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Mini-Review

Theme: ADME of Therapeutic Proteins
Guest Editors: Craig Svensson, Joseph Balthasar, and Frank-Peter Theil

ADME of Biologics—What Have We Learned from Small Molecules?

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Abstract. Thorough characterization and in-depth understanding of absorption, distribution, metabolism, and elimination (ADME) properties of a drug candidate have been well recognized as an important element in small molecule (SM) drug discovery and development. This has been the area of focus for drug metabolism and pharmacokinetics (DMPK) scientists, whose role has been evolving over the past few decades from primarily being involved in the development space after a preclinical candidate was selected to extending their involvement into the discovery stage prior to candidate selection. This paradigm shift has ensured the entry into development of the best candidates with optimal ADME properties, and thus has greatly impacted SM drug development through significant reduction of the failure rate for pharmacokinetics related reasons. In contrast, the sciences of ADME and DMPK have not been fully integrated into the discovery and development processes for large molecule (LM) drugs. In this mini-review, we reflect on the journey of DMPK support of SM drug discovery and development and highlight the key enablers that have allowed DMPK scientists to make such impacts, with the aim to provide a perspective on relevant lessons learned from SM drugs that are applicable to DMPK support strategies for LMs.

KEY WORDS: ADME; biologics; drug discovery and development; large molecules; small molecules.

INTRODUCTION

Over the past decade, there has been increased investment to the development of biotechnologically derived drug products or biologics in pharmaceutical companies. While these LM drugs, including but not limited to peptides, proteins and monoclonal antibodies (mAbs), currently comprise a small portion of the pipelines of the 50 largest pharmaceutical firms, the number of LMs under development has increased dramatically in recent years (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. Unlike the discovery and development of SM drugs, where the sciences and the functional role of DMPK in studying and understanding ADME processes have been well recognized as an indispensable and integral discipline spanning from early discovery to development and post-marketing spaces (3), the function of DMPK in support of LM drug development, especially for the more commonly known therapeutic proteins, including mAbs, is somewhat limited to mostly *in vivo* pharmacokinetics (PK) and/or pharmacokinetics-pharmacodymanics studies, typically after candidate selection and primarily in the clinical space. For this reason, these molecules will be the primary focus of this review. Reports on mechanistic investigation of ADME processes for LMs are sparse and our current understanding of the associated mechanisms and key determinants of PK properties is scant (4). 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 (5), many decades after numerous SM drugs were on the market. It is also noteworthy that the regulatory oversight of LMs has recently been put under the Center for Drug Evaluation and Research (CDER), the same governance as for SMs, following the FDA's decision in 2003. Therefore, 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 mini-review, we present a brief historical perspective on how the roles of DMPK have evolved over time, and highlight the key enablers for studying the ADME processes of SM drugs and their underlying mechanisms in order to influence internal de-risking strategy and decisions. External factors, such as changing regulatory environments and

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evolving LM discovery and development landscape, are briefly reviewed. We also present an overview of a DMPK concept analogy between SMs and LMs. Mirroring from SM experiences, we put forward a glimpse of similarly integrative DMPK roles and key enablers for earlier and more in-depth characterization of the ADME properties of LM drug candidates to help enhance the probability of success in their development.

SM DRUG DISCOVERY AND DEVELOPMENT: HISTORICAL PERSPECTIVE

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 complex clinical trials (1,6–8). It is estimated that the success rate for SMs is about 5–10 %, typically requiring more than ten clinical candidates entering Phase I trials to yield only one successful drug to market. The overall attrition rate would be much higher if one takes into account the total number of SMs synthesized during discovery phase, which is generally in thousands before narrowing down to one preclinical candidate with all desired properties, including *in vitro* potency/selectivity, *in vitro*/*vivo* DMPK, efficacy and safety, suitable for the first-in-human trial (9). Prior to 1990, poor human pharmacokinetics and bioavailability were the most significant cause of attrition, accounting for ~40 % of all attrition in development. This number was dramatically reduced to ~8 % by 2000 (9). Interestingly, it was not a coincidence that starting in the late 1980s, DMPK became involved in SM drug discovery as opposed to primarily after candidate selection, and that this *Paradigm shift* in the roles of DMPK contributed largely to this drastic difference (3). Previously, compounds were selected based primarily on *in vitro* potency and *in vivo* efficacy in animal studies, with little attention being paid to the exposure (PK) as an important measure connecting 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 (10–13). 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.

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): (1) rapid advancement of Cytochrome P450 (CYP) science and (2) availability of liquid chromatography–mass spectrometry (LC-MS).

