breathers like rats, dogs, sheep) tend to overpredict the CNS delivery potential. Also, the manner in which drug is dosed, i.e., flooding the nasal cavity vs. aerosolization can yield false-positive results. Poor absorption of an NME is likely to rule out the nose-to-brain transport pathway, whereas a positive result signals potential for the route that should be carefully explored [25].

IN RoA Biopharmaceutical Assessment

Assessment of IN delivery feasibility is typically accomplished with in vitro and in vivo models. In vitro cell cultures provide useful information about a drug candidate's permeability in airway epithelia, it's metabolic liability, and irritancy potential in acute cytotoxicity assays [26, 27]. However, the ultimate proof of systemic IN deliverability is obtained from pharmacokinetic studies in animal models such as rat, rabbit, or dog. In situ perfusion models estimate nasal absorption by measuring loss of drug from a buffered drug solution introduced to the nasal cavity via a cannulated nasopharyngeal duct and exiting via the nares [22, 28]. Importantly, methodology that mimics IN dosing in humans as closely as possible is likely to be the most informative. This includes bolus aerosol spray in a reasonable volume (up to 1–2 μl/cm^2 nasal surface area in the test species) in transiently anesthetized animals to assure drug deposition in the nasal cavity [20]. The PK profile and overall bioavailability are typically compared against a reference route (e.g., injectable or oral route). Successful demonstration of systemic deliverability should be further supported by data that shows no safety, irritancy, or compromised barrier integrity issues at the nasal epithelium. For the sensitive nasal mucosa, a lack of irritancy with the drug candidate and formulation composition

can be assessed with cell cultures or excised tissue models with measurements such as transepithelial electrical resistance, transepithelial permeability of typically poorly permeable model compounds, and biomarker of epithelial damage (secretion of inflammatory mediators, release of cytosolic-enzyme lactate dehydrogenase (LDH), or release of membrane-bound enzymes). In vivo nasal lavage studies in a rat model can also indicate sensitivity to mucosal damage or vascular leakage in nasal secretions [22, 29]. Taken together, a combination of these studies can provide a good mechanistic understanding of the physical and biochemical response to the NME and formulation prior to human trials.

13.2.1.2 Buccal/Sublingual (B/SL) Delivery

The general patient acceptance and ease of delivery for noninvasiveness and painless oral cavity drug dosing make B/SL delivery an attractive non-oral delivery option. Absorption via the oral cavity can greatly benefit drug candidates that are extensively metabolized through GI first pass, irritate the GI mucosa, evoke nausea and vomiting, exhibit poor oral permeability, or show slow-onset pharmacokinetic profile [30]. Even though the permeability barrier in the oral cavity is significantly more difficult to overcome relative to the nasal mucosa, drugs with the necessary physicochemical attributes can be rapidly absorbed [15, 31]. The main differentiating factor between the two mucosae being the approximately 200–500 μm-thick stratified squamous epithelia with varying degrees of keratinization is the oral cavity that confers skin-like properties, but is also less prone to mucosal damage compared to the nasal mucosa [31, 32].

The following sequence of events is necessary to systemically deliver a drug candidate via the B/SL route: first, the compound must be released from the dosage form (tablet, patch, lozenge, film, or gel formulation); second, it must rapidly dissolve in the 0.7–0.9 ml saliva present and be distributed throughout the oral cavity within a few minutes; third, it must partition into the epithelial lining; forth, it must diffuse across the epithelial barrier; and lastly, absorbed into the systemic circulation. Systemically absorbed drug candidates drain into the internal jugular vein, bypassing gut and hepatic first-pass metabolism which can lead to fairly rapid-onset PK profile [32].

Despite the formidable mucosal barrier, intraoral drug permeation follows the same basic principles of drug permeation (pH-partition theory) as the oral epithelium. In general, BCS I and II compounds have the best probability of achieving significant systemic absorption. The preferred physicochemical attributes that maximize delivery include small molecular size (MW typically <500 g/mol), log $P \sim 2$–4, and high solubility at a pH that maximizes the fraction unionized in saliva (Table 13.3). Deviation from these parameters results in a significant drop-off in systemic absorption. Small molecules between 400 and 700 g/mol typically have bioavailabilities ranging from 15% to 70%, while peptides are generally $<25\%$ [30]. Although aqueous-based systems are preferred, non-damaging, solvent/cosolvent, and permeation enhancer-based approaches (e.g., alcohol/glycol solutions, hydroalcoholic solutions, or mixed surfactant micellar systems) and

mucoadhesive buccal delivery systems can maximize the thermodynamic potential of lipophilic drugs and facilitate diffusion into the intraoral mucosa [33]. In all cases, the irritation potential or mucosal damage induced by the drug formulation and the recovery of the mucosa or reversibility of the epithelial damage is critical to assure adequate safety/tolerability and good patient compliance [34]. For B/SL delivery, the transcellular pathway is the shortest and most efficient mechanism of absorption, while the paracellular pathway involves a more tortuous route leading to longer lag times. Small molecule drug candidates with balanced hydrophilic-hydrophobic characteristics are likely to be absorbed by both pathways and show the best potential for systemic exposure [33].

The primary challenge for B/SL delivery is retention of the drug candidate at the mucosal surface to facilitate efficient partitioning into the mucosal lining. Several approaches have been devised to overcome the short residence time limitations:

1. Lozenges/lollipops (e.g., Actiq®) and gums (e.g., Nicorette®) physically remain in the oral cavity, prolonging drug release over the duration.
2. Mucoadhesive patches/tablets (e.g., Striant®) establish an intimate contact of the drug/polymer matrix with the absorptive buccal/sublingual mucosa.
3. Sublingual, orally disintegrating tablets release drug candidates in a highly permeable region.
4. Multiple sprays of a solution formulation maximize deposition throughout the oral cavity surface area.

Hydrophilic macromolecules show reduced permeability in the B/SL mucosa as is also the case with other absorptive mucosa. Larger molecular size results in greater restricted diffusion into the paracellular spaces or aqueous pores. In B/SL mucosa, a threshold of approximately 20 kDa has been proposed for the epithelial barrier [35]. Permeation enhancers and mucoadhesive drug retention strategies (patches, tablets, lozenges) can enhance macromolecular B/SL absorption [34, 35].

B/SL RoA Biopharmaceutical Assessment

Evidence of a drug candidate's potential for B/SL absorption can be obtained from in vitro and in vivo models. In vitro cell culture systems and excised tissue in diffusion chambers provide valuable information regarding the mechanism and rate of transmucosal drug permeation and stability [36, 37]. For in vivo assessments, both rabbit and dog are practical animal models with anatomical characteristics (i.e., keratinization levels) and absorption characteristics that mimic that of humans [38, 39]. Rat and hamster oral cavities are small and heavily keratinized which leads to underestimation of absorption, hence a nonideal delivery model [37]. As with the nasal in vivo assessment, studies should be conducted with either lightly anesthetized or immobilized conscious animals to assure better dose delivery and retention in the oral cavity. Long-acting anesthesia has been shown to influence PK, resulting in overestimated bioavailability due to lowered blood flow in the vasculature and/or altered salivary secretion [40]. A drug candidate can be presented to the oral cavity

as a solution spray, loaded onto a substrate, or as a powder/solid dosage form. Employing a dosing technique that simulates the anticipated human dosing scenario is most likely to provide the most relevant insight. Absorption from different sites in the oral cavity (sublingual, buccal, or gums) may differ, so will the residence times. Consequently, the extent of clearance from the oral cavity in the form of swallowed fraction should be deconvoluted from the PK profile by measuring the PK from orally dosed drug.

The irritation or sensitizing potential of the drug candidate and dosage form in the oral cavity is essential to prove safety and tolerability for an intraoral product. In vitro cell culture and excised tissue experiments can effectively reveal the potential for a drug formulation to compromise epithelial integrity. These experiments include transepithelial electrical resistance measurements, transmucosal flux measurement of a poorly permeable paracellular marker, and analysis of biomarkers of oral cavity mucosal damage such as intracellular enzymes or lipids that are extracted from the tissue [31, 35, 36]. In vivo, irritancy signals such as erythema, edema, and eschar formation should be explored for the drug candidate and dosage form, especially for chronically administered formulations. An added but often overlooked factor is the taste implications of the drug candidate. Highly bitter taste-inducing drugs may be difficult to mask for B/SL absorption. Therefore, learning about the organoleptic properties of the drug candidate in humans is necessary to guide dosage form and flavor selection in formulation composition design.

13.2.1.3 Inhalation (INH) Delivery

Lung delivery is an attractive RoA offering a pain-free, non-invasive alternative to deliver drug to a much larger surface area (>100 m^2) compared to the IN and B/SL cavities. The alveolar epithelial barrier being thin relative to other mucosal barriers can exhibit good permeability properties and modest metabolic capacity [48]. In general, the lungs are believed to be more permeable to small molecules than the oral gastrointestinal mucosa [41, 42]. In a series of studies, Schanker and coworkers have shown that the lung is capable of exhibiting high bioavailability and rapid absorption for small lipophilic drugs. Small hydrophilic compounds (MW <1000 g/mol) with a log $P < 0$ have a mean absorption half-life of approximately 1 h, whereas lipophilic small molecules with a log $P > 0$ generally have an absorption half-life of about 1 min [43]. Molecular size also appears to play a role for molecules >1000 g/mol. The mechanistic basis for rapid absorption from the alveolar region and distal airways follows a similar pattern to IN and B/SL delivery where transcellular diffusion across the large surface area leads to systemic absorption. Hydrophilic compounds that tend to favor paracellular pathway show slower transport through the aqueous pores in the regions between cells, where molecular size and degree of ionization determine the rate of transport [41].

