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# ADME FOR THERAPEUTIC BIOLOGICS: WHAT CAN WE LEVERAGE FROM GREAT WEALTH OF ADME KNOWLEDGE AND RESEARCH FOR SMALL MOLECULES

Weirong Wang[1] and Thomayant Prueksaritanont[2]

[1] *Janssen Research and Development, LLC, Spring House, PA, USA*

[2] *Merck Research Laboratories, West Point, PA, USA*



## 1.1 INTRODUCTION

Over the past decade, there has been increased investment to the development of biotechnologically derived drug products or biologics (including peptides, proteins, and monoclonal antibodies, mAbs, aggregately referred as large molecule (LM) drugs) in pharmaceutical companies [1, 2]. These are attributable to the reported therapeutic success of this modality thus far, together with the rapid advancement and breakthroughs in the fields of recombinant DNA biotechnology and molecular biology. However, reports on mechanistic investigation of absorption, distribution, metabolism, and excretion (ADME) processes for LMs are sparse and our current understanding of the associated mechanisms and key determinants of pharmacokinetic (PK) properties is scant [3]. Conceivably, these are related to the fact that the biopharmaceutical industry is still at an early stage, relative to the traditional pharmaceutical counterpart; the first approved LM drug product was in 1980s [4], several decades after many small molecule (SM) drugs were on the market. In addition, unlike the discovery and development of SM drugs, where the sciences and the functional role of drug metabolism and pharmacokinetics (DMPK) in studying and understanding ADME processes have been well recognized as an indispensable and integral discipline spanning from early discovery to development and postmarketing spaces [5], the function of DMPK in support of LM drug development is somewhat limited to mostly *in vivo* PK and/or pharmacokinetics/pharmacodynamics (PK/PD) studies, typically after candidate selection and primarily in the clinical space. Despite the intrinsic difference between SM and LM drugs, it should be of particular interest to appraise the relevance and applicability of what we have learned over the past few decades from the discovery and development of SM drugs to the same process of LMs. Thus, in this chapter, a brief historical perspective is presented on how the roles of DMPK and the key enablers for studying the ADME processes of SM drugs and their underlying mechanisms have evolved over time in order to influence internal de-risking strategy and decisions. External factors, such as changing regulatory environments and evolving LM discovery and development landscape, are briefly reviewed. Also presented is an overview of a DMPK concept analogy between SMs and LMs, as well as case examples to demonstrate the applicability of SM DMPK knowledge and experiences to LM drug discovery and development.

## 1.2 SM DRUG DISCOVERY AND DEVELOPMENT: HISTORICAL PERSPECTIVE

### 1.2.1 Evolving Role of DMPK: Paradigm Shift

It has long been well recognized that the drug discovery and development process is very expensive, largely due to a high development attrition rate and prolonged development time to meet the requirement for more extensive and

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complex clinical trials [1, 6–8]. In 1990s, poor human PK and bioavailability were the most significant cause of attrition for SM drugs, accounting for approximately 40% of all attrition in development. This number was dramatically reduced to approximately 8% by 2000 [7]. Such a drastic difference has been attributable primarily to a *Paradigm shift* in the roles of DMPK from little involvement decades before 1990 to active participation in SM drug early discovery starting in late 1980s [5]. Previously, compounds were selected mainly based on *in vitro* potency and *in vivo* efficacy in animal studies, with little attention being paid to the exposure or PK as an important measure connecting pharmacodynamics (PD)/efficacy/safety profiles, or consideration to commonly observed differences in these profiles between animals and humans. The integration of DMPK support as a key component of the overall drug discovery process helped to better understand ADME properties and filled these gaps, thus enabling proper data interpretations and rationale-based predictions of DMPK-related properties in humans [9–13]. As a result, potential liabilities of new chemical entities *in humans* were dialed out as early as possible, leading to increased likelihood for preclinical candidates to be developed successfully as therapeutic agents.

### 1.2.2 Key Enablers to Successful DMPK Support

The aforementioned successful DMPK support would not have been possible without numerous advances over the past few decades in drug metabolism sciences and technologies, which have provided powerful tools to enable DMPK scientists to shape SM drug metabolism research. Of special note are two key enablers, signifying game changers within the time period of interest (late-1980s to late-1990s): (i) rapid advancement of cytochrome P450 (CYP) science and (ii) availability of liquid chromatography–mass spectrometry (LC–MS). As will be described in later sections, these elements and associated wealth of information generated over the last few decades can be leveraged and applied to support LM drug development.