The CYP enzymes play central roles in the metabolism of SMs; it is estimated that over 70 % of marketed SM drugs were eliminated primarily by CYPs (13). CYP enzymes were discovered in 1958 and research on their structure and function, as well as their role in drug metabolism, were rapidly expanded in the 1980s–1990s (13–16). 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 (3,14–16). Specifically, the comprehensive understanding of the similarities and differences in CYP expressions and functions across animal species and humans (11,12) has aided in successful prediction of human PK properties and selection of a development candidate with desired human PK. The knowledge of CYP substrate specificity, multiplicity and responses to factors, such as inducers and inhibitors, has provided a means to select candidates that do not bear considerable liability to serious DDI, either as perpetrators or victims (16–19). It is noteworthy that CYP-mediated DDI has been one of the major causes for drug withdrawal from the market. Work that identified CYP2D6, 2C19, and 2C9 polymorphisms as primary causes for the most frequent variations in phase I metabolism of drugs, and along with the availability of a number of methodologies and reagents for CYP-mediated reaction phenotyping (20,21), has prevented candidates that were primarily metabolized by one of those polymorphic CYP isoforms from entering into development. 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 marriage of high-performance liquid chromatography (HPLC) with mass spectrometry (MS) has provided unprecedented sensitivity, selectivity, and high throughput that have facilitated rapid assessment of ADME properties and the multiplicity of their governing factors for SM candidates in animals and humans (22–25). Prior to the introduction of this powerful technology, drugs and their metabolites in biological fluids were mainly quantified using HPLC with ultraviolet–visible or fluorescence detection, which requires good separation of the analytes from one another and from endogenous components with similar spectral absorption properties. As a result, the capacity of the analysis was limited by the lengthy separation and sample clean-up. Another type of complimentary bioanalyses was immunological assays, such as radioimmunoassay. They offered a higher throughput and better assay sensitivity but suffered from low selectivity. Furthermore, the task of metabolite characterization was painstaking, as it usually was very time-consuming and involved many steps, including radio-labeling of the parent compounds, isolation of a considerable quantity of metabolites of interest and structure elucidation by stand-alone MS and nuclear magnetic resonance analyses.

Capitalizing on chromatographic separation and mass selectivity, the LC-MS technology enables the quantitation of co-eluting 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 (22–24). 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 re-shaped 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 (3,10,25). With the recent advent of new chromatographic techniques, such as ultra performance liquid chromatography, and more sophisticated MS, such as high resolution mass spectrometry (26), this technology will continue to be the most powerful tool for drug discovery and development for SMs, and potentially for LMs alike.

Case Examples

In the current state, the scope of DMPK spans the continuum of SM drug discovery and development, and is even expanded into the post-marketing phase. In the discovery stage, the primary DMPK role is to help minimize development risks by identifying DMPK-related issues during lead optimization and integrating DMPK information in the selection of high quality drug candidates for entry into development. In the development space, DMPK scientists typically help plan development programs and manage risks following thorough ADME characterization of the clinical candidates. Two examples presented below illustrate the impact of DMPK efforts and the two key enablers in the discovery through early development in de-risking and enhancing the possibility of success of drug development.

Example 1: DMPK Optimization Led to Successful Candidate Selection

MK-4305 (Fig. 1) is a potent dual orexin receptor antagonist currently in phase III clinical trials for the treatment of primary insomnia. Identification of this compound exemplifies how effective DMPK support has facilitated addressing two critical issues, namely poor oral availability and bioactivation (27). These two issues presented a big hurdle to the optimization of a leading diazepane class, derived from high-throughput screening.

To address the first issue, mechanistic DMPK studies were conducted, including *in vitro* metabolism in hepatic preparations and *in vivo* disposition in animal studies. The *in vivo* results indicated that the poor oral availability was primarily attributed to extensive intestinal and hepatic first-pass metabolism, largely through CYP-mediated oxidative reactions. Subsequent *in vitro* metabolism studies confirmed similar metabolic pathways in animals and in humans. In parallel, the effort to address the issue of bioactivation revealed that reducing the metabolic liability plus blocking a key site of bioactivation was an effective approach to minimize the formation of reactive species. As a result, screening metabolic stability across species using liver microsomal preparations was set up as a key element of the research operational plan to guide compound selection and structure modification. The structure activity relationship built up from the data obtained from these *in vitro* studies allowed chemists to make informed decisions on structure design and thus allowed identification of potent compounds with significantly improved metabolic stability and reduced bioactivation potential, especially in humans. Of note, LC-MS played a pivotal role in dealing with both issues, enabling rapid and specific analysis of large number of samples with quick data turnaround.

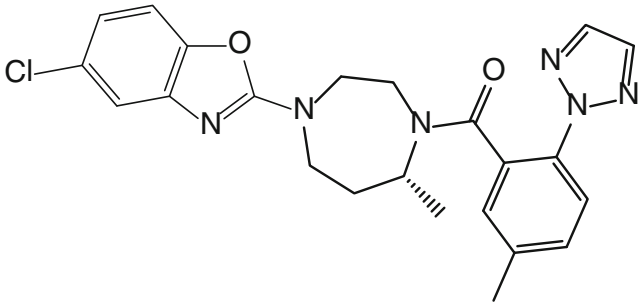

Fig. 1. Structure of MK-4305

Efforts dealing with these two issues were rewarded with identification of a lead structure with a 7-methyl substitution on the diazepane core, and ultimately selection of MK-4305 (27). Coupled with the good potency, the improved PK properties of this compound have warranted the low efficacious dose range in patients.