Dissolution and absorption of mild-to-moderately lipophilic drugs are aided by lung surfactants naturally present in the lung lining fluid [44]. Drugs with very high log P can become entrapped for hours, days, or weeks, if the solubilizing capacity

of the lining fluid is overwhelmed [43]. For example, fluticasone propionate (log $P = 3.5$) demonstrated central lung tissue concentrations 30–40 times higher than systemic blood levels 16 h post-dose [45]. Along the same lines, moderately lipophilic compounds that carry a net positive charge such as pentamidine [46] and tobramycin [47] can bind to negatively charged proteins on cell surfaces leading to retention and prolonged release profiles. Evaluating the structure-absorption relationship and physicochemical profiling of marketed pulmonary products, Tronde et al. [48] found that passive diffusion accounted for most of the absorption in the lung, which correlated well with polar surface area (PSA) and hydrogen-bonding potential. Drugs with high PSA (>120 Å^2) are usually excluded from intestinal and the blood-brain barrier, but readily absorb across the pulmonary epithelium.

An inhalation drug product represents perhaps the most complicated development path of all the routes discussed in this chapter. Accurate dose delivery for consistent and deep deposition in the lungs is not easily achieved and may not be applicable in all situations. For an inhalation product, the following sequence of events must occur: first, an appropriate device needs to be identified early in product development; the device should generate an aerosol in the 1–5 μm mean aerodynamic diameter range to facilitate deep lung deposition; the emitted dose should reproducibly deposit in the lung with little influence from patient breath; solid drug particles (powders and suspension formulations) should dissolve readily, or solution droplets diffuse readily into the lung lining fluid; and, finally, the solubilized drug should partition and permeate the epithelial barrier to reach the systemic circulation.

Three major types of inhalation devices are typically used each with unique dosing characteristics, deposition profiles, and patient handling requirements: (1) nebulizers for solution and suspension formulations, (2) metered-dose inhalers (MDIs) for solution or suspension formulations, and (3) dry-powder inhalers (DPIs). Nebulizers are commercially available devices that deliver the dose as an aerosolized mist that is passively inhaled over 5–10 min. The aerosol is typically generated by an air compressor or ultrasonic piezo-electronics. Nebulizer devices are available in a wide variety: more affordable, bulky, non-portable pump devices or more expensive, discrete, portable, handheld devices with integrated electronics. MDIs deliver a metered dose of solution or suspension of drug dispersed in a propellant such as hydrofluoroalkanes (HFAs). Basic devices deliver a high-velocity spray to the oral cavity, while newer devices minimize oropharyngeal deposition and provide a more uniform lung deposition profile by using the patient's breath to actuate drug dispersion. Dry-powder inhalers deliver micron-sized aerodynamic powder particles to the deep lung region, either by patients' breath activation or active dispersion of drug doses. They are available as unit dose or multidose unit with multiple packaging configurations (capsules for capsule-based devices, e.g., Aerolizer; blister packs for blister-based inhalers, e.g., Advair®; or drug powder reservoir for reservoir devices, e.g., Turbuhaler®). Each device type

has newer generation devices that focus on miniaturization for portability, improved reproducibility in dose delivery or improved patient compliance.

INH RoA Biopharmaceutical Assessment

Several in vitro, in vivo, and ex vivo models are available to assess pulmonary route delivery feasibility, providing information on lung permeability, absorption, clearance, or metabolic capacity. Primary cell cultures of airway or alveolar epithelium and well-established cell lines such as Calu-3, A549, and 16HBE14o- provide good insights toward permeability characteristics and potential for lung absorption through established in vitro-in vivo correlations [49, 51]. More complex models such as the isolated perfused rat lung (IPRL) take into account lung absorption and elimination under controlled conditions [52]. However, the mainstay for lung delivery assessments is in vivo rodent studies with drug candidates introduced via intratracheal or nasal aerosol administration [53]. Intratracheal instillation is a practical method to introduce a known amount of drug to the lung, while aerosolization (from nose cone or aerosol chambers) provides better deep lung deposition as well as a realistic estimate of the inhalable dose fraction from an aerosol [43]. Simple aerosolization devices such as a syringe microsprayer or syringe powder insufflator can effectively achieve suitable dose delivery in the rodent model for pharmacokinetic assessment of an NME's pulmonary delivery feasibility.

Alveolar, bronchial, or tracheal epithelial cell cultures are effective models to demonstrate safety at the site of deposition. Common tests include assessing the capacity of a drug candidate to irreversibly disrupt epithelial tight junctions or the permeability barrier and testing for inflammatory signals via biomarkers released from the cells over time [50, 54]. Bronchoalveolar lavage studies provide an additional measure of the biochemical response to an NME with assays for pro-inflammatory cytokines, polymorphonuclear cell infiltration, and cytosolic lactate dehydrogenase release as evidence of tolerability of the drug [54].

13.2.1.4 Transdermal (TD) Delivery

Transdermal dosing is an attractive non-invasive alternative to medicines that require frequent administration or those that elicit strong side effects in the GI tract or erratic efficacy due to extensive first-pass metabolism. With products like TD nitroglycerine approved more than 30 years ago, TD delivery has gained momentum due to the non-invasiveness of the route, ease of administration, and improved patient compliance especially for elderly or the young that have difficulty swallowing pills. Two of the most common hurdles for TD delivery are the ability to deliver meaningful amounts of drug across the stratum corneum at a reasonable rate and the risk of incompatibility of the NME formulation at the site of application due to the presence of immune and inflammatory cells in the epidermal/dermal

tissue. The stratum corneum, the outer cornified layer of the skin, is the toughest barrier in the body, designed to keep the body safe from the environment and external material.

For passive TD delivery, the following sequence of events occurs: drug candidate is released from the device or patch system; it needs to partition into the SC or skin appendages (sweat glands or hair follicles); the drug then diffuses across the lipidic barrier into the dermal tissue along a concentration gradient; en route, it resists metabolic inactivation and provoking an irritation/sensitization response; and, finally, it passes into the capillary bed to enter the systemic circulation. Oftentimes the skin itself acts as a drug depot site gradually releasing drug over an extended duration which leads to the typical slow sustained blood PK profile. Continuous resupply of drug from the patch or device to the skin ensures delivery over a long time frame (hours or days). To control the rate of input and eliminate variability due to patient's skin characteristics (thickness, permeability, or hydration level), patch systems may incorporate rate-controlling membranes to provide uniformity in delivery [55].

The formidable stratum corneum barrier is approximately 10–20 μm thick comprised of keratin bundles, lipid-filled corneocytes, and intercellular lipid lamellae tightly packed together. Transport across this barrier is achieved either by transcellular, intercellular, or trans-appendageal mechanisms. The tortuous intercellular lipidic pathway typically accounts for majority of drug transported [55]. Transcellular diffusion is more direct, but requires a challenging sequence of partitioning between alternating protein and lipid domains resulting in poor overall efficiency. Similarly, low-density and limited surface area of trans-appendageal pathway tends to result in low delivery efficiency [55].

Transdermal drug products can be assigned to one of three categories:

1. Passive, reservoir, or drug/matrix patches.
2. Second-generation TD products utilize chemical penetration enhancers or an energy source such as current (iontophoresis) or ultrasound (sonophoresis) to drive drug across the SC.
3. Third-generation TD products use novel SC disruption mechanisms, poration, thermal ablation, radio frequency, cavitational ultrasound, lasers, or microneedles to create microchannel disruptions in the SC to augment passive drug permeation [56].

Passive TD patches release drugs from a solution or gel reservoir across a rate-controlling semipermeable membrane (reservoir TD systems) or from rate-controlled polymer matrix (matrix TD systems). Chemical penetration enhancers are used in creams, ointments, and TD patches to temporarily increase the skin permeability [57, 58]. Specialized TD delivery strategies such as metered liquid sprays and topical gel rub-ons deliver solubilized drug formulated with penetration enhancers to achieve a depot in the skin. For the active systems, iontophoresis utilizes electrical charge from wearable batteries to drive drugs with the same charge into the stratum cornuem [59]; ultrasound softens cutaneous lipids with high frequency waves; cavitational ultrasound, thermal ablation, and poration-based mechanisms bore small holes or microchannels in the SC. Drug passively

diffuses across these channels to create a localized depot for both small molecules and macromolecules. The challenge for a number of these novel delivery technologies has been to balance the delivery efficiency with safety/tolerability to the stratum corneum and dermal tissue.

TD RoA Biopharmaceutical Assessment

TD feasibility can be demonstrated with a combination of mathematical prediction estimates, in vitro skin diffusion studies, and in vivo PK studies. Complex quantitative structure-permeability relationship (QSPR) analyses, multivariate principal component analyses, neural network, and probabilistic modeling have been used to parameterize the preferred physicochemical properties for TD delivery in humans [60]. In vitro skin diffusion studies in Franz-type diffusion chambers determine the extent of drug transport across pig, rabbit ear, or human cadaver skin. These tissues mimic the thickness, lipid content, and permeability characteristics of the human skin. With careful experimental design measure, the drug flux across the skin, metabolic stability, and skin irritation potential can be assessed under conditions that resemble human dosing [61]. The preferred animal models for TD studies are the pig and rhesus monkey being generally more representative of the human skin [62]. However, commonly available laboratory animal models such as rat (hairless or shaved) or rabbit may be adequate to demonstrate initial feasibility of the RoA. Standard in vitro skin transport experiments can also determine the potential for achieving therapeutic drug candidate levels. If the target therapeutic plasma level and plasma clearance of the compound are known (i.e., from IV studies), the target flux rate to achieve therapeutic TD delivery can be determined [63].

The skin irritation/sensitization potential of drug and formulation components are important to validate the TD strategy. Dermatitis (contact or allergic) is the most common dosing site adverse effect as a result of an inflammatory response to drug- or product-induced challenge. In vitro keratinocyte cell culture or human cadaver skin can be used to assay for biomarkers of immune response (pro-inflammatory cytokines) and intracellular enzymes (lactate dehydrogenase) to gauge the extent and mechanism of damage [64, 65]. More recently, genomic and proteomic studies have been adopted to better understand its biochemistry at the cellular level [65]. These models serve as effective screening tools early in formulation development and can be combined with more traditional skin tests such as Draize test, transepidermal water loss, SC conductance, etc., to provide an overall assessment of skin tolerance.