The CYP enzymes play central roles in the metabolism of SMs; it is estimated that more than 70% of marketed SM drugs were eliminated primarily by CYPs [13]. CYP enzymes were discovered in 1958, and research on their structure, function, regulation, and tissue expression levels, as well as their role in drug metabolism, was rapidly expanded in the 1980–1990s [14–16]. Such rapid advancement provided fundamental concepts and important tools that helped leverage preclinical/*in vitro* results as a bridge to clinical outcomes, consequently enabling one to predict, understand, and manage clinical findings, particularly with respect to human clearance and PK variability due to factors such as CYP-mediated drug–drug interaction (DDI) or CYP polymorphism [13, 16–18]. Specifically, for compounds with CYPs as the major or sole contributor to their metabolism, human metabolic clearance can be reasonably predicted based simply on *in vitro* metabolism studies with recombinant CYP isoforms, corrected for relative expression levels of each isoform in tissues [19]. In addition, the knowledge of CYP substrate specificity, multiplicity, and responses to factors, such as inducers and inhibitors, has provided a means to quantitatively predict, based on *in vitro* studies with specific CYP marker substrates or inhibitors/inducers, the magnitude of DDI, thus enabling a selection of candidates at discovery stage that do not bear considerable liability to serious clinical DDIs, either as perpetrators or victims [16–18, 20]. The DDI prediction results have also been used (and accepted by regulatory agencies) to inform inclusion and exclusion criteria for clinical programs, decide whether a clinical DDI study is needed, and inform product labeling with respect to dosage adjustment and warning/contraindication when used with other medications [21, 22]. Collectively, advances in understanding CYPs, the primary determinant for clearance mechanism of majority of SM drugs, has helped reduce drug development failure rate due to undesirable human PK properties.

In the area of tools and technologies, the successful coupling of high performance liquid chromatography with mass spectrometry (MS) has provided unprecedented sensitivity, selectivity, and high throughput that has facilitated the rapid assessment of ADME properties and the multiplicity of their governing factors for SM candidates in animals and humans [23–26]. Capitalizing on chromatographic separation and mass selectivity, the LC–MS technology enables the quantitation of coeluting or overlapping analytes, which otherwise would be constrained by chromatographic resolution. A dramatic outcome of this feature is the various *in vivo* and *in vitro* cassette studies in which more than one compounds were administered or incubated for the screening of DMPK properties, including metabolic stability, DDI liability, and plasma protein binding [23–25]. Along with the accelerated method development similarly attributed to the extraordinary selectivity and sensitivity of LC–MS, this practice has tremendously facilitated the speed and throughput of analyses of samples of low concentrations or of small volumes. Likewise, LC–MS technology has reshaped the business of metabolite characterization, allowing rapid detection and identification of major metabolites of drug candidates so that the result can be fed back into the cycle in time to influence the synthetic chemistry effort. Together, this powerful technology has enabled informed decisions to be made rapidly on a large number of candidates, each available in a small quantity, during the discovery stage. It has also enabled other in-depth mechanistic investigations into the governing factors of ADME processes, as well as detailed and accurate characterization of ADME properties of development candidates required for risk mitigation and regulatory submission [5, 10, 26]. With the recent advent of new chromatographic techniques, such as ultraperformance

liquid chromatography, and more sophisticated MS, such as high resolution MS [27], this technology will continue to be the most powerful tool for drug discovery and development for SMs, and potentially for LMs alike.

### 1.2.3 Regulatory Considerations

Successful development of a drug candidate requires the right set of high quality data to help inform decisions not only internally, but also decisions by regulatory authorities. In-depth industry analysis by PhRMA has attributed much of the increasing R&D costs to the extending development times in clinical phases (10–15 years), greatly influenced by the increased regulatory demands in today's low risk, low tolerance environment, and stemmed primarily from the withdrawal of several prominent prescription drugs from the market over the past decades for safety reasons. Of special note was the withdrawal of the drugs from the U.S. market in 1990s, half of which due to serious and unmanageable safety issues as a result of PK and/or PD DDIs. These occurrences prompted the FDA to publish guidance documents for industry to encourage the characterization of DDI potential for a new molecular entity early in the drug development process [21]. The first two guidance documents: one on *in vitro* DDI, published in 1997, and the other on *in vivo* DDI, published in 1999, focused on metabolic DDI due to CYPs, and was based primarily on considerable advances in our understanding of roles of the CYP family at the time. In the latest draft DDI guidance recently issued [22], there are recommendations to conduct many additional drug transporters, and drug interaction studies for LMs have been included for the first time. Given the current status and understanding of drug transporter sciences relative to the CYPs [28], the inclusion of drug transporters in the latest guidance suggested that the FDA has become more proactive in embracing evolving sciences in their decision making. Likewise, much less is known about LM drugs in their DMPK properties and underlying DDI mechanisms in comparison with SM drugs. Consistent with this, the time span between the first approved LM drug in 1986 and the anticipated DDI guidance is much shorter than the corresponding time span of many decades for SM drugs. This apparently speedy process for LMs may be attributable to the decision of the 2003 FDA to transfer the regulatory responsibility from the Center for Biologics Evaluation and Research (CBER) to Center for Drug Evaluation and Research (CDER), who has been overseeing the regulatory approval of SM drugs and has provided more comprehensive information on ADME properties and associated DDI implications. It is conceivable that there will be increasing regulatory demands for other DMPK-related information for LMs in the near future. In fact, the CDER Science Prioritization and Review Committee has recently highlighted several relevant LM DMPK aspects warranting additional research and further understanding [29], suggesting that the most relevant factors that affect the PK/PD determinants of LMs, such as a variety of specific receptors that can influence protein $t_{1/2}$ and distribution (e.g., delivery of therapeutic enzymes to the correct cellular compartment), should be identified.