Example 2: DMPK Aided in Risk Management Decision in Early Development Space

Compound A is a dual alpha/gamma peroxisome proliferator-activated receptor (PPAR α/γ) agonist with comparable potency for both subtype receptors (EC_{50} of 70 nM and 50 nM for the α and γ, respectively). According to the prevailing hypothesis at the time, dual activity was required for the efficacy. However, characterization of the metabolism of Compound A revealed a para-hydroxylated metabolite in human liver microsomes which was selective for the PPAR γ receptor (EC_{50} of 30 nM for γ *versus* 800 nM for α). In addition, formation of this metabolite was catalyzed by two polymorphic enzymes, CYP2C8 and 2C19. Furthermore, significant circulating levels of this metabolite were found in preclinical species with varying AUC ratios of the metabolite to Compound A (0.2–0.4 in rodents, 0.5 in monkeys and 11 in dogs). These findings raised a concern over the suitability of Compound A to test the working hypothesis in humans given the likelihood of significant but also variable formation of the metabolite. To mitigate the risk, the exposure levels of the

metabolite relative to Compound A in plasma samples from the first-in-human study was proposed as the early Go/No Go criteria. With the readiness of LC-MS bioanalysis, the exposure of the metabolite in those human plasma samples was quickly determined (28). It was found that the exposure ratio of the metabolite to Compound A was about 3 from a single dose human study. This finding facilitated a quick decision to discontinue the development of Compound A, avoiding further costly development.

Regulatory Considerations

Successful development of a drug candidate requires the right set of high quality data to help inform decisions not only internally, as exemplified by the above two case examples, 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 from the market over the past decades of several prominent prescription drugs for safety reasons. This is not unexpected considering the regulatory history. Following major safety incidents, including the well-publicized thalidomide case, the Federal Food, Drug and Cosmetic Act was introduced in 1938, and the Drug Amendments Act of 1962 was passed by Congress requiring the FDA to approve all new drug applications based on proven efficacy and safety. Of special note are the withdraw of the drugs from the US market between 1999 and 2003, half of which due to serious and unmanageable DDIs over the period 1995 to 2002. 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 (29). 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. These two guidance documents were later revised and combined into one comprehensive document in 2006, which includes both *in vitro* and *in vivo* CYP and drug transporter studies. The revision was said to be based on additional scientific understanding in the role of drug-metabolizing enzymes and transporters, particularly P-glycoprotein (P-gp), in drug interactions. Although it is apparent that the advent of technology/understanding of sciences led to development of regulations and Guidance, given the current status and understanding of drug transporter sciences relative to the CYPs, the inclusion of P-gp in the latest guidance suggested that the FDA has become even more proactive in embracing evolving sciences in their decision making. This is even more evident in the latest draft DDI guidance recently issued (30,31), where recommendations to conduct many additional drug transporters and drug interaction studies for LMs have been included for the first time. It is widely recognized that unlike the CYP-mediated drug interactions that can be readily defined by inhibition or induction of CYP enzymes, the evidence for the so-called transporter-mediated drug interactions is often less conclusive (32). 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 2003 FDA's decision to transfer the regulatory responsibility from the Center for Biologics Evaluation and Research to CDER, who has been overseeing the regulatory approval of SM drugs and is used to 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.

LM DRUG DISCOVERY AND DEVELOPMENT: LINKING TO SM DRUG EXPERIENCE

High-Level DMPK Support: Current State

From a DMPK perspective, the current state for a LM support paradigm is similar to where we were with SM drugs a few decades ago. This notion is supported by several lines of evidence. 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 (4: informal survey at June 2011 International Consortium for Innovation and Quality in Pharmaceutical Development meeting with members from over 20 pharmaceutical firms). This conventional mindset, especially widespread in many pharmaceutical firms, resembles what was practiced for SMs prior to the 1990s, and may stem from a wide belief that PK of Biologics 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 mAbs is well behaved due primarily to their on-target specificity has recently been challenged. For example, a specific off-target interaction of an anti FGFR4 monoclonal antibody candidate has been identified as the cause for its rapid clearance, poor target tissue biodistribution and limited efficacy (33). The authors concluded that screens typically developed to identify general non-specific interactions are likely to miss the rare and highly specific cross-reactivity observed in this study. This case highlights the importance of more mechanistic studies in avoiding unexpected clinical outcomes. Similarly, we found several of our early mAb candidates that displayed much shorter half-life ($t_{1/2}$) than anticipated (34). This less than desirable DMPK property, likely linked to off-target interactions, was recognized only after DMPK involvement following candidate selection. Some of the candidates were eventually terminated due to the poor PK behavior and safety concerns. It is also notable that the relatively low attrition rate of LMs that is often referred to is based on compounds that entered clinical development over two decades ago. This good record may not be replicated going forward, considering the recent examples above, and an evolving LM landscape enriched with a variety of new and untested engineering technology platforms (see below).

Secondly, 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. Prediction of human PK is typically done using an empirical allometric scaling approach, without mechanistic understanding or molecular consideration for possible species differences (35), similar to the much earlier practice with SMs. In the case of mAbs, it has been widely accepted that non-human primate (NHP) is a representative animal model for human PK, and human PK prediction is heavily dependent on this single species (35). Not surprisingly, 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 3–6 of primate amyloid beta, exhibited faster clearance, with a much shorter $t_{1/2}$ of <2.5 days, compared to ~13 days for a control antibody (no affinity to the target) in monkey (36). 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. Clearly, without appropriate DMPK input and mechanistic insights, this compound would have been precluded from further development. A few other examples along this line have also been highlighted (Dr. Frank-Peter Theil, personal communication), where NHP PK failed to inform human PK correctly, due to either under- or over-prediction. 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. As a case in point, the neonatal Fc receptor (FcRn), a key determinant of IgG homeostasis, was proposed in 1964, but not until 1996 was it shown to be a key determinant of IgG pharmacokinetics (37–40). In agreement, FcRn related research publications have also been relatively sparse since its discovery in 1958, as compared to those on CYPs over a similar 30-year period.