13.2.2 Injectable Drug Delivery

Over the past decade, the injectable drug market has grown several-fold with product sales expected to reach US$ 326 billion in 2015 [66]. This began approximately 30 years ago with the advent of recombinant insulin and has since grown

rapidly due to the advances in non-oral therapies such as recombinant proteins, peptides, monoclonal antibodies, and antibody drug conjugates. The ability to provide therapies for chronic diseases such as diabetes, rheumatoid arthritis, and osteoporosis, among others, that substantially improve morbidity of the disease state and quality of life and lifesaving therapies in the areas of virology (hepatitis C, HIV) and cancer has driven increased investment by pharmaceutical and biotech companies leading to over 200 marketed products in these areas.

Non-oral, injectable delivery offers a practical and efficient way to deliver drugs with direct access to the systemic circulation. Many of the biopharmaceutical factors that are required to understand drug absorption across a mucosa as with the noninvasive, non-oral routes described above may not fully apply. Instead, drug delivery devices provide the means to administer drug directly into the intradermal (ID) space, subcutaneous (SC) space, intramuscular (IM) space, or intravenous (IV) space. Biopharmaceutical assessment of these injectable routes is almost entirely dependent on in vivo PK and injection site safety studies in rodent or a larger, non-rodent species (e.g., dog, monkey, or pig). Consequently, this section does not focus on building rationale for assessing an injectable RoA and the biopharmaceutical assessment methodology, but highlights the delivery features and delivery devices that are most frequently used in enabling testing for clinical trials.

A cross-sectional overview of the skin is shown in Fig. 13.2. Through the use of needle-based delivery devices, drugs can be delivered to the appropriate area of the

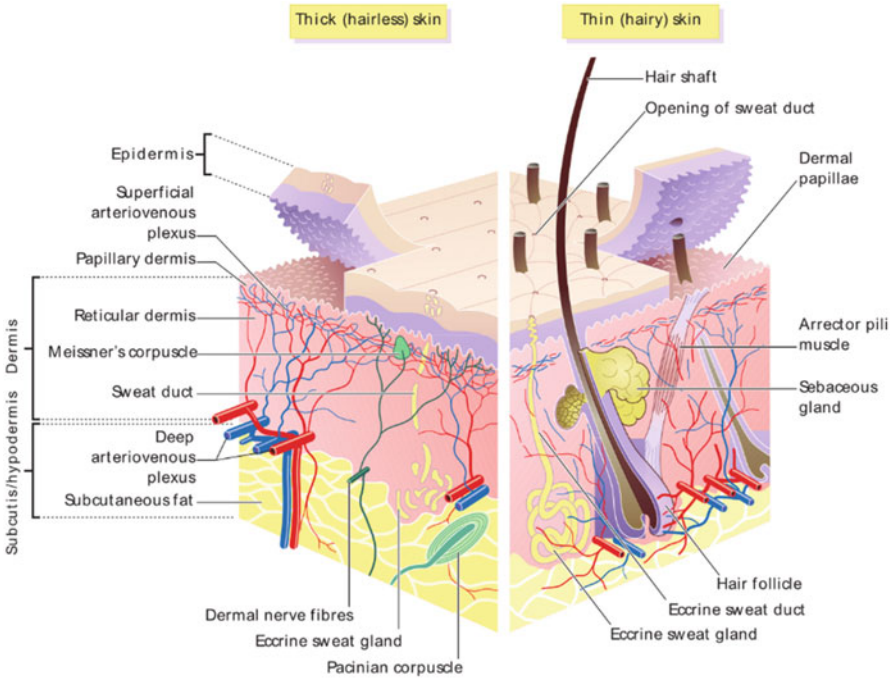

Fig. 13.2 Cross section of skin [67]

skin, ranging from intradermal to intramuscular, by controlling the depth and angle of insertion of the needle.

13.2.2.1 Intradermal (ID) Drug Delivery

As with TD, intradermal delivery (ID) requires drug candidate delivery past the stratum corneum, which is the outermost layer of the epidermis. This can be achieved with a regular hypodermic syringe and needle penetrating the skin at a very shallow angle (approximately 5–15°) and injecting the drug formulation into the skin at a depth of 1 mm, a procedure known as the Mantoux technique. Molded guides are available, such as the ID adapter from West Pharmaceuticals which make this process easier for patients [68].

An alternate approach is to use microneedles (MNs) to penetrate and deliver the formulation through the skin. The needle diameters are typically in the micrometer (μm) range; lengths are approximately 1 mm or less and available as either single needles or arrays, with the latter much more common. Advances in materials, design, and manufacturing have led to the development of needles strong enough to penetrate the skin without losing the drug payload [69]. A key limiting factor to this type of approach is the amount of formulation that can be delivered without leakage as the space for bolus delivery is extremely limited in this space. Typical ID delivery volumes are 0.1 ml or less.

ID delivery is a very popular technique for administering the purified protein derivative (PPD) skin test for tuberculosis. The antigen is administered into the ID layer with a tuberculin syringe containing a small volume of the antigen. Administration of vaccines and hormones has also been driving the growth of ID delivery. Additionally, in certain populations such as geriatrics or infants where it is difficult to give the vaccine through intramuscular injections, intradermal delivery is a viable RoA.

13.2.2.2 Subcutaneous (SC) Drug Delivery

Subcutaneous delivery is a rapidly growing technique largely due to the convenience provided to patients for self-administering therapies in a reliable and cost-effective manner. Since these injections can be administered by the caregiver or the patients, the usual administration areas are the abdomen, the upper arm, and sometimes the upper thigh. The products intended for subcutaneous delivery are primarily needle-based delivery devices which will be described later. Growth of this procedure began with the ability of diabetic patients to administer insulin to themselves and has since become a key factor for drug product differentiation. Subcutaneous injections require the needle to penetrate the stratum corneum and the dermis layers to enter the subcutaneous fat layer and be distributed systemically.

The key challenges for subcutaneous administration are delivery depth and volume. Needle penetration must not be too shallow or too deep to avoid delivering

drug candidate into either the ID or intramuscular (IM) space, respectively. Typical penetration depths are around 6–8 mm, with a generally accepted minimum of at least 4 mm. The thickness of the dermis and subcutaneous layers also varies considerably between patients [70]. Typically the user performs a "skin pinch" to ensure sufficient subcutaneous space is presented for injection. The volume of deliverable medication as a bolus injection is typically 1 ml or less. The SC space cannot hold volumes larger than 2 ml, and the pain felt from the injection increases with volume. This presents a significant challenge for biologic drug candidates, as the often higher doses require an increase in either dose volume or concentration, with the latter risking protein instability and agglomeration.

13.2.2.3 Intramuscular (IM) Delivery

IM is the most common injection practice for delivery beyond the subcutaneous space. In order to ensure full penetration of the subcutaneous fatty layer, the injections are typically delivered at an angle of 75–90° to the skin surface at depths of ¾ in. or greater. In some larger patients, depths up to 3 in. may be necessary to ensure sufficient IM penetration. Typical areas for injection include the upper arm, hips, and gluteal muscle area. These are chosen as a preferred route over intravenous injection as it is faster to administer with lower complications. Relative to SC route the absorption rates are faster due to greater blood supply in the muscles. The volumes delivered by this technique are typically larger than for SC, with volumes of up to 5 ml possible in the gluteal muscle area [71].

13.2.2.4 Intravenous (IV) Drug Delivery

IV is a faster method to deliver medication to the body with 100% bioavailability, but can be considered a much more complicated injection process compared to ID, SC, and IM. It is typically performed in a hospital or surgical center setting utilizing aseptic techniques, for treatments requiring direct delivery into the bloodstream. It is also necessary for large volume infusions with drug candidates diluted into normal saline, dextrose, lactated Ringer's solution, etc.

Delivery is usually accomplished with an IV access device, which can be connected to an infusion pump for prolonged administering, or to a closed valve for bolus administration with a syringe push. There are several types of devices used for venous access. The simplest accesses a vein with a hypodermic syringe and needle to penetrate the vein and push the medication. This technique requires training to ensure that the needle is placed accurately within the vein rather than spearing through the vein. Short peripheral IV catheters with a one-way access valve can also be used to minimize pain and provide continuous venous access for infusions or multiday injections.

Table 13.4 Summary of different invasive delivery modes

Route of administration	Device examples	Drug examples	Administration sites	Depth of penetration	Volumes delivered
Intradermal	Tuberculin syringes Microneedles Needle-free injectors	Hyaluronic acid (anti-wrinkle) Botox	Lower arms	<1 mm	≤0.1 ml
Subcutaneous	Prefilled syringes Pens Auto-injectors Needle-free injectors Patch pumps	Insulin GLP-1 analogs Abatacept Herceptin	Upper arms Abdomen Thigh	4–8 mm	<2.0 ml bolus
Intramuscular	Hypodermic syringes Needle-free injectors	Penicillin Methotrexate	Upper arms Hips Gluteal muscles	15–75 mm	≤5 ml typically
Intravenous	IV catheters	Nivolumab	Veins (wrists, upper arms, central access)	Into veins	Very large infusions

Central venous catheters (CVCs) are used for delivery to larger veins, such as the superior or inferior vena cava or to access the heart directly. This is necessary for certain medications, such as chemotherapy treatments, where rapid dilution of the medicine by blood flow prevents drug-induced irritation in peripheral veins. A CVC can remain in the body for an extended period of time (i.e., multiple months) for continued therapy. Typically these catheters are implanted through a surgical procedure with local anesthesia and often have multiple lumens to enable delivery of multiple drugs simultaneously. Peripherally inserted central catheters (PICCs), midline catheters, are gaining popularity since they are easier to insert, can be inserted at the patient's bedside, and are less intrusive in a patient's life.

Bacterial infections with such catheters are a potential risk since these catheters remain in the body for long periods of time. Tunneled catheters, wherein the catheter is inserted under the skin for some distance prior to venous entry, have been shown to reduce infection rates.