## 1.3 LM DRUG DISCOVERY AND DEVELOPMENT

### 1.3.1 Role of DMPK: Current State

From a DMPK perspective, the current state for an LM support paradigm and ADME knowledge is similar to where we were with SM drugs a few decades ago. First, DMPK is involved primarily in the development space after a preclinical LM candidate has already been selected, and much less at the early drug discovery stage of the optimization and selection of LM candidates. This conventional mindset, widespread in many biotech and pharmaceutical firms, resembles what was practiced for SMs before the 1990s, and may stem from a wide belief that PK of biologics, especially mAbs, is well behaved/predictable and that this property is not known to be a major success-limiting factor, based on a historical record of relatively low attrition rate for LMs versus SMs. However, the view that PK of all mAbs is well behaved and the deviation of typical mAb PK properties is due primarily to their intended target binding has recently been challenged. For example, a specific off-target interaction of an anti-FGFR4 mAb candidate has been identified as the cause for its rapid clearance, poor target tissue biodistribution, and limited efficacy [30]. The authors concluded that screens typically developed to identify general nonspecific interactions are likely to miss the rare but highly specific off-target binding observed in this study. Similarly, we found that several of our early mAb candidates displayed much shorter half-life ($t_{1/2}$) than anticipated [31]. This less than desirable DMPK property was recognized after DMPK involvement following candidate selection. Some of the candidates were eventually terminated due to the poor PK behavior and safety concerns. In addition, an examination of the clinical PK of approved mAbs clearly showed that mAbs can exhibit different PK at their saturated dose [32]. It is also notable that the relatively low attrition rate of LMs that is often referred to may not be replicated going forward, considering the increasingly competitive LM landscape and an evolving LM pipeline enriched with a variety of new and untested engineering technology platforms [33].

Furthermore, current DMPK approaches for LM support in preclinical development is usually limited to *in vivo* PK studies in laboratory animals, including mice, rats, dogs, and monkeys. In the case of mAbs, it has been widely accepted that nonhuman primate (NHP) is a representative animal model for human PK, and human PK prediction is typically performed using an empirical allometric scaling approach

heavily dependent on this single species [34]. However, recent publications suggest potential issues with this approach. Vugmeyster et al. have shown that an anti-amyloid beta Ab2, a humanized mAb against amino acids three to six of primate amyloid beta, exhibited faster clearance, with a much shorter $t_{1/2}$ of less than 2.5 days, compared to approximately 13 days for a control antibody (no affinity to the target) in monkeys [35]. Additional mechanistic studies revealed that the fast elimination of Ab2 was linked to off-target binding to fibrinogen specific to monkeys and not humans, and thus provided a basis for a projected much slower elimination of Ab2 in humans. The prediction was later proven in a clinical trial [36]. Clearly, without appropriate DMPK input and mechanistic insights, this compound would have been precluded from further development. There are few other examples along this line (Merck internal database; Dr. FP. Theil, personal communication), where NHP PK failed to inform human PK correctly, due to either under- or overprediction. Collectively, these cases underscore our currently limited knowledge about the ADME processes of LMs and their determinants, which are even less adequate than those we knew for SMs in the 1980s.