Finally, a major barrier 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 currently available and commonly used bioanaytical methods for determination of LMs in biological fluids are ligand-binding assays that are immunological in nature. These assays usually have an associated degree of non-specificity. For example, multiple forms of mAb and ligand can exist *in vivo*, including free mAb, free ligand, and mono- and/or bi-valent 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* (41). 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 (42), will certainly complicate data interpretations and hamper in-depth understanding of underlying mechanisms (43). Although LC-MS is emerging as a tool for qualitative and quantitative applications of LMs (44–46), this technology is still in its infancy for the applications to the quantification of LM drugs. The routine use of LC-MS is mainly 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 (46).

Evolving Biologics Landscape: Trend

The clinical success of mAbs and other therapeutic proteins continues to reinforce protein engineering research with a focus on discovering the next generation of therapeutics that are beyond conventional forms (47–50). Recent advances in selection processes using display platforms and in maturation development have been reported to facilitate this evolution. Coming with these new technological progresses are developments of more unnatural LMs with concomitantly greater unknown properties, necessitating more extensive research. Additionally, many LMs are being developed for several new target indications, including neurological diseases. These new endeavors yield increasing complexity beyond life-threatening diseases that have traditionally been the area of focus for Biologics in the past. Noticeably, the reportedly higher success rate of clinical development of biotech drugs as compared to SMs was based on these traditional biotech drugs, and may not hold true for future LM generations. Moreover, the expansion to non life-threatening diseases will likely stipulate a higher bar for regulatory approval, which in turn would necessitate more studies to ensure safety and efficacy of a drug product, especially under the current regulatory environment. Consistent with this, the CDER Science Prioritization and Review Committee has recently highlighted several relevant LM DMPK aspects warranting additional research and further understanding (51). These include key ADME 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). Finally, the heightened competition for the global market share and reimbursement challenges will also raise the bar to drive best-in-class with respect to safety, efficacy and compliance (52). These factors, although not all unique to LM development, collectively provide a strong case for more investigations to gain insight into ADME of Biologics.

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 (Ce), which is related to systemic concentrations (Cp)), following certain relationships defined by molecular mechanisms of action of a drug, irrespective of its modality (53 and Fig. 2). 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 (Fig. 2). For SMs, the

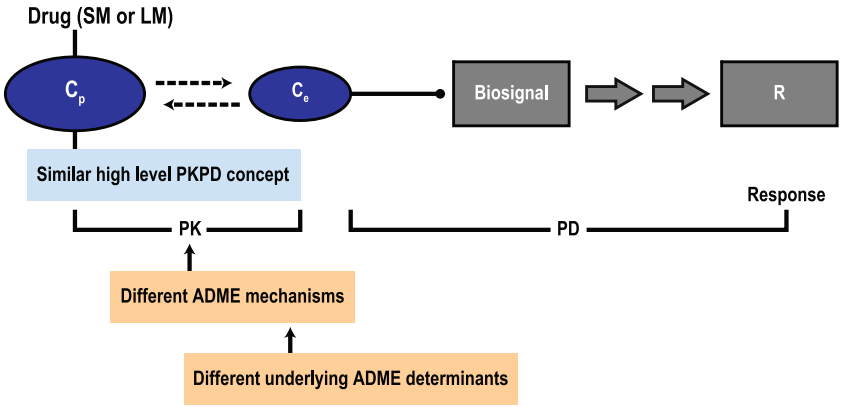

Fig. 2. An illustration of pharmacokinetics and pharmacodynamics and DMPK concept analogy

ADME processes are relatively well studied and are mainly governed by (1) specific characteristics of a compound, including its physico-chemical properties and ability to interact with transporters, drug-metabolizing enzymes and binding proteins, and (2) physiological factors which govern the exposure of the compound to those proteins, such as distribution, tissue localization, and organ blood flow (54). Not only have the nature of these interactions and their governing factors been largely characterized, appropriate tools required for the studies have also been largely available. As illustrated in Fig. 3, following a typical oral administration, a 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, prior to reaching systemic circulation for distribution to tissues and other organs of elimination, including kidney. The majority of SMs enter tissues by passive diffusion, and the key determinant of tissue distribution includes non-selective binding to tissue proteins. Many SM drugs have also been reported to enter tissues via active transport, and the transporters involved have been identified. As described earlier, SMs are eliminated from the body predominantly via metabolism with CYPs as the major metabolizing enzymes. Biliary and renal excretion is the two major pathways for the elimination of a variety of compounds that escape metabolism. Systemic bioavailability (F), a PK parameter central to efficacy and safety of a drug candidate, is a product of these processes (Fig. 3).