A summary of the more invasive delivery modes and the features associated with each of these is shown in Table 13.4.

13.3 Self-Administration of Therapy by Patients

This section discusses primarily the different types of devices available for subcutaneous injections by patients and the factors that are driving the adoption of such devices. With diabetes reaching epidemic proportions, more and more patients are dependent on insulin to manage their disease [72]. This has led to self-administration of insulin as a subcutaneous injection by patients, spurring to innovation by drug delivery companies to make the process easier and more intuitive for patients. Furthermore, patients administering self-therapy lead to a decrease in the healthcare-associated costs and improved compliance.

The change in the user population from trained healthcare professionals to patients has led to manufacturers focusing on human factors and interactions with the device to make the injection process intuitive. Health authorities are increasingly scrutinizing human factor testing before approving injectable products. The US Food and Drug Administration (FDA) has issued guidance on human factor testing that includes use-related hazard evaluation [73]. It is now requiring device and combination product manufacturers to assess the type of user (healthcare provider, caregiver, trained patient, and untrained patient) and the use environment (at a doctor's office, at home) along with the device and interface to establish usability criteria during design validation activities for inclusion in the new drug application filing.

13.3.1 Prefilled Syringes (PFSs)

This is the most commonly used type of device for self-administration. Typically, these syringes are supplied with a pre-attached needle for accurate penetration depth with the correct dose metered into the syringe by the manufacturer. The user only needs to uncap the syringe, insert the needle into the appropriate injection site, and press the plunger to deliver the medication. This market is predominated with glass PFS as they ensure stability of the drug at an affordable price point due to the relatively inert nature of glass combined with its excellent resistance to moisture and oxygen permeability. Other materials such as cyclic olefin polymers (COPs) and cyclic olefin copolymers (COCs) are also used by a few manufacturers, as they are easier to manufacture through injection molding and offer better breakage resistance compared to glass. While these plastics offer good moisture resistance, oxygen permeability is an issue which can lead to instability of the prefilled drug. This often requires manufacturers to test the stability of the drug in the PFS from the very early stages. Adoption of these newer materials for PFS is gradually increasing in the marketplace. PFSs are used as single-use disposable products with the user discarding the used syringe into a sharps receptacle after injection. With the advent of the Needlestick Safety and Prevention Act (the Act) (Pub. L. 106–430) and the

Fig. 13.3 UnoPen™ from Ypsomed (Courtesy of Ypsomed)

Fig. 13.4 LyoTwist™ pen from Ypsomed (Courtesy of Ypsomed)

growing preference from manufacturers, health authorities, and patients/caregivers to prevent needlestick injuries, additional sharps protection is usually provided for these syringes. Since the manufacturers must perform extensive shelf life and stability testing with the drug candidate and formulation in the syringe prior to product approval, changing the syringe after product approval is an expensive and time-consuming process. Innovative sharps safety devices in the form of housings that fit over an existing syringe and plunger rod that can house a retracted needle are available.

13.3.2 Pens

Pens have grown in popularity as a convenient way of delivering insulin for diabetic patients. Single-dose delivery pens generally have a permanently attached needle, whereas removable needles allow for multidose delivery of insulin for the same patient. The primary container for storing the drug is usually a glass cartridge with a stopper and a crimp seal at one end and a plunger at the other end. Prefilled cartridges are loaded into the pen, and the user attaches a fresh needle for each injection. After dialing in the dose, delivering the injection then removing and discarding the needle, the user retains the pen and cartridge for the next dose. The delivered dose is controlled by the calibrated movement of a plunger rod pushing the stopper a set distance, either dialed in by the user or preset to a fixed dose by the manufacturer. A typical pen is shown in Fig. 13.3. More sophisticated designs, such as pens that can hold a lyophilized drug and a diluent separately, until the time of mixing, have also been developed as shown in Fig. 13.4. Over the years, additional

Fig. 13.5 YpsoMate® auto-injector (Courtesy of Ypsomed)

features such as dose counting, electronic pens, and automatic injection have been added to the pens to drive differentiation in a crowded market. Such advances have blurred the line between pens and auto-injectors.

13.3.3 Auto-Injectors

These are a further simplification of the injection process of self-administration by patients. Auto-injectors are powered usually by springs which store mechanical energy and release it to fully automate the process of injection. Activating the device merely requires pressing it against the skin and/or depressing a button. Similar to pens, battery-driven electronic auto-injectors are also available, but are more expensive and typically single-use disposable devices. These devices are primarily used by patients for administering growth hormones, fertility treatments, and biologics intended for subcutaneous delivery. One example of an auto-injector using a syringe as the drug container is shown in Fig. 13.5.

13.3.4 Patch Pumps

Once again, the needs of diabetic patients for a long-term constant insulin infusion have led to the innovative development of patch pumps. These devices consist of a reservoir to store the drug candidate and formulation, which are infused into the body through an infusion tubing or soft cannula. The soft cannula or infusion tubing is initially inserted by a needle, which is then retracted to leave only the tubing in place. For patients who wear this over multiple days, the softer tubing provides

Fig. 13.6 OmniPod® patch pump from Insulet (Courtesy of Insulet)

better comfort compared to a steel needle. One example of a patch pump is shown in Fig. 13.6.

13.3.5 Needle-Free Injectors

Needle-free injectors have evolved as an alternate way of delivering injections to the ID, SC, and IM spaces [74]. These were driven initially as a way to avoid issues associated with needle-based delivery technologies. The drug formulation is propelled to a high-velocity jet stream that penetrates the skin to the desired depth. Spring-based systems as well as gas cartridge-based systems have been used to propel the liquid medication, and by controlling the energy, the depth of penetration is achieved. They can also be used to deliver powder or liquid formulations.

13.4 Vision for the Future

When challenges to oral administration are encountered, or non-oral delivery is required, non-invasive or invasive delivery options need to be investigated. Although these methods are more complicated from a patient perspective compared to oral delivery, engineering and scientific advances are making such delivery modes more intuitive for patients. While injectable products are the most practical way to dose drugs with direct access to the systemic circulation, non-injectable alternate RoAs (such as IN, B/SL, INH, TD) continue to gain strong interest in clinical development. As more complex NMEs emerge, innovative ways to deliver drugs through new RoAs or technological advances in formulation and drug product design become paramount. These new delivery strategies take advantage of advances in understanding the patient and disease state, opening new doors into novel product options for each of the RoAs. There has been a steady trend over the past decade toward self-administration becoming more prevalent among patients. As a result, devices are being developed with more patient-focused human factor considerations such as portability, intuitiveness, and ease of use. In addition, with the growth of mobile health applications, delivery devices are becoming more connected with mobile devices, thereby enabling better patient compliance with treatment regimens and advancement in standards of care.

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Chapter 14
Outlook for the Future

John S. Morrison and Michael J. Hageman

Abstract The nature of the pharmaceutical industry is constantly evolving. There is currently a strong demand for new therapeutics to be simultaneously safer, more effective, and less expensive. These seemingly incompatible expectations will likely increase over time, and balancing them will require continuous innovation, such as novel technologies to expand the so-called "druggable" chemical space and new insights to reduce the number and severity of costly clinical failures. As a result, the industry has begun pivoting away from the traditional therapeutic product profile: once-a-day, orally administered small molecule drugs intended for large patient populations. Instead, there is a renewed focus toward more niche or specialty areas, using alternative molecular therapeutic modalities administered with novel delivery technologies, often via non-oral routes to smaller and more specific patient populations.

The shift from traditional to specialty drug candidates has also resulted in larger organizations focusing less on establishing new internal drug discovery expertise and more on leveraging the existing clinical expertise toward new therapies discovered by external partners. Such partners include specialized smaller pharmaceutical organizations or academic groups with novel molecular assets, targeting capabilities or even whole drug discovery platforms. The size and lack of experience of these small entities often necessitate that their technologies be developed with the assistance of larger, more established pharmaceutical organizations. The successful execution of this approach requires a collaborative mind-set to collectively overcome the interconnected drug discovery and development challenges. This includes assessing the progressability and developability of promising drug candidates as well as ensuring pertinent clinical information is translated upstream into continuing discovery efforts.

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Despite implementing significant changes, a number of challenges continue to impede the industry's productivity improvement efforts. These include significant knowledge gaps, effective decision-making in an uncertain environment, and competing stakeholder interests. This chapter explores how these issues were, are, and may be addressed in the past, present, and future.

Keywords Pharmaceutical industry productivity • Scientific knowledge gap • Drug candidate selection • Risk assessment • Stakeholder interests • Drug discovery • Effectiveness • Efficiency • Attrition

14.1 Introduction

The growth of the pharmaceutical industry, often referred to as "big pharma," originated as part of the post-World War II boom [1]. Wartime efforts focused on soldier/patient treatment for infectious diseases provided a vision for establishing an improved postwar quality of life for everyone. Many chemical companies saw the opportunity to shift resources and expertise toward the manufacture and supply of drugs which during the war were truly lifesaving. These companies began to grow quickly by capitalizing on the extension of so-called home remedies into products for manufacture and sale. The earnings of these larger firms were then used to build research divisions to improve efficiencies of scale and quality control. In fact, that became one of the key sales pitches and ever-widening distribution of their products. With even greater financial success, these companies began to explore opportunities for more innovative research based on improved understanding of disease and its causes [2].

By the mid-1960s, growth was well underway, and research efforts had identified molecules demonstrating the ability to modify disease processes, either through serendipity or screening in phenotypic disease models. Once identified, these molecules were advanced to other parts of the company for manufacture and sale. The very nature of the screening process yielded molecules with the attributes necessary for progression into successful therapeutics (i.e., appropriate ADME, toxicological, and pharmaceutical properties). These early screening models therefore provided a dual role, assessing the potential for efficacy against a disease as well as indirectly selecting for what is contemporarily known as "developability" properties.