One of major barriers limiting our understanding in ADME properties of LMs may be related to lack of appropriate analytical tools. It is well known that the structural complexity of LMs has posed formidable bioanalytical challenges. The commonly used bioanalytical methods for the determination of LMs in biological fluids are ligand-binding assays that are immunological in nature. These assays usually have an associated degree of nonspecificity. For example, multiple forms of mAb and ligand can exist *in vivo*, including free mAb, free ligand, and mono- and/or bivalent complexes of mAb and ligand. Given the complexity of the dynamic binding equilibrium occurring in the body after dosing, and multiple sources of perturbation of the equilibrium during bioanalysis, *ex vivo* quantification of the forms of interest (free, bound, or total mAb and ligand) may differ from the actual ones *in vivo* [37]. Several other possible weaknesses that may result in erroneous characterization of drug disposition have also been identified and recognized by regulatory agencies. These shortcomings, which include interferences from structurally related compounds such as endogenous proteins, degraded or catabolic products that are immunoreactive but may or may not be active or may elicit activity with different potencies, will certainly complicate data interpretations and hamper in-depth understanding of underlying mechanisms [38, 39]. LC–MS is emerging as a highly useful complementary tool for qualitative and quantitative applications to LMs [40–42]. However, the routine use of LC–MS is still hampered by the relatively time-consuming development process due to complex sample preparations, such as immunocapture and enzyme digestion of LMs, and limited sensitivity as compared to a typical immunoassay [42–44].

### 1.3.2 SM/LM DMPK Analogy

On a high level, PK/PD models and concepts are generally similar between SMs and LMs. In other words, PD is linked to PK (or specifically drug concentrations at biophase ($C_e$), which is related to systemic concentrations ($C_p$)), following certain relationships defined by molecular mechanisms of action of a drug, irrespective of its modality [45]. Similarly, PK is a collective depiction of ADME processes for both SMs and LMs. However, at the next level down, including ADME processes and associated underlying determinants, there are differences between the two modalities. For SMs, the ADME processes are relatively well studied and are mainly governed by (i) specific characteristics of a compound, including its physicochemical properties and ability to interact with transporters, drug-metabolizing enzymes, and binding proteins and (ii) physiological factors that govern the exposure of the compound to those proteins, such as distribution, tissue localization, and organ blood flow [46]. Not only have the nature of these interactions and their governing factors been mostly characterized, appropriate tools required for the studies have also been largely available. As illustrated in Figure 1.1 following a typical oral administration, an SM drug is absorbed either via passive diffusion and/or active transport, and then subjected to first-pass metabolism in the intestine and/or liver, before reaching systemic circulation for distribution to tissues and other organs of elimination, including kidney. Systemic bioavailability (F), a PK parameter central to efficacy and safety of a drug candidate, is a product of these processes.

In general, the ADME processes for LMs are much less characterized, as compared to SMs, even though their ADME processes are similar in concept. Unlike SMs, oral administration is precluded by molecular size, hydrophilicity, and gastric degradation of LMs. LMs are administered intravenously, intramuscularly, or subcutaneously (SC). As illustrated in Figure 1.1, following an SC administration, an LM is absorbed and potentially subjected to metabolism/catabolism at the injection site as well as during transport through the lymphatic system before reaching blood circulation. This is based largely on limited studies in sheep [47], and more recently in rats and dogs [48]. Analogously to SM drugs, systemic bioavailability, F, is a product of these processes. But unlike SMs, there are no established methods to measure the extent of absorption or presystemic catabolism. There is also little knowledge on the factors that can impact these parameters in animals or humans [3, 48]. Not surprisingly, it remains a challenge to extrapolate the SC absorption results in preclinical species into humans for LMs.

The majority of SMs enter tissues by passive diffusion, and the key determinant of tissue distribution includes nonselective binding to tissue proteins. Many SM drugs have also been reported to enter tissues via active transport, and the transporters involved have been identified. Similar to