Unlike SMs, LMs are administered intravenously, intramuscularly or subcutaneously. Oral administration is precluded by molecular size, hydrophilicity, and gastric degradation of LMs. In general, the ADME processes for LMs are much less characterized, as compared to SMs. Nevertheless, their ADME processes are similar in concept. As illustrated in Fig. 3, following a subcutaneous (SC) administration, a 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, and more recently in rats and dogs (55). There still remain many unknowns, including impact of physiological factors on SC absorption, which can be compound-dependent, that will require further studies to resolve (4,55). Similar to SMs, once entering the blood circulation, they 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

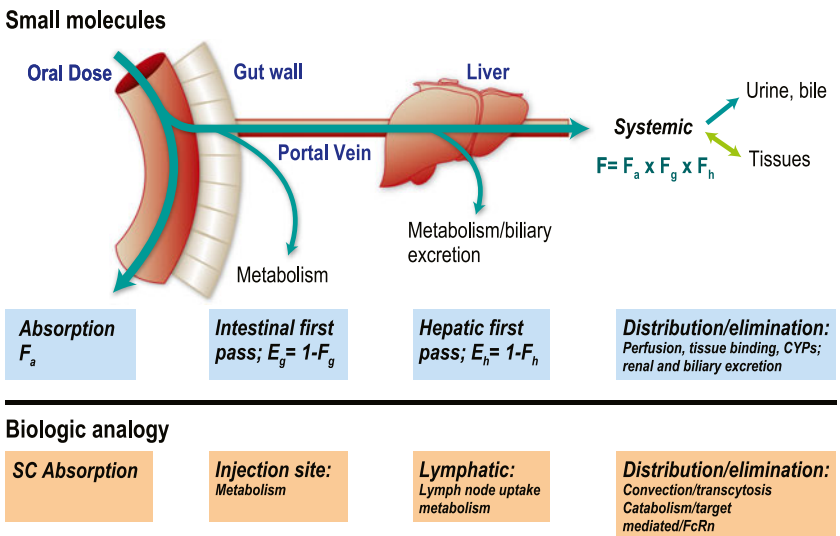

Fig. 3. ADME and pharmacokinetic concept analogy between SMs and LMs

the extracellular space (56,57). This fundamental difference between the two modalities is in line with the location of their respective biological targets. Namely, the targets are mostly on the cell surface for LM drugs, which are in contrast to the intracellular location for most SM drugs. This difference also explains why tissue distribution studies have been more frequently conducted (*versus* other ADME related types of studies) for LMs (58). However, in-depth studies on these processes are scarce, and currently it 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 (4). In comparison, LMs are also subjected to elimination, involving metabolism and excretion via kidney and bile. Contrary to SMs, LMs are typically not subjected to metabolism by CYP enzymes, but generally metabolized to peptides and amino acids in several tissues, by circulating phagocytic cells. A unique elimination mechanism for LMs is associated with the binding to their targets on the cell surface. Subsequent to the internalization, the LMs inside the cells may be degraded, a process typified by target-mediated disposition. On the other hand, antibodies and endogenous immunoglobulins are protected from degradation by binding to FcRn, resulting in relatively long elimination half-lives (4,56). 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.

Key Focus Examples: Following SMs Path

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 important, 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 two examples analogous to the aforementioned SM examples to illustrate how the same principles can be applied to LMs, exemplifying the impact of early and better understanding in ADME processes in the discovery and early preclinical development spaces.

Example 1: FcRn Tool Aided in mAb Lead Optimization and Product Qualification

Although the impact of the FcRn salvage pathway on IgG PK has been established since the early 1990s, our understanding of the relevant molecular mechanisms and implications is still limited. For example, since FcRn is an Fc receptor, it had been commonly assumed that IgGs with the same Fc sequences would bind to FcRn equally and be protected by FcRn similarly. Until our recent publication, this assumption has not been systematically challenged, even though marketed therapeutic mAbs with wild-type human Fc sequence can exhibit a wide range of $t_{1/2}$ and clearance values. Wang *et al.* (34) 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, indicating that the Fab domain may also impact the FcRn interaction. Importantly, these *in vitro* FcRn binding differences, especially at neutral pH, were correlated to the PK properties of these mAb in humanized FcRn mice, where a target-mediated mechanism was expected to be insignificant (34). A similar *in vitro–in vivo* correlation was also observed for a limited number of mAbs having PK datasets available for NHPs and humans, under conditions where the target-mediated clearance mechanism was minimized. 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. It is noteworthy that *in vitro* FcRn binding properties have been used extensively to aid in Fc engineering of mAbs with varying Fc sequences (Fc mutants) for their optimal $t_{1/2}$ potential in humans, and that for these Fc mutants, there is a good correlation between FcRn binding affinities (primarily at acidic pH) and mAb PK (59,60).

