Special Section on Pharmacokinetic and Drug Metabolism Properties of Novel Therapeutic Modalities—Minireview

Mechanisms Influencing the Pharmacokinetics and Disposition of Monoclonal Antibodies and Peptides

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ABSTRACT

Monoclonal antibodies (mAbs) and peptides are an important class of therapeutic modalities that have brought improved health outcomes in areas with limited therapeutic optionality. Presently, there are more than 90 mAb and peptide therapeutics on the United States market, with over 600 more in various clinical stages of development across a broad array of therapeutic areas, including diabetes, autoimmune disorders, oncology, neuroscience, and cardiovascular and infectious diseases. Notwithstanding this potential, there is high clinical rate of attrition, with approximately 10% reaching patients. A major contributor to the failure of the molecules is often times an incomplete or poor understanding of the pharmacokinetics (PK) and disposition profiles leading to limited or diminished efficacy. Increased and thorough characterization efforts directed at disseminating mechanisms influencing the PK and disposition of mAbs and peptides can aid in improving the design for their intended pharmacological activity, and thereby their clinical success. The PK and disposition factors for mAbs and peptides are broadly influenced by target-mediated drug disposition and nontarget-related clearance mechanisms related to the interplay between the relationship of the structure and physiochemical properties of mAbs and peptides with physiologic processes. This review focuses on nontarget-related factors influencing the disposition and PK of mAbs and peptides. Contemporary considerations around the increasing in silico approaches to identify nontarget-related molecule limitations and enhancing the druggability of mAbs and peptides, including parenteral and nonparenteral delivery strategies that are geared toward improving patient experience and compliance, are also discussed.

Introduction

Over the last several decades, there has been a staggering increased investment by the medicinal community in the discovery and development of protein-based drugs or biologics. The most common structural forms of biologics are peptides and monoclonal antibodies (mAbs). There are currently more than 90 mAb- and peptide-based therapeutics on the United States’ market, with over 600 more in various stages of clinical development (Recio et al., 2016; Grilo and Mantalaris, 2019). Combined, these two biologic modalities also encompass greater than 30% of the number of molecules investigated in clinical trials (Mohs and Greig, 2017; Grilo et al., 2016; Grilo and Mantalaris, 2019). The rise in the number of clinical investigations and approvals of mAb- and peptide-based biologics warrants the concomitant growth around the science of dissecting factors influencing the pharmacokinetics (PK) and disposition of these molecules. This review focuses on the current knowledge around the molecule-centric physiochemical properties and physiologic mechanisms related to the PK, metabolism, and disposition of mAbs and peptide molecules. With regard to peptides, the content herein focuses on peptide molecules that are non-mAb domain-based entities with a molecular weight from ~1 to ~10 kDa and peptidic protein molecules that are non-mAb entities with larger molecular weight in the range of ~10–50 kDa.

Historically, the realization of the potential of protein-based drugs was spurred by the discovery of recombinant DNA technology, and further facilitated by advances in protein engineering, synthetic synthesis technologies, and directed-evolution strategies encompassing protein expression and purification, as well as the realization of the unique target interaction specificity of biologics relative to nonprotein-based modalities. These advances have led to the term biologics being used to encompass a burgeoning structurally diverse array including peptides, larger proteins, cytokines, replacement enzymes, and mAbs. Combining these protein structures with native and non-native elements lends to additional structural diversity including fusion proteins (e.g., Fc- or albumin-fusion constructs), conjugated molecules (e.g., PEGylated, acylated, and other time-extension technologies, where PEG denotes polyethylene glycol), domain-based biologics (e.g., antigen-binding fragments, nanobodies, and single-chain variable fragments), and bispecific antibodies and antibody-drug conjugates. The flexibility in structural design of biotherapeutic modalities has led to a simultaneous increase within the last decade in the clinical development of biologics across a number of therapeutic areas, including (but not limited to) diabetes, autoimmune disorders, oncology, neuroscience, and cardiovascular and infectious diseases (Recio et al., 2016; Grilo and Mantalaris, 2019). While exceptionally