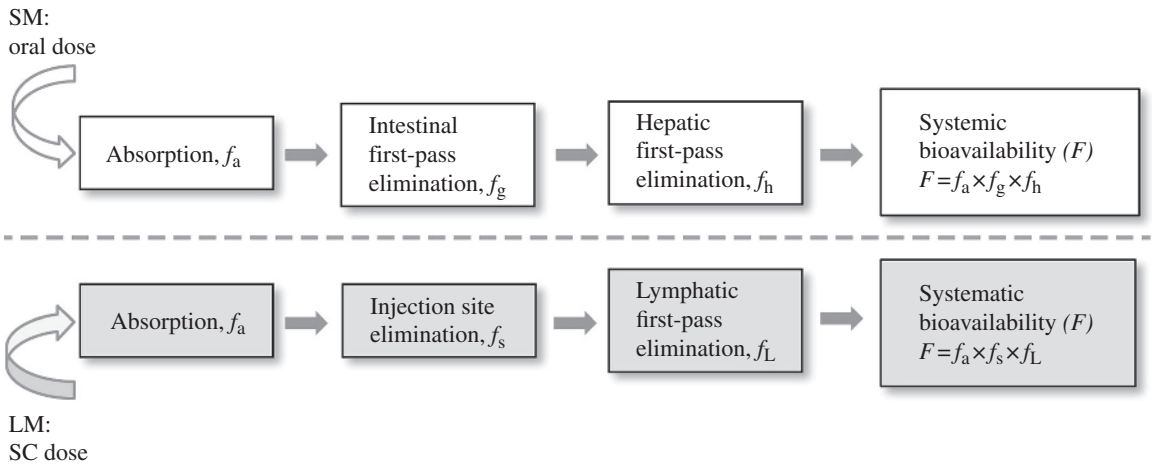


**FIGURE 1.1** The ADME concept analogy between SMs and LMs. $f_a$ = fraction absorbed; $f_g$ = fraction-escaped gut first-pass elimination; $f_h$ = fraction-escaped hepatic first-pass elimination; $f_S$ = fraction-escaped degradation/catabolism at the injection site; $f_L$ = fraction-escaped degradation/catabolism in the lymphatic system.

SMs, once entering the blood circulation, the LMs must cross the vascular wall to reach the site of action in target tissue(s) in order to exert their pharmacological activity before being eliminated via metabolism or other elimination pathways. Because of their molecular size, distribution of LMs into tissues is generally slow, and via the so-called convective transport through pores on capillary walls, as well as transcytosis from circulation to the extracellular space [49, 50]. Unlike SMs, distribution of LMs is usually limited to extracellular fluids due to their size and hydrophilicity. This fundamental difference between the two modalities is in line with the location of their respective biological targets. Namely, the targets are either soluble or on the cell surface for LM drugs, which are in contrast to the intracellular location for most SM drugs.

Presence of target in peripheral tissues can significantly change the tissue distribution of LMs, and leads to potential disconnect between plasma and tissue levels for LM. Given the importance of understanding the distribution to target tissue for LMs and the availability of related tools, tissue distribution studies have been more frequently conducted (vs other ADME-related types of studies) for LMs [51]. However, despite the wealth of LM (especially mAbs) tissue distribution data that had been accumulated [51, 52], in-depth mechanistic studies on these processes are scarce, and it currently is still unclear which transport pathway, convective transport or transcytosis, would be quantitatively more important in terms of extravasation of protein drugs from blood circulation [3].

As described earlier, SMs are eliminated from the body predominantly via metabolism with CYPs as the major metabolizing enzymes. Direct excretion into bile and/or urine is the other major elimination pathway for a variety of compounds that escape metabolism. For LM, common mechanisms of elimination include filtration (e.g., into urine), secretion (e.g., into the bile), and biotransformation (e.g., metabolism or catabolism). Smaller size LMs are subjected to elimination via kidney. Contrary to SMs, LMs are typically not subjected to metabolism by CYP enzymes, but generally believed to be catabolized to peptides and amino acids via proteolysis throughout the body, either extracellularly or intracellularly following fluid-based pinocytosis/receptor-mediated endocytosis. Subsequent to pinocytosis/endocytosis, the LMs usually are catabolized inside the cells. A notable exception for this process is with mAbs and endogenous immunoglobulin Gs/albumin, which are protected from degradation by binding to Fc receptor of the neonatal (FcRn) (at acidic pH) with subsequent dissociation (at neutral pH) to recycle back into circulation. As a result, these molecules display a distinct key feature of relatively long elimination half-lives usually in weeks [3, 49]. Although the impact of the FcRn salvage pathway on IgG PK has been established since the early 1990s [53–55], our understanding of the relevant molecular mechanisms and implications is still limited. For examples, until our recent publication showing that Fab domain may also impact the FcRn interaction [31], it had been commonly assumed that IgGs with the same Fc sequences would bind to FcRn equally and be protected by FcRn similarly. We have shown that mAbs with wild-type human Fc sequences interacted with FcRn with considerable differences in both binding at acidic pH and dissociation at neutral pH, thus exhibiting a wide range of $t_{1/2}$ and clearance [31]. Based on these results, we have implemented *in vitro* FcRn binding/dissociation assays, and *in vivo* human FcRn mouse studies, as useful screening and funneling tools for PK assessment of mAbs with wild-type Fc sequences. Fortunately, we have witnessed a rapid rise in FcRn-related researches over the past few years [56–61]. This increasing trend, which is reminiscent to what happened with CYPs decades ago, is an important

step toward full integration of DMPK to IgG drug discovery and development.