Additionally, we have recently applied this concept and the *in vitro* and *in vivo* FcRn tools to aid in mAb product qualifications under various storage conditions. Specifically, we showed that methionine (Met) oxidation of mAbs resulted in a significant reduction of their serum circulation $t_{1/2}$ in human FcRn mice, and that the magnitude of the change correlated well with the levels of Met oxidation and changes in FcRn-IgG binding affinities (61). We also demonstrated for

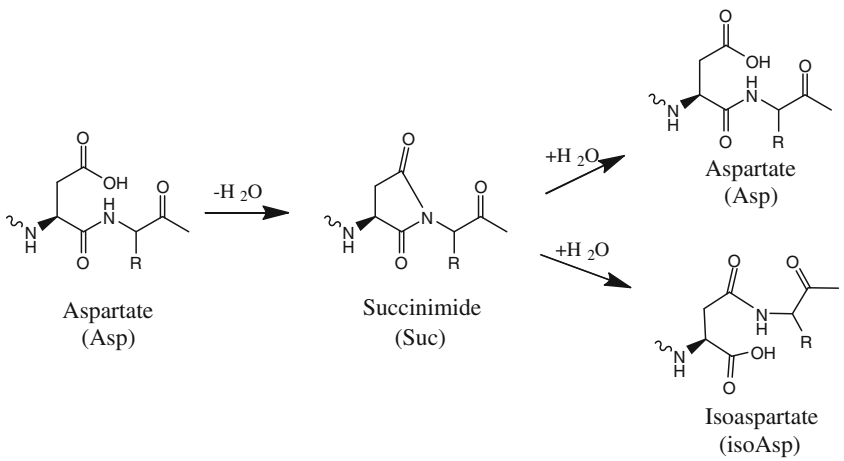

Fig. 4. Isomerization of aspartate in the solution

a mAb of interest that relatively low levels of Met oxidation accumulated during extended refrigerated storage did not have a significant impact on FcRn binding and serum $t_{1/2}$ in the human FcRn mouse model. Based on the correlation established above between the mouse model and human, we anticipated similar results in humans.

Example 2: LC-MS - Key Enabler in Candidate Selection: In Vivo Transformation of mAb

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 (62). LC-MS is commonly used to detect Asp isomerization in proteins during stability testing at relatively high protein concentrations (mg/ml levels). To our knowledge, there have been no reports on the detection of Asp isomerization 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.

Presented here is the first demonstration of *in vivo* Asp isomerization with significant impact on the function of a model mAb (mAb X).[1] 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 (Fig. 4) in the CDR region (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. Unfortunately, an immunoassay used for the pharmacokinetic evaluation of mAb X was incapable of distinguishing the parent compound from its inactive isomer, thereby necessitating a more specific LC-MS assay. To overcome the issue of sensitivity, analytes in plasma samples from mouse PK studies were enriched via immuocapture, using biotinylated mouse anti-human IgG (Fc) antibody coupled to magnetic beads. 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) were separated and detected by LC-HRMS. The isoAsp-mAb X/parent ratio was found in mouse serum to increase from 1:10 at day 2 to 1:2 at day 14 and 1:1 at day 28, representing an increase in the absolute levels of isoAsp-mAb X of ~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.

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. We submit that 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, be followed in the endeavors to increase the possibility of success of a safe and efficacious LM candidate.

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REFERENCES

1. DiMasi JA, Feldman L, Seckler A, Wilson A. Trends in risks associated with new drug development: success rates for investigational drugs. Clin Pharmacol Ther. 2010;87:272–7.
2. Kaitin KI, DiMasi MA. Pharmaceutical innovation in the 21st century: new drug approvals in the first decade, 2000–2009. Clin Pharmacol Ther. 2011;89:183–8.
3. Baillie TA. Metabolism and toxicity of drugs. Two decades of progress in industrial drug metabolism. Chem Res Toxicol. 2008;21:129–37.
4. Lin JH. Pharmacokinetics of biotech drugs: peptides, proteins and monoclonal antibodies. Curr Drug Metab. 2009;10:661–91.
5. Waldmann TA. Immunotherapy: past present and future. Nat Med. 2003;9:269–77.
6. Paul SM, Mytelka DS, Dunwiddie CT, Persinger CC, Munos BH, Lindborg SR, *et al.* How to improve R&D productivity: the pharmaceutical industry's grand challenge. Nat Rev Drug Discov. 2010;9:203–14.
7. Kola I, Landis J. Can the pharmaceutical industry reduce attrition rates? Nat Rev Drug Discov. 2004;3:711–5.
8. Sung NS, Crowley Jr WF, Genel M, Salber P, Sandy L, Sherwood LM, *et al.* Central challenges facing the national clinical research enterprise. JAMA. 2003;289:1278–87.
9. MacCoss M, Baillie TA. Organic chemistry in drug discovery. Science. 2004;303:1810–3.
10. Lin JH, Lu AYH. Role of pharmacokinetics and metabolism in drug discovery and development. Pharmacol Rev. 1997;49:403–49.
11. Lin JH. Species similarities and differences in pharmacokinetics. Drug Metab Dispos. 1995;23:1008–21.
12. Tang C, Prueksaritanont T. Use of *in vivo* animal models to assess pharmacokinetic drug–drug Interactions. Pharm Res. 2010;27:1772–87.
13. Wienkers LC, Heath TG. Predicting *in vivo* drug interactions from *in vitro* drug discovery data. Nat Rev Drug Discov. 2004;4:825–33.