As the analytical tools to probe biology, biochemistry, and molecular properties improved and permitted assessing responses at a molecular level, the industry embarked on a scientifically rational reductionist-based path, albeit one that lost sight of the larger biological system [3]. Current engineering and analytical technologies have advanced to the point where it is possible to carry out hundreds to thousands of experiments in high-throughput screening (HTS) and molecular synthesis (combinatorial synthesis). However despite significant advances, the complexities of biological interactions and redundancies are still somewhat outside the

grasp of the current state of science and require further computational abilities as well as advanced in vivo experimental techniques to understand these interrelated events.

The pharmaceutical industry is approaching a critical productivity threshold in delivering safe and efficacious new therapies, which has been well documented in the literature [4–7]. Despite advances in understanding disease biology and committing resources to exploit those advances, fewer new drugs are being approved while development costs continue to escalate [8–10]. These problems stem from the difficulties inherent in the drug discovery process [11]:

1. An incomplete understanding of endogenous protein function and activity, as well as how these proteins can be modulated and whether these efforts translate to ameliorating human disease.
2. Discovering and optimizing "lead" compounds to specifically interact with the desired target while simultaneously avoiding unintended off-target interactions.
3. Structurally modifying the "lead" compounds to yield drug candidates with suitable physicochemical and ADMET (absorption, distribution, metabolism, excretion, and toxicological) properties while simultaneously maintaining potency such that the molecules are compatible with the intended delivery strategy and able to reach the site of action.

Figure 14.1 summarizes the preclinical drug discovery and development process to help elucidate the inherent difficulties. As a result of these challenges, there is a high demand for innovative, novel, affordable, and effective therapeutics to treat unmet and poorly met medical needs. The following work explores the challenges, typical resolution measures, and alternative options for the future sustainability of the pharmaceutical industry.

14.2 Causes of Poor Productivity in the Pharmaceutical Industry

The specific mechanisms of many disease pathologies are poorly understood [10, 12–16]. Drug discovery efforts venture into the scientific wilderness with many possible paths but limited resources. Decisions must be made balancing perseverance versus pragmatism: where to expend resources, how long to continue the efforts, and when to stop [17]. Against this backdrop, it is unsurprising that many drug candidates ultimately result in failure. Specifically, success rates for Phase 1 through regulatory approval vary somewhat but were reported to be 14–18% between 1995 and 2005 for the 50 largest pharmaceutical companies [4], <5% for anticancer drugs [18], 9.6% between 2006 and 2015, and 11.6% between 2011 and 2014 [17, 19–23].

The rate of decline for pharmaceutical innovation depends upon the particular definitions and assessment criteria employed [24]. For example, nearly as many

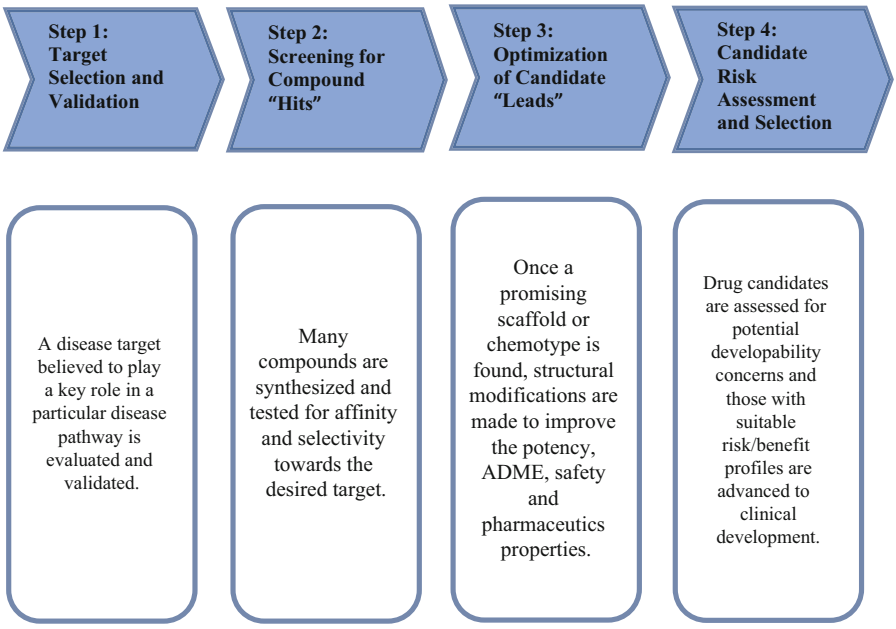

Fig. 14.1 The preclinical drug discovery/development process

studies observed an increase in innovation as those determining that innovation was declining based upon objective measures such as the number of new drug approvals or patents issued. However, for more subjective assessments of either therapeutic value or financial outcome, the majority reported a negative trend in innovation.

Regardless of the innovation trend, development costs are definitely increasing. It has been estimated that producing one drug approval requires screening 5000–10,000 compounds, between 8 and 12 years of intensive R&D, and a total cost of approximately $1 billion [25]. Morgana reviewed published development cost estimates between 1980 and 2009 and found a range of $92 million to $884 million [26]. Mullard found that by 2014, the estimated out-of-pocket cost to develop a successful drug was $1.4 billion [14]. Jogalekar provides a broader estimate of between $1 billion and $5 billion [27]. The number of new drug approvals per billion dollars spent (inflation adjusted) has reportedly halved every 9 years since 1950, a sort of reverse Moore's law which has been dubbed "Eroom's law" [10].

Resolving this poor productivity requires defining the problems clearly and completely. Einstein reportedly stated that if he "had an hour to solve a problem, [he]'d spend 55 min thinking about the problem and five minutes thinking about solutions" [28, 29]. The issues can be categorized as resulting from both a lack of effectiveness resulting in drug candidate attrition and a lack of efficiency or cost containment [4, 30]. In the current environment, the risk/benefit ratio has become imbalanced, with an ever-present drive to completely eliminate risk often losing

sight of valuable potential benefits. These issues are discussed in further detail in the next few sections.

14.2.1 Poor Drug Candidate Development "Effectiveness"

Although universally low, approval rates for drug candidates vary by therapeutic area [4, 5, 22], organizational size [4], modality [22, 23], and the availability of biomarkers [22, 23]. As the successful innovative drugs become generic and less expensive, any new therapeutics must be demonstrably better to be competitive. Consequently pharmaceutical R&D becomes crowded into smaller regions of hard-to-treat unmet medical needs with greater failure risks. This cumulative effect has been termed the "bigger than the Beatles" problem [10].

Increased drug candidate attrition has also been frequently associated with physicochemical properties [7, 31–35]. For example, a recent review of data from four major pharmaceutical companies linked poor physicochemical properties with safety-related clinical failures [7]. Over the past two decades, many pharmaceutical organizations have emphasized potency and structural uniqueness over other properties such as lipophilicity and molecular size which influence drug candidate absorption, distribution, metabolism, excretion, and toxicity (ADMET) [31, 33, 34].

14.2.2 Poor Drug Candidate Development "Efficiency"

While the rate of drug candidate approvals has either stagnated or declined, development costs have continued to increase [10, 13]. Several causal factors have been implicated: a risk/benefit imbalance, late-stage commercial/financial decisions, biased decision-making, and increased regulatory hurdles involving more costly and complex clinical trials [10, 12–16, 36]. The lowering of regulatory risk tolerance has been termed a "cautious regulator" problem, and while increased regulatory requirements may not necessarily improve safety, they definitely increase R&D costs [10]. Furthermore, the increased costs can occur in a greater than proportionate fashion.

Efficiency metrics also counterintuitively depend upon the number of drugs a company successfully launches [37]. The median development cost for a company with a single successful drug was $350 million, but this cost continued to increase to $5.5 billion for companies with more than eight successful drugs on the market. The reason for this discrepancy is that larger organizations must recoup the costs of both successes and failures, whereas failed smaller companies disappear and do not pass along the failure costs. Furthermore, being first to launch is often perceived as more lucrative and thus encourages greater resource allocation, which has been termed a "throw money at it" problem [10].

14.3 Key Challenges to Improving Productivity

Improving both the effectiveness and efficiency of the drug discovery and development process requires a better understanding of the key productivity challenges, which are complex and multifaceted. Significant scientific knowledge gaps hinder the translation of biomedical research into clinically proven therapies. Collaboration between individuals, groups, and organizations requires bridging very different goals, cultures, and work processes. Additionally, decision-making in the face of many unknown variables and with limited resources is also a significant challenge. These factors are further complicated by incompatibilities and incongruencies as discussed further below.

14.3.1 Scientific Knowledge Gaps

The term translational medicine began appearing in the literature in the early 2000s, with the goal of better linking therapeutic targets to human disease causality and transferring this information from "bench to bedside and back again" [38–40]. A key challenge is the cycle time between initiating a promising new disease target program and receiving clinical feedback to validate targets and preclinical models [39, 41]. This has been estimated to consume 15–17 years of intensive R&D [42, 43], with preclinical discovery typically requiring 3–6 years [42] and average clinical cycle times of approximately 9 years [10].

Given such long feedback times, the frequent irreproducibility of initially exciting biomedical research in new disease areas is extremely frustrating [39, 41]. This irreproducibility has been reported across the industry by research scientists at Bayer, Amgen, and MD Anderson Cancer Center [44, 45] as well as the Center for Open Science in Charlottesville, Virginia [46]. It has been estimated that 50–70% of initial research findings could not be reproduced by either the original investigator or another researcher [46, 47]. Such faulty execution of science has been estimated to waste approximately $28 billion annually of critical drug R&D resources as well as "undermining cumulative knowledge production" [46, 47]. Several causes have been proposed for this phenomenon: errors or flaws in reference materials, insufficient facilities and resources, poor study designs/training/laboratory protocols, pressure to publish, inadequate animal models, insufficient validation and problems with data collection/handling/analysis, and selective reporting [48–51]. Animal models of disease and their translation to humans can represent another source of variability and lead to clinical trial failures [52–54], stemming from simple gender differences [55] to phenotypical differences in spite of identical genotypes [56].