Another unique elimination mechanism for LMs is target-mediated drug disposition (TMDD) [62]. Even though TMDD was first described for conventional SM drugs by Dr. Gerhard Levy in 1994 [63], the PK of conventional SM drugs is usually independent of their targets because the fraction of SMs involved in the target binding is usually negligible. In contrast, TMDD is common for LM drugs, especially mAbs, due to their relatively low nonspecific systemic clearance and extremely high target-binding affinity. The target–drug interaction and subsequent degradation thus contribute significantly not only to the PD, but also to the PK of LM drugs [62]. A resulting key feature of TMDD is nonlinearity in PK with higher clearance observed at lower doses. In addition, at a given dose, PK of LMs with TMDD can also be altered with changes in PD reflective of target expression-level alterations. As is the case for their absorption, distribution, and metabolism, the underlying mechanisms and factors influencing elimination of LMs have not been extensively investigated, especially as compared to SMs.

### 1.3.3 Leveraging SM Experience: Case Examples

Given all of the considerations above, there is a need to advance LM ADME sciences and develop enabling tools/technologies for ADME studies of LMs, similar to the two fundamental elements vital to the successful SM discovery and development. Equally importantly, realization of these two elements requires more active and timely participation of DMPK scientists over the entire continuum of LM drug discovery and development. In this section, we present examples to illustrate how the same principles and knowledge gained from SMs can be applied to LMs, exemplifying the impact of early and better understanding in ADME processes in the discovery and early preclinical development spaces.

***1.3.3.1 Example 1: LM-SM DDIs—Leveraging Knowledge on CYPs*** Recently, CYP-mediated DDI observed when LMs were coadministered with SMs has been a subject of increasing interest for LM drugs across industry and regulatory scientists [64–66]. These DDIs typically involves LMs that target cytokines and/or treat inflammatory diseases, both of which can impact CYPs [67]. Along the same line with SM–SM DDIs, but with an added consideration of altered levels of endogenous cytokines in disease settings, the CYP knowledge and tools could potentially be applied to explore the utility of *in vitro* CYP studies to quantitatively predict the LM–SM DDI risk. Indeed, as a first step toward the prediction, a model has been recently developed using *in vitro* CYP suppression data with interleukin-6 (IL-6) from hepatocytes to simulate the disease–drug interactions reported in clinical studies with sensitive CYP3A SM substrates [68]. The results were encouraging and clearly highlighted the complexity associated with underlying pathological factors. This is not surprising given the nature of interactions that are primarily disease state dependent and/or drug target dependent, which are different from and more complicated than typical DDIs observed between SMs. Nevertheless, by leveraging the existing knowledge on CYPs, and particularly their regulation factors, it has been possible to provide insights into the underlying mechanisms for the observed DDIs [64, 65, 67]. More importantly, the wealth of information on CYPs has enabled a consensus framework to be developed in a relatively short time among industry and regulatory agencies that entails a general approach for LM–SM DDI assessment during drug development [67]. There remains, however, a need for additional research in disease biology and physiologically relevant *in vitro* systems to facilitate *in vitro–in vivo* extrapolations of the impact of LMs or diseases on CYPs, and eventually successful prediction of LM–SM DDIs.

***1.3.3.2 Example 2: LC–MS to Characterize In Vivo Transformation of mAb—Key Enabler in Candidate Selection*** In this example, we show that LC–MS can provide invaluable information to aid in the understanding of LM disposition important to candidate selection, similar to its role in SM drug discovery support. Therapeutic proteins are subjected to transformation mechanisms such as deamidation, oxidation, and isomerization. These processes usually result in relatively small structural changes in the parent drugs. Such small structural changes may be difficult for a conventional immunoassay to differentiate, but they can still affect biological activity, PK, and immunogenicity of a therapeutic protein [69]. LC–MS is commonly used to detect Asp isomerization in proteins during stability testing at relatively high protein concentrations (mg/mL levels), but not in plasma from *in vivo* studies, due in part to the difficulties in sample analysis resulting from the complex matrix and requirement for high sensitivity.