[1] Wang *et al.*, Poster presented at the ADME Protein Therapeutics Introductory Workshop in August, 2011 at the State University of New York at Buffalo

14. Guengerich FP. Cytochrome P450s and other enzymes in drug metabolism and toxicity. AAPS J. 2006;8:E101–11.
15. Estabrook RW. A passion for P450s (remembrances of the early history of research on cytochrome P450). Drug Metab Dispos. 2003;31:1461–73.
16. Lin JH, Lu AYH. Inhibition and induction of cytochrome P450 and the clinical implications. Clin Pharmacokinet. 1998;5:361–90.
17. Honig PK, Wortham DC, Zamani K, Conner DP, Mullin JC, Cantilena LR. Terfenadine–ketoconazole interaction: pharmacokinetic and electrocardiographic consequences. J Am Med Assoc. 1993;269:1513–8.
18. Schmassmann-Suhijar D, Bullingham R, Gasser R, Schmutz J, Haefeli WE. Rhabdomyolysis due to interaction of simvastatin with mibefradil. Lancet. 1998;351:1929–30.
19. Prueksaritanont T, Ma B, Tang C, Meng Y, Assang C, Lu P, *et al*. Metabolic interactions between mibefradil and HMG-CoA reductase inhibitors: an *in vitro* investigation with human liver preparations. Br J Clin Pharmacol. 1999;47:291–8.
20. Ingelman-Sundberg M. Implications of polymorphic cytochrome P450-dependent drug metabolism for drug development. Drug Metab Dispos. 2001;29:570–3.
21. Zhou SF, Liu JP, Chowbay B. Polymorphism of human cytochrome P450 enzymes and its clinical impact. Drug Metab Rev. 2009;41:89–295.
22. Hopfgartner G, Bourgogne E. Quantitative high-throughput analysis of drugs in biological matrices by mass spectrometry. Mass Spectrom Rev. 2003;22:195–214.
23. Korfmacher WA. Principles and applications of LC-MS in new drug discovery. Drug Discov Today. 2005;10:1357–67.
24. Castro-Perez J, Plumb R, Granger JH, Beattie L, Joncour K, Wright A. Increasing throughput and information content for *in vitro* drug metabolism experiments using ultra-performance liquid chromatography coupled to a quadrupole time-of-flight mass spectrometer. Rapid Commun Mass Spectrom. 2005;19:843–8.
25. Korfmacher WA. Use of mass spectrometry for drug metabolism studies. Curr Drug Metab. 2006;7:455–563.
26. Plumb R, Castro-Perez J, Granger J, Beattie I, Joncour K, Wright A. Ultra-performance liquid chromatography coupled to quadrupole-orthogonal time-of-flight mass spectrometry. Rapid Commun Mass Spectrom. 2004;18:2331–7.
27. Cox CD, Breslin MJ, Whitman DB, Schreier JD, McGaughey GB, Bogusky MJ, *et al*. Discovery of the dual orexin receptor antagonist [(7*R*)-4-(5-chloro-1,3-benzoxazol-2-yl)-7-methyl-1,4-diazepan-1-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone (MK-4305) for the treatment of insomnia. J Med Chem. 2010;53:5320–32.
28. Riffel KA, Polinko MA, Song HC, Rippley RK, Lo MW. Quantitative determination of a novel insulin sensitizer and its para-hydroxylated metabolite in human plasma by LC-MS/MS. J Pharm Biomed Anal. 2004;35:523–34.
29. Huang SM, Strong JM, Zhang L, Reynolds KS, Nallani S, Temple R, *et al*. New era in drug interaction evaluation: US Food and Drug Administration update on CYP enzymes, transporters, and the guidance process. J Clin Pharmacol. 2008;48:662–70.
30. Girish S, Martin SW, Peterson MC, Zhang LK, Zhao H, Balthasar J, *et al*. AAPS workshop report: strategies to address therapeutic protein–drug interactions during clinical development. AAPS J. 2011;13:405–16.
31. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Guidance for Industry Drug Interaction Studies — Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations, Draft Guidance, February 2012.
32. Lin JH. Transporter-mediated drug interactions: clinical implications and *in vitro* assessment. Expert Opin Drug Metab Toxicol. 2007;3:81–92.
33. Bumbaca D, Wong A, Drake E, Reyes 2nd AE, Lin BC, Stephan JP, *et al*. Highly specific off-target binding identified and eliminated during the humanization of an antibody against FGF receptor 4. mAbs. 2011;3:376–86.
34. Wang W, Lu P, Fang Y, Hamuro L, Pittman T, Carr B, *et al*. Monoclonal antibodies with identical Fc sequences can bind to FcRn differentially with pharmacokinetic consequences. Drug Metab Dispos. 2011;39:1469–77.
35. Wang W, Prueksaritanont T. Prediction of human clearance of therapeutic proteins: simple allometric scaling method revisited. Biopharm Drug Dispos. 2010;31:253–63.
36. Vugmeyster Y, Szklut P, Wensel D, Ross J, Xu X, Awwad M, *et al*. Complex pharmacokinetics of a humanized antibody against human amyloid Beta Peptide, anti-abeta ab2, in nonclinical species. Pharm Res. 2011;28:1696–706.