Safety issues can arise from either an unintended target effect [57] or polypharmacology involving an off-target [58]. A true preclinical/clinical translational approach requires better fusion of nonclinical and clinical data to understand

the contributing factors for adverse effects across chemotypes [59, 60]. For instance, Wager found that drug candidate efficacious concentrations below a specific threshold were more likely to survive toxicological studies across all CNS-focused programs [61].

The drug design process itself represents another productivity challenge. Target-based drug discovery is scientifically and logically attractive, utilizing high-throughput screening to evaluate the affinity and selectivity of thousands of compounds [10, 62]. This focus on target binding does not however represent the complexity of cellular processes [63, 64]. In contrast, phenotype-based drug discovery (also known as network or systems biology or even classic pharmacology) has historically been more productive for first-in-class therapies, and many drugs currently on the market were discovered with this lower-throughput approach [64–73].

Despite amassing large datasets, the ability to effectively and reproducibly translate this information into successful therapies remains elusive [74]. Rigid and risk averse organizational structures, continuing to employ legacy procedures and punishing inevitable failures, also bear some responsibility for the lack of innovation [75]. Cross-disciplinary discovery teams can be further hindered by a functional silo mentality, unclear accountability and governance, as well as infrequent or ineffective evaluation and feedback [76].

14.3.2 Decision-Making in a Resource-Constrained and Uncertain Environment

With ultimately finite resources and many unknowns, good decision-making requires the proper balance between perseverance and pragmatism [17]. The need to quickly terminate projects likely to fail (the so-called "fail fast" strategy) is widely understood by researchers; however project teams are often loss adverse (the "sunk cost fallacy") [21, 77]. Within a smaller team environment, the loss of both past resource investment and a potentially marketable medicine appears wasteful relative to continuing a project to the next decision point. Furthermore, at larger organizations, the addition of one more risk to the pipeline appears small in the broader context.

The pharmaceutical industry has shifted to focus more R&D upon unmet medical needs [9, 78]. However, these novel biological mechanisms are often not well understood, and this lack of scientific knowledge represents even greater failure risks. On the other hand, attractive and proven targets can represent a different type of risk if pursued concurrently by too many organizations. For instance, multiple PD1/PDL1 programs are currently undergoing clinical evaluation, which presents an additional external risk factor [20]. Furthermore, although clinical trial subject risks are assessed by institutional review boards (IRBs), the

individuals comprising these boards often perceive risk in different ways which can affect how clinical trials proceed or whether they proceed at all [79].

Accurate decision-making can be hindered by internal cognitive biases [36, 80]. The most common biases applicable to drug discovery and development include "overconfidence" in a project's ultimate success, "expectation" of results leading to poor judgment calibration and forecasting, the "availability" of more recent and/or big results, "pseudocertainty" or excessive focus on apparent certainty, "interest" in incentives resulting from a successful drug candidate, "pattern recognition" when evaluating datasets, and "social groupthink".

Despite acknowledging the existence of these biases, they can be difficult to counteract in complex fields such as biomedicine and drug discovery due to the many inherent variables [81]. Researchers attempt to compensate with tighter experimental controls, which consequently shifts away from the real system under investigation. The result according to Sarewitz is a failure of internal controls, over-selection of data, and overreporting of false-positive results. While research scientists generally appreciate that existence of inconsistencies, including the ambiguities and uncertainties inherent in complex datasets, the current research paradigm provides no incentives to report negative results or internally replicate and validate experiments [81].

14.3.3 Incompatible Stakeholder Interests

The key stakeholders in the discovery and development of new medicines are scientists, business people, and the public, each of which has very different interests. Pharmaceutical companies must generate enough revenue to remain profitable and sustainable. They must fund both business expenses in the short term, as well as make wise R&D investments that balance risk and reward in order to generate a return on investment over the long term. Scientists on the other hand seek to generate and apply new knowledge. Investments in science may be "knowledge productive" but not immediately "asset productive." In other words, the new knowledge gained improves the understanding of whether and how new medicines may be created but may not immediately provide clinically successful drug candidates. In contrast the public wants therapies that improve health but also with the contradictory expectations that new drugs be readily accessible, inexpensive, and completely safe.

Unfortunately the premise that drug companies provide highly valuable products benefiting patients, the economy, and society in general is not well appreciated. The cost of a hospital stay or a sub-productive life due to illness is often not sufficiently taken into account when assessing the value and ultimately the price of a new medicine [82]. The costs of drug development are also misunderstood and quite different from manufacturing costs [83]. These development costs are paid up front before any revenue is generated and continue to increase due to greater scientific challenges, patent time frames, and regulatory hurdles. Additionally, more testing

for individual specific efficacy (i.e., personalized medicine) and safety requires more R&D efforts which further increase costs, sometimes disproportionately so.

The ultimate success or failure of a drug development program cannot be predicted beforehand but requires significant financial and temporal investment to determine the answer. Both the successful and unsuccessful programs must be paid for, and the costs of both are included in successfully marketed drug prices. It has been argued that this is simply the cost of doing business and a former head of Pfizer R&D stated "pricing should be based not on R&D costs but on the value a drug delivers to patients" [84]. Despite this, it is difficult for the public to understand why innovative medicines are priced much higher than generics, with the latter widely available for many diseases [85]. The pharmaceutical industry's public image would also benefit from better explanations for price increases occurring over the lifetime of the patent, in some cases increasing twofold or more [86]. Such increases have been taken to the extreme for some orphan indications by companies with little to no internal research and development and thus no R&D costs [87], which tarnishes the reputation of the entire industry in the public eye.

14.4 How the Productivity Problem Has typically Been Addressed

There have been many advances in the pharmaceutical sciences since the mid-twentieth century, notably in the areas of drug discovery, ADME, PK/PD, formulation, regulation, and drug utilization [2]. The so-called big pharma has played a large role in supporting these advances as the "leading engines" of innovation [88]. However, productivity has slowed, and the industry perspective and business models are changing in an attempt to correct this decline [89]. R&D costs have also increased due to greater regulatory burdens from "societal risk aversion" as well as the pursuit of high-risk/high-reward therapeutic areas [90]. No single business entity has the ability or resources to provide the necessary financial, scientific expertise and bioinformatic investments to interpret and utilize the large and increasing amount of data collected [91]. As a result, many of the productivity improvement initiatives undertaken by the industry involve some form of interorganizational interaction focused on efficiency as discussed further below.

14.4.1 Consolidations and Adopting "Best Practices"

One of the major responses to the drop in productivity has been mergers and acquisitions. In fact, 42 members of PhRMA in 1988 had been reduced to 11 by 2012 [92]. For smaller companies, the major fate after receiving approval for a new medicine is to become acquired [93]. Furthermore, since the year 2000, a fairly

small number of companies control the majority of new molecular entities (NMEs), including mostly marketing organizations with little to no internal R&D capabilities [93].

A former president of Pfizer argues that the breadth and diversity of drug candidate portfolios across many major pharmaceutical companies were historically the key to generating valuable new medicines, and the decline in drug approvals correlates with the decreasing number of these companies [94]. Mergers and acquisitions make apparent short-term business sense with respect to raising stock prices, producing synergies, and removing duplication to decrease costs. However, LaMattina notes that this short-term gain is at the expense of long-term and ultimately more valuable R&D productivity. The "R&D engine" is critical to a sustainable business as well as benefiting the public but is "especially vulnerable" to the negative consequences of consolidation. "Lean-focused" reorganizations from acquisitions, downsizing, and restructuring have had the unintended collateral effects of employee demoralization and knowledge drain, despite the obvious caveat that "the process should not contribute to the problem" [42].

Best practices are often enacted to prevent wasting valuable resources by "reinventing the wheel" [95]. Myatt notes that innovation requires creativity and haphazardly applying so-called best practices is not a substitute for "wisdom, discernment, discretion, subject matter expertise, or intellect." As an example, attempts to increase R&D productivity by front-loading development tasks in a parallel fashion in order to reduce cycle times and increase speed paradoxically have the opposite effect when attrition rates are high [96].

14.4.2 Partnerships and Collaborations

A poor understanding of the complexities of human biology and disease contributes to the high failure rates in modern drug discovery and development. As a consequence pharmaceutical companies have evolved from a completely independent and vertically integrated model to a more collaborative and interactive one [97]. Such partnerships bring together greater expertise and experience, as well as efficiencies from pooling resources. However different academic and biotech outfits as well as small, midsized, and large pharmaceutical organizations have their own independent operating processes, experience, and expertise [6, 98]. Appropriate consideration and planning as well as a "collaborative mind-set" and appreciation for diverse approaches can maximize the benefits and offset the difficulties, risks, and complexities to ultimately create a "symbiotic model of innovation" [6, 99].

This approach allows pharmaceutical companies to diversify and balance their drug portfolios with the goal of developing more cost-effective products [6]. In fact, the increase in collaboration efforts over the past two decades is evident by the shift in number of pharmaceutical publications from large pharmaceutical companies to external collaborators [100]. Rafols notes that "big pharma" is transforming from the primary driver of R&D to the role of "network integrator." Schumacher defines

several new types of innovators which have become differentiated in the current environment: knowledge creators, integrators, translators, and resource leveragers [101].

Public/private partnerships (PPPs) across academia, industry, patient advocacy groups, and government organizations have been successful in combating HIV/AIDS as well as exploring the Human Genome Project [102]. Another example is the European Innovative Medicines Initiative (IMI) which began in 2008 to boost drug development by facilitating collaboration, and the president's Council of Advisors on Science and Technology has advocated for a similar model in the United States [91, 103]. These endeavors have been critical in overcoming challenges such as accessing large populations to test statistical significance, resolving complex regulatory issues, and providing novel insights. They also provide multidisciplinary expertise and often complex or novel technologies to evaluate new mechanisms and large complex datasets. Additionally, they can rapidly address significant public health threats [102].

Outsourcing is another collaboration strategy aimed at reducing costs and accessing external expertise, which can be used to complement or substitute for internal R&D [104]. Outsourcing works best when employed as a collaborative knowledge-sharing endeavor rather than a unidirectional "numbers-only" approach [105].