The first demonstration of *in vivo* Asp isomerization with significant impact on the function of a model mAb (mAb X) has been recently shown [33]. In this case, liquid chromatography with high resolution mass spectrometry (LC-HRMS) provided qualitative and quantitative information on the structurally modified products of therapeutic proteins in biological matrices. It was found that this mAb completely lost its target-binding ability due to isomerization of a single Asp in the complementary determining region (CDR) (isoAsp-mAb X) following an accelerated stability test at 40 °C over 3 months. This raised a question with respect to the *in vivo* relevance of this *in vitro* occurrence and the developability of this mAb. For this, an LC–MS assay was needed since the immunoassay used for the PK evaluation of mAb X was incapable of distinguishing the parent compound from its inactive isomer. Coupled with immunocapture, using biotinylated mouse antihuman IgG (Fc) antibody to enrich analytes and following trypsin digestion of mAb X, a unique 43-amino

acid peptide that contains the Asp of interest (Pep A) and isoPep A (surrogates for the parent and isoAsp-mAb X, respectively) was separated and detected by LC–HRMS. The isoAsp-mAb X/parent ratio was found in mouse serum with an increase in the absolute levels of isoAsp-mAb X of approximately 45% from Day 2 to Day 28. The result from this work provided direct evidence of Asp isomerization *in vivo* and thus disqualified mAb X from further development consideration.

***1.3.3.3 Example 3: Mechanism-Based Human PK Prediction*** For SM drugs, knowledge gained over recent decades has established a foundation for a "bottom-up" physiologically-based PK (PBPK) modeling approach to integrate drug-specific parameters obtained *in vitro* using human tissues or, for the majority of SMs, the major PK determinant CYP systems to predict and provide mechanistic insights into the PK properties in humans under various intrinsic and extrinsic factors [19, 70]. For LM drugs, owing to limited understanding of the ADME determinants and especially with regard to scalability from *in vitro* systems to *in vivo* and/or from preclinical species to humans, a fully bottom-up PBPK-based model has either yet to be completely validated or widely applied for prospective human PK prediction. Nevertheless, there has been some recent progress of PBPK models with mAbs, with potential translational utility to human situations [71–74]. For example, Abuqayyas and Balthasar have recently developed a PBPK model with TMDD components in the tumor compartment to predict the disposition of mAbs *a priori* in plasma and in tissues, including tumors that express target antigens in mice [74]. In addition to FcRn–IgG interaction, the model structure included the following determinants: antibody–target-binding affinity, target expression levels, rates of internalization of mAb–target complexes, plasma and lymphatic flow rates, and the tumor vascular volume. Two mAbs, one with high tumor target antigen-binding affinity and one nonbinding control, were examined. The exposure of both mAbs in plasma, tumor, and other tissues was predicted reasonably well in the xenograft-bearing SCID mice [74]. It is anticipated that further development of LM PBPK models, coupled with better understanding in target–LM interaction biology and improved experimental methods to characterize target expression and dynamics, will eventually allow *a priori* prediction of LM plasma and tissue disposition in humans. A dedicated chapter (by Yanguang Cao and William Jusko) in this book focusing on PBPK for therapeutic mAbs provides more details about how to apply this technique during drug development.

Currently, human PK for LMs not subject to nonlinear or species-specific clearance mechanisms is predicted reasonable well from preclinical PK with the principle of allometry [34, 75–77]. This is because certain general elimination processes of LMs are governed primarily by physiological parameters, which can be scaled between species in a compound-independent manner. However, for LMs subject to nonlinear clearance, a more mechanism-based modeling approach incorporating the impact of targets on PK is needed for human PK prediction. A nice example was reported by Luu et al. recently on how mechanistic modeling can be used to predict human PK of a mAb exhibiting TMDD [78]. PF-03446962 is a human mAb against ALK1 (activin receptor-like kinase 1) that exhibited nonlinear PK, a hallmark of TMDD, in monkeys. A TMDD model as depicted in Figure 1.2 was used to capture the plasma PK profiles of PF-03446962 following single and multiple doses. The mechanism-related parameters, such as $k_{on}$, $k_{off}$, $k_{deg}$, and $k_{int}$ rates were experimentally determined for both monkeys and humans. Together with allometric scaling of monkey PK parameters (e.g., $k_{el}$, $k_{12}$, and $k_{21}$), the model successfully predicted the plasma PK profile of PF-03446962 in humans.

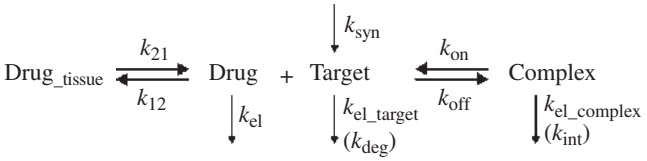


**FIGURE 1.2** Schematic representation of a TMDD model for description of the interaction between a drug and its target. $k_{12}$ = transfer rate constant of drug from central to peripheral (tissue) compartment; $k_{21}$ = transfer rate constant of drug from peripheral (tissue) to central compartment; $k_{el}$ = elimination rate constant of drug; $k_{syn}$ = synthesis rate of target; $k_{el\_target}$ ($k_{deg}$) = elimination rate constant of target; $k_{on}$ = association rate constant; $k_{off}$ = dissociation rate constant; $k_{el\_complex}$ ($k_{int}$) = elimination rate constant of drug/target complex.