37. Burmeister WP, Huber AH, Bjorkman PJ. Crystal structure of the complex of rat neonatal Fc receptor with Fc. Nature. 1994;372:379–83.
38. Junghans RP, Anderson CL. The protection receptor for IgG catabolism is the beta(2)-microglobulin-containing neonatal intestinal transport receptor. Proc Natl Acad Sci U S A. 1996;93:5512–6.
39. Ghetie V, Hubbard JG, Kim JK, Tsen MF, Lee YF, Ward ES. Abnormally short serum half-lives of IgG in beta 2-microglobulin-deficient mice. Eur J Immunol. 1996;26:690–6.
40. Israel EJ, Wilsker DF, Hayes KC, Schoenfeld D, Simister NE. Increased clearance of IgG in mice that lack beta(2)-microglobulin: possible protective role of FcRn. Immunology. 1996;89:573–8.
41. Lee JW, Kelley M, King LE, Yang J, Salimi-Moosavi H, Tang MT, *et al*. Bioanalytical approaches to quantify "total" and "free" therapeutic antibodies and their targets: technical challenges and PK/PD applications over the course of drug development. AAPS J. 2011;13:99–110.
42. European Medicines Agency (EMA), Committee for Medicinal Products for Human Use (CHMP). Draft Guideline on the Clinical Investigation of the Pharmacokinetics of Therapeutic Proteins. July 2005.
43. Tang C, Prueksaritanont T. Theoretical analysis of interplay of therapeutic protein drug and circulating soluble target: Temporal profiles of 'free' and 'total' drug and target. Pharm Res. 2011;28:2447–57.
44. Ezan E, Dubois M, Becher F. Bioanalysis of recombinant proteins and antibodies by mass spectrometry. Analyst. 2009;134:825–34.
45. Hall MP, Gegg C, Walker K, Spahr C, Ortiz R, Patel V, *et al*. Ligand-binding mass spectrometry to study biotransformation of fusion protein drugs and guide immunoassay development: strategic approach and application to peptibodies targeting the thrombopoietin receptor. AAPS J. 2010;12:576–85.
46. Campbell, Yves Le Blanc JC. Peptide and protein drug analysis by MS: challenges and opportunities for the discovery environment. Bioanalysis. 2011;3:645–57.
47. Nelson AL, Dhimolea E, Reichert JM. Development trends for human monoclonal antibody therapeutics. Nat Rev Drug Discov. 2010;9:767–74.
48. Yamaguchi A, Usami K, Masuda K. Current technological development of antibody therapeutics. Immun Endoc Metab Agents Med Chem. 2011;11:21–32.
49. Jing LI, Zhenping ZHU. Research and development of next generation of antibody-based therapeutics. Acta Pharmacol Sin. 2010;31:1198–207.
50. Beck A, Cochet O, Wurch T. GlycoFi's technology to control the glycosylation of recombinant therapeutic proteins. Expert Opin Drug Discov. 2010;5:95–111.
51. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), The CDER Science Prioritization and Review Committee (SPaRC). Identifying CDER's Science and Research Needs Report, July 2011.
52. Aggarwal S. What's fueling the biotech engine—2010 to 2011. Nat Biotech. 2011;29:1083–9.
53. Mager DE, Wyska E, Jusko WJ. Diversity of mechanism-based pharmacodynamic models. Drug Metab Dispos. 2003;31:510–8.
54. Prueksaritanont T, Lin JH, Baillie TA. Complicating factors in safety testing of drug metabolites: kinetic differences between generated and preformed metabolites. Toxicol Appl Pharmacol. 2006;217:143–52.
55. Wang W, Chen N, Shen X, Cunningham P, Fauty S, Michel K, *et al.* Lymphatic transport and catabolism of therapeutic proteins following subcutaneous administration to rats and dogs. Drug Metab Dispos. Published ahead of print February 10, 2012. doi:10.1124/dmd.111.043604.
56. Lobo ED, Hansen RJ, Balthasar JP. Antibody pharmacokinetics and pharmacodynamics. J Pharm Sci. 2004;93:2645–68.

57. Wang W, Wang EQ, Balthasar JP. Monoclonal antibody pharmacokinetics and pharmacodynamics. Clin Pharmacol Ther. 2008;84:548–58.
58. Vugmeyster Y, Defranco D, Szklut P, Wang Q, Xu X. Biodistribution of [(125)I]-labeled therapeutic proteins: application in protein drug development beyond oncology. J Pharm Sci. 2010;99:1028–45.
59. Dall'Acqua WF, Kiener PA, Wu H. Properties of human IgG1s engineered for enhanced binding to the neonatal Fc receptor (FcRn). J Biol Chem. 2006;281:23514–24.
60. Igawa T, Tsunoda H, Tachibana T, Maeda A, Mimoto F, Moriyama C, *et al*. Reduced elimination of IgG antibodies by engineering the variable region. Protein Eng Des Sel. 2010;23:385–92.
61. Wang W, Vlasak J, Li Y, Pristatsky P, Fang Y, Pittman T, *et al*. Impact of methionine oxidation in human IgG1 Fc on serum half-life of monoclonal antibodies. Mol Immunol. 2011;48:860–6.
62. Filpula D. Antibody engineering and modification technologies. Biomol Eng. 2007;24:201–15.