In contrast to "closed" outsourcing collaborations, consortia or "open" collaborations with academia and biotech start-ups allow access to external innovation [106]. This precompetitive information sharing or pooling has resulted in new insights and discoveries in biomarkers, disease progression trajectories (DPTs), and clinical outcome assessments (COAs) [107]. Performing more early-stage research in the precompetitive public arena can free up resources of larger pharmaceutical companies to focus on later-stage discovery and development activities [108].

Companies have also downscaled internal discovery efforts and partnered with academic groups to fill the gap [109]. Academic and industry collaborations can synergistically combine the curiosity-driven research culture of academia with the more rigorous discovery practices from industry [110]. For example, academia provides a unique opportunity to identify, validate, and apply new biomarkers [111]. Academia is driven to answer "hypothesis-driven" problems in order to advance scientific knowledge, whereas industry seeks to provide safe, effective, and commercially viable products. The goals, drivers, cultures, risk/reward assessments, and career advancement considerations of each are unique and if not properly taken into account can undermine the endeavor [42].

However, these collaborations require aligning incentives, investments, and efforts to overcome cultural differences and R&D bottlenecks in order to translate research discoveries into practical treatment therapies [102, 112]. Challenges include misaligned strategies and objectives with respect to resource allocation, loss of decision-making autonomy, differing expectations and cultures, as well as intellectual property allocation [102]. Academia can benefit from a better appreciation of the risks involved with translational research in target selection, assay

design, medicinal chemistry practice, and preclinical pharmacology [110]. Successful execution will also require better target validation, control over false-positive hit rates, and improved informatics infrastructure [97].

14.4.3 The Results of Productivity Improvement Efforts

Clearly the pharmaceutical industry must deliver safe, efficacious, and cost-effective therapeutics which bring value to patients, physicians, payers, and government agencies [113]. The industry also has a responsibility to provide sustainable revenue to support the continued development of new therapeutics as well as a return on investment for shareholders. To do so, the industry must become more effective and efficient, improving the seemingly contrary speed, quality, and cost aspects of developing new drugs.

Typically, companies have focused on cutting R&D timelines and cost, with the unfortunate and unanticipated outcome of reducing quality [113]. This has resulted from conducting more drug development procedures in parallel, more quickly and with fewer people. Opportunities to halt unsuccessful molecules before they incurred large later-stage development costs are lost, resulting in a "development speed paradox" [90]. While cycle times for successful molecules halved, the cost of terminating the unfortunately more prevalent "unmarketable molecules" disproportionately increased. As Lendrem states, "the pharmaceutical industry simply became really slick at delivering late-stage failures to the market place" [90].

The pharmaceutical industry "industrialized" R&D with the intent to improve efficiency and retaining quality but instead made changes which resulted in increased implementation of quantity-based metrics [114]. Such metrics lead to poor outcomes when they are overabundant or inappropriately focused. The simple adage "you get what you measure" must be aligned with the desired outcomes [113]. Quantification metrics can also impede more subjective, but equally valuable, aspects of innovation [28]. Alternatively, failures can result from focusing on the wrong problem. Groups responsible for solving an issue are pressured to do so quickly, often without spending sufficient time understanding the problem [29].

It has been noted that the decline in productivity and innovation correlates with the concentration of big pharma R&D within a smaller number of companies. Following mergers, the subsequent removal of duplications, overlaps, and redundancies reduces the odds of finding successful therapeutics by eliminating differentiated "parallel paths" [88]. Downsizing also frequently results in the loss of talent, either voluntarily or otherwise [89]. In general, acquisitions over the past decade have been perceived as not representing an efficient use of capital [115].

The wide adoption of target-based high-throughput screening was due to the apparent advantages of speed and cost over previous phenotypic-based screening efforts [10]. Unfortunately, this "serial search, filter, and selection process" did not increase the "signal-to-noise" ratio for assessing drug candidate pharmacokinetics, pharmacology, and safety. The lack of improvement in drug approval success rates

indicates "companies [may have] industrialized the wrong set of activities" [10]. Focusing on drug/target affinity is better for validated targets, while a phenotypic screening may be much more suitable for new or polypharmacology-focused targets.

Additionally, screening libraries produced by combinatorial chemistry generally overlap due to limited number of starting materials, reagents, and reactions amenable toward high throughput processes [10]. There is an inherent bias for more compounds over better compounds as the latter are more difficult to specifically define at the discovery stage. And finally, the large number of hits are reduced in serial fashion beginning with potency, since assessing a molecule's full "biological potential" is much more challenging and impractical in any sort of high-throughput fashion.

A review of 142 AstraZeneca development programs (2005–2010) found safety was the main reason for preclinical IND toxicology and clinical Phase 1 failures, while lack of efficacy failures was more common in Phase 2 trials [114]. Current clinical trial design may also be partially responsible for the general industry productivity decline [10]. This includes (1) narrow clinical search parameters that may miss other valuable indications; (2) poorly designed, large expensive clinical trials conducted as a compromise between science, regulation, public relations, and marketing; and (3) the regulatory push for more clinical trials than in the past.

Artemisinin, an antimalarial therapy, represents an example of a successful drug for a deadly disease which would be considered undevelopable under the modern industry paradigm [116]. Although safe and effective, it demonstrated enzyme induction, cell line toxicity, and neurotoxicity during early drug discovery and development. Gordi argues that the use of prespecified charts and deselecting molecules based on undesired properties may lead to "missed opportunities in bringing best-in-class medications to patients" [116].

Current drug discovery and development processes are not as rational as they appear [10]. Although each successful therapeutic has a "biology story" in which the mechanistic understanding appeared to predict the outcome, there are many more failures than successes with this apparently logical approach. Despite the industry's best efforts, productivity may continue to fall if the intersection between commercial success and approval of drugs continues to diverge [10]. All of this leads to the key question: what other valuable medicines may current pharmaceutical industrial practices be missing, and how can the industry become more productive at selecting winners?

14.5 Pathway to a More Successful Future

The pharmaceutical industry is approaching a critical productivity threshold. In order to remain sustainable and meet the unmet medical needs of today and the future, the industry must become more effective in developing successful new medicines as well as more efficient in reducing development costs. To do so, a

number of contradictions and incompatibilities must be resolved: significant knowledge gaps, drug candidate selection processes, accurate and appropriate risk assessment and management, as well as alignment of disparate stakeholder interests. These are discussed further below.

14.5.1 Bridging Knowledge Gaps

Translational science is a term frequently invoked as a means to bridge the so-called valley of death for preclinical to clinical translation [117]. It requires a network or systems biology approach to understand the normal versus disease state and how a drug candidate molecule functions within the "global physiological environment" from both a safety and efficacy standpoint [118–120]. Less discussed, but equally important, is the concept of "reverse translation" whereby knowledge gained in the clinic is used to inform drug candidate selection decisions in the drug design phase and help prevent costly clinical failures [10, 59]. Effective, early, and rapid bidirectional preclinical/clinical translation requires developing and incorporating advanced technologies, including wearable tracking devices, biomarkers, models, and knowledge management systems.

The current explosion of wearable technology and "personal environment monitoring" is also being employed in the drug development process [121]. The wealth of continuous and real-time data collection can help stratify patient populations with respect to both efficacy and safety [122]. This information can be further augmented by incorporating social media feedback to understand patients' needs and wants with respect to treatment progress and off-target issues [117].

Biomarkers are also a critical drug development risk mitigation tool being integrated into the drug discovery and development process [123]. They provide confidence in the relevance of a molecular target to human disease and, along with surrogate end points, can be utilized in early proof-of-concept studies, potentially even in Phase 1 studies [118]. Earlier efforts to establish biomarkers in preclinical stage increase the likelihood of success in the clinic [113].

The use of wearable tracker and biomarker data along with efficacy and safety readouts as well as drug candidate exposures can be implemented into predictive models to better understand the interaction of exposure, target engagement, and safety and efficacy relationships [121, 123, 124]. These models can be used to simulate pharmacology, disease progression, and pharmacokinetic/pharmacodynamic relationships to understand the disease state and the effect of treatment options [116, 118]. As an example, it was recently reported that implementation of a PK/PD model for safety and efficacy assessments of CNS and pain programs at AstraZeneca resulted in decreased animal usage and better understanding of in vitro and in vivo relationships [125].

Automation may also reduce clinical cycle times, allowing for more rapid feedback and decision-making. For instance, a new instrument capable of onsite chemical synthesis, purification, and formulation of drug candidates was recently

reported [126]. With further development and regulatory acceptance, such an instrument could be used in the future for onsite compounding simultaneously at multiple locations, precluding the need for costly and time-consuming scale-up and stability studies.

The ultimate beneficiary of improved translational science may be the concept of personalized medicine, an area of increasing focus for both the industry and regulators [113, 127]. An improved understanding of the heterogeneity of treatment effect (HTE) will provide a better assessment of the value or benefits and risks of treatment options [128]. However, the paradox of precision medicine is that finding the right drug for the right patient at the right time is an intensive effort, benefiting a small population and at a much higher cost [129, 130].

The translational science initiative will also require interpretation of ever-larger amounts of data from different patients, geographies, and multiple time points. Better information and knowledge management systems are needed to integrate and leverage this collective data [131, 132]. Implementing these knowledge management systems will require significant financial, scientific expertise and informatics investments to effectively utilize the large datasets. Successfully implementing translational science will also require cross-disciplinary collaboration to develop new understandings, tools, and regulatory guidances [91, 129, 130]. To share these burdens and leverage the necessary scientific expertise, expansion of precompetitive public/private collaborations (PPPs) further into preclinical space and better translational informatics across all clinical studies to improve modeling and predictive tools has been recommended [117, 120].

A translational approach will also benefit safety science by incorporating all the data collected for safety assessments across the industry [59]. This in turn would support a transition from simple toxicological assessment to drug development enablement earlier in the discovery space and help move beyond traditional pharmacovigilance to a broader pharmacosafety vision [120, 133, 134].