***1.3.3.4 Example 4: PK/PD Modeling for LMs*** A mechanistic PK/PD modeling approach has been increasingly used to help define and better understand systemic exposure–effect (efficacy or safety) relationship, a key element to successful SM drug development. This approach can similarly be applied to the development of LM drugs. In fact, of all DMPK aspects of LMs, this is the area that has been relatively well developed and received great attention from DMPK scientists supporting LM drug development. As described earlier, unlike most of SM drugs, the PK and PD of LM drugs are often interrelated. For these molecules, TMDD model has not only been an important tool to characterize the PK of LMs, it has also been incorporated widely into the PK/PD modeling of LMs to characterize PD effect and dose–response relationship for LMs, delineating the impact of target engagement (TE) on downstream pharmacological effects.

One such example was presented by Ng et al., for TRX1, an anti-CD4 mAb [79]. TRX1 exhibited typical target-mediated nonlinear PK characteristics in humans. Binding of TRX1 to CD4 receptors on circulating T cells leads to down-modulating the CD4 receptors in a dose- and concentration-dependent manner, which in turn changes

the extent of target impact on TRX1 PK. Therefore, a receptor-binding-based PK/PD model as depicted in Figure 1.2 was also used to describe the PK and PD (CD4 target binding) of TRX1. Serum TRX1 concentration and total and free CD4 levels were measured and fitted into the model simultaneously to account for the effect of PD on PK. This mechanism-based PK/PD model was later used to simulate PK/PD-time profiles after different dosing regimens to help guide the dose selection in future clinical studies.

For LM drugs against soluble targets, the impact of target binding on drug PK may not be as apparent, depending on whether the elimination rate for drug–target complex is similar to that of the free drug [80, 81]. Nevertheless, understanding the interplay between drug and target is essential in determining the dosing regimen for LMs. Following LM treatment, there is often a rapid accumulation of drug/target complex due to dramatic differences in the elimination rates of free target and LM drug/target complex [81]. Dissociation of the accumulated LM drug/target complex will result in the return of free target to baseline while free drug levels are still orders of magnitude higher than the free target levels. As shown by Wang et al., following treatment of siltuximab, an anti-IL-6 mAb, in cynomolgus monkeys, total IL-6 levels reached 10,000–100,000-fold above the IL-6 baseline, and free IL-6 returns to baseline when siltuximab levels were $>10^6$-fold higher than the IL-6 baseline (also 100–1000-fold higher than the highest total IL-6 levels [81]). A quantitative PK/TE model that takes into account the production rate of IL-6, elimination rates of IL-6 and siltuximab/IL-6 complex, equilibrium dissociation constant between siltuximab and IL-6, as well as the PK characteristics of siltuximab was established via simultaneous fitting of total siltuximab, total IL-6, and free IL-6 concentration profiles [81]. The model provided estimation of all model parameters and was used successfully to predict the free IL-6 profiles at higher siltuximab doses, where the accurate determination of free IL-6 concentration became technically too difficult. This kind of integrated PK/TE/PD modeling approach provided a framework for prediction of efficacious dose levels and duration of action for mAbs against soluble ligands with rapid turnover.

## 1.4 CONCLUSIONS

Over the past few decades, a better understanding of ADME processes, brought about by participation of DMPK scientists in early discovery through late development, has been crucial to enhancing the possibility of success of SM drugs. We attribute the success of DMPK involvement to the combination of substantial progresses in the drug metabolism sciences, particularly in the area of CYPs, the major enzymes responsible for clearance mechanisms of a large number of SMs, with the availability of powerful tools, notably the LC–MS technology. Compared to SMs, the role of DMPK in supporting LM drug discovery and development is far behind and should be increased and expanded to cover the entire process. This point of view is underpinned by a number of factors, including the evolving and competitive biotechnology landscape, and imminent/growing regulatory pressure. A few case examples are presented to illustrate the relevance and transferability of strategies and experiences of DMPK support for SM drugs to LM drugs. A similar path used for SM drug discovery and development, especially with respect to establishing mechanistic understanding in ADME properties and associated determinants, as well as developing necessary tools and technology, can be followed in the endeavors to increase the possibility of success of a safe and effective LM candidate.

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