14.5.2 Improving Drug Candidate Selection

A better understanding of how biomedical research translates to treating human disease will also enable better drug candidate selection, minimizing the risks of pursuing suboptimal candidates [33, 135]. This will require better early screening to select compounds with efficacy against disease rather than potency against a target, through a combination of target- and phenotypic-based screening [136]. Historically, phenotype-based drug design strategies have been more successful and would complement the current industry standard target-based drug design approach, especially for novel or polypharmacology targets [10, 63, 109, 136, 137]. Virtual screening may also prove valuable to augment standard high-throughput approaches [138, 139].

Improving drug candidates also requires greater molecular diversity beyond typical small molecular therapeutic or “druggable” space from both a “nature”

(chemical starting points) and "nurture" (more informed optimization) standpoint. Doing so will require advancing beyond "rule of 5" restrictions and greater exploration of alternative molecular modalities [33, 135, 140]. A better appreciation of the role of physicochemical properties in drug candidate design and attrition' is also recommended [141].

Organs on a chip represent a promising time and cost-saving technology to assess tissue exposure-response and reduce dependence on animal testing [142–146]. These microfluidic cell cultures, generated using microchip manufacturing methods, mimic tissue-tissue interfaces as well as physiological microenvironments of the human lung, intestine, liver, kidney, and heart. These systems can be used for target identification and structure property relationship assessments.

Complex diseases such as those in neurodegeneration and oncology may have several interacting pathways or nodes of a network, requiring that multiple targets be addressed simultaneously [136, 147, 148]. Such polypharmacology can "intentionally" improve efficacy, prevent drug resistance, and reduce target-related adverse events for multigenetic diseases but also may "unintentionally" increase off-target adverse events [58]. A convenient starting point for polypharmacology-focused efforts is the repurposing of existing drugs to take advantage of small molecule drug promiscuity as well as known physicochemical properties and similarities in protein family binding sites [58, 149].

14.5.3 Better Risk Assessment and Management

Along with improvements in bidirectional knowledge translation and drug candidate selection, critical and accurate decision-making is necessary for appropriate risk assessment and management. However people require proper tools and information to make good decisions and overcome biases and unconscious assumptions [80, 150]. Often the focus of productivity improvement is on efficiency enhancements, rather than the more difficult challenge of improving effectiveness with fewer clinical failures [136]. Doing the "right science" to effectively prevent failures is more important than doing "the wrong science quickly" to efficiently minimize the consequences of failure [90]. Several strategies have been proposed to mitigate the risk of clinical trial failures:

1. A "five-dimensional framework" or five R's strategy to improve drug candidate decision-making and the potential for project success—selecting the right target, tissue, safety, patient, and commercial potential [114].
2. A "Three Pillars" strategy to reduce cancer drug attrition rates: better preclinical models with greater clinical predictability, more predictive and pharmacodynamically revealing biomarkers, and better collaboration to leverage the independent strengths of industry, academia, and regulators [18].
3. A "Three Pillars" strategy to improve clinical drug candidate survival: integrated understanding of fundamental PK/PD from exposure at the site of action,

confirmation of target binding, and expression of functional pharmacological activity [35].

Taken together, these strategies recommend gaining early knowledge of the disease and therapy, developing better preclinical to clinical translation, greater collaboration between subject matter experts, and establishing early proof of concept for the proposed therapeutic efficacy and mechanism.

In addition to the unknown development path for so-called "next-generation" therapeutics, gaps between bench discoveries and successful new therapeutics have widened due to the unknown regulatory requirements [91]. Reforms of the regulatory and patent systems are needed if critical new drug development for unmet medical needs is to remain sustainable [91, 151].

14.5.4 Aligning Stakeholder Interests

The future of the pharmaceutical industry also requires aligning the disparate interests of the key stakeholders to discover the intersection of utility, value, and sustainability (Fig. 14.2). This necessitates better understanding and communication of the full drug development costs (including the failures) versus the simple manufacturing costs of a final product. The development costs also include meeting regulatory demands, which should ensure sufficient patient safety without unduly adding to the cost of life-improving therapies [91, 151]. Ultimately minimizing both safety risks and development costs represents a continuum, and a compromise between the two must be reached since completely precluding any potential safety liabilities would incur unacceptably high development costs (Fig. 14.3).

Pay for performance has also been cited as a potential remedy to improve quality, both from the discovery to development transition and the clinical to commercial transition. Incentives for academia must change to ensure the quality and robustness of data in order to resolve the irreproducibility crisis responsible for significant resource waste and opportunity cost in the drug discovery [152]. Concurrently, payers are increasingly asking for proof of efficacy before

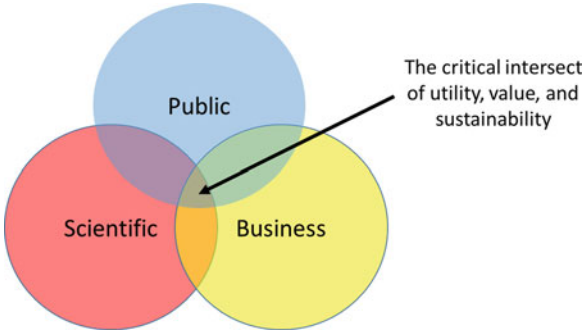

Fig. 14.2 The intersection of key stakeholder interests

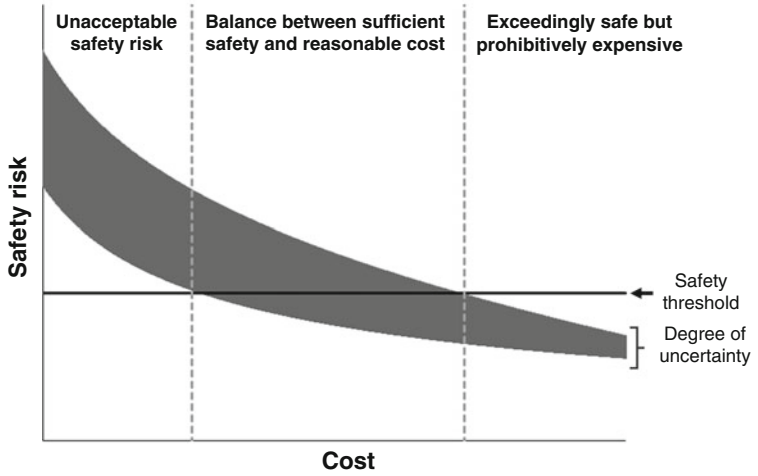

Fig. 14.3 The drug development cost versus safety trade-off

compensating for costly therapeutics that may only be effective in some patients or subpopulations [153].

Operational effectiveness or performance requires everyone within an organization to collaboratively and collectively work together toward the same goal ensuring quality medicines are produced as rapidly and efficiently as possible [154, 164]. The Ringel group identified several factors correlating with improved performance: greater focus on traditional versus rare diseases or personalized medicines, geographic centralization including less outsourcing, and a functional rather than business unit-focused structure. Interestingly, the Tollman group arrived at a different conclusion regarding the latter factor, advocating that program teams be asset rather than functional focused. Ultimately such discrepancies likely indicate the problem is more nuanced and requires further study.

There is also a difference of opinion as to whether R&D efficiency can be improved with further standardization, simplification, and removal of duplication from the drug development process ([154], [164]). Previous efforts focused on efficiency improvements such as Lean Six Sigma or mergers, and acquisitions have failed to reduce costly clinical failures [136]. Instead, improving effectiveness is recommended through executing on strategy in a repeatable and quality producing manner to create value [155]. Furthermore, any efficiency-focused processes necessary to support critical activities and business needs must be balanced with the freedom for scientific exploration to ensure the creativity, intuition, and serendipity necessary for innovation in drug discovery are not lost [113, 156]. Process implementation requires effective management (doing things right), while innovation requires effective leadership (doing the right things and creating a clear and

compelling vision). Ultimately, "the process should not contribute to the problem" [42].

Organizational cultures (including team or department subcultures) are also an important factor in performance, requiring time and energy investment, whereas changing a nonproductive culture can be a slow and challenging process [114, 157, 158]. Fostering an optimal and organizational specific culture improves employee engagement, retention, and productivity but requires trust, transparency, and rewarding the right behaviors [159, 160, 164]. Learning and development are also critical cultural elements to ensure employees are able to meet the ever-changing challenges both today and in the future, including both current and future employees [44, 161, 162].

14.6 Summary

Resolving the pharmaceutical industry's productivity crisis will require overcoming multiple contradictions and incongruencies. Just as the concept of translation science recommends a network or systems biology approach, drug discovery and development may need to pursue a similar systems network approach. In such a system, expanded flexibility and knowledge sharing would be prioritized over efficiency measures which have had a negligible effect on improvement and may be the enemy of innovation [28, 136]. Knowledge would be accumulated and shared early to minimize risk, both in preclinical to clinical translation and the reverse.

While the next scientific breakthrough cannot be predicted, pharmaceutical scientists' contributions are critical, and organizational flexibility is necessary to accommodate creativity and serendipity [2]. The decline in productivity and innovation has been correlated with the concentration of big pharma R&D within a smaller number of companies, with differentiated "parallel paths" being eliminated as unnecessary duplication [88]. However the "parallel paths" strategy to diversify and balance the risk of failure in the face of uncertainty has been historically successful: the British Longitude Prize, the Manhattan Project, the DuPont's synthetic nylon fibers, the incandescent light bulb, and the integrated circuit, not to mention the success of smaller biotechnology-focused companies. Although many individual efforts may be unsuccessful, the larger collective effort increases the odds of creating a successful therapeutic.

Ultimately, the contradictions within the current drug discovery/development paradigm may be unresolvable and require a "scientific revolution" in which completely new and noncumulative ideas are established and implemented to solve contemporary challenges [163]. Einstein famously said that if he "had an hour to solve a problem, [he]'d spend 55 min thinking about the problem and five minutes thinking about solutions" [29]. Stated alternatively, if a solution to a problem is not readily apparent, then the problem is not sufficiently understood and requires further study.

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