ABBREVIATIONS: CDR, complementarity-determining region; FcRn, neonatal Fc receptor; Fv, fragment variable; GLP-1, glucagon-like peptide-1; mAb, monoclonal antibody; PD, pharmacodynamics; PEG, polyethylene glycol; pI, isoelectric point; PK, pharmacokinetics.
promising, there is a confounding rate of clinical attrition for protein-based therapeutics, estimated at 8%–10% (depending on the disease indication) achieving clinical success and reaching patients (Mohs and Greig, 2017). The causalities of clinical failure for biologics are related to a myriad of reasons (e.g., insufficient safety margins and strategic industry decisions), but frequently have been attributed to poor exposure-efficacy or pharmacokinetics (PD)/pharmacodynamics profiles (Mohs and Greig, 2017; Recio et al., 2016; Grilo and Mantalaris, 2019).

Given the critical link between exposure (PK) and response (PD or pharmacology), an important attribute proposed for enhancing the clinical success of mAbs and peptides involves augmenting their disposition and PK attributes. Tuning the PK and disposition properties of these agents toward their targets in combination with efforts to optimize their PD relationships can lead to molecules having improved safety and efficacy profiles. In addition, optimizing the PK and disposition properties can facilitate reducing the dose and/or dose frequency, thereby improving patient convenience, increasing compliance, and yielding better therapeutic outcomes with pragmatic clinical success. Toward that end, there has been a recent surge in interrogations that have focused on dissecting and understanding the mechanisms and factors influencing their PK and disposition in order to advance improvements in this science. The PK and disposition properties of mAbs and peptides are influenced by two broadly categorized phenomena: target-mediated drug disposition and nontarget-related clearance mechanisms. This review focuses on nontarget-related factors influencing the disposition and PK of mAbs and peptides.

Peptides and mAbs have overlapping, yet distinct, considerations with regard to mechanisms influencing their clearance. Dissecting the nontarget-mediated mechanisms affecting the PK and disposition of either mAbs or peptides is a complex endeavor, involving a deep-rooted understanding of protein structure and dynamics and the intrinsic physiologic mechanisms influencing the peripheral clearance and tissue disposition of these biologic modalities. Mechanistically, recent reports have highlighted the intricate balance in the physicochemical properties of mAbs and peptides as an important aspect in determining these factors. Physicochemical attributes such as the molecular weight, secondary and tertiary structure, charge- and hydrophobicity-related attributes, post-translational modifications (e.g., glycosylation, deamidation, and methylation), and thermal and catabolic stability, each play a role in the clearance of mAbs and peptides to varying degrees. Some peptides may also encompass the added feature of conjugation (e.g., PEGylation, acylation, and polysialic acid) or fusion to larger domains (e.g., the Fc region of mAb or albumin moieties) that are intended to influence their PK and disposition. Thus, in addition to molecule-centric physicochemical properties, several studies have reported physiologic-based protein interactions with circulating serum albumin and the neonatal Fc receptor (FcRn) as key pillars affecting PK and disposition in mAb and peptide biologics.

Beyond understanding factors that influence the peripheral clearance of mAbs and peptides, there is a need to augment the druggability of these modalities in terms of administration in order to improve patient experience and compliance. Optimizing the druggability of mAbs and peptides requires knowledge of the practical application to the therapeutic area and the patient population in which these modalities will be applied with regard to dose volume, route, and frequency. Historically, mAbs (especially those developed for oncology) have been administered via the intravenous route, which can be potentially less convenient and more costly for patients, payers, and providers. As mAbs are being developed for indications that patients and providers want and need, many nonintravenous delivery approaches are being interrogated for mAbs in order to facilitate the flexibility of consistent self-administration. Some of these approaches include other forms of parenteral delivery, such as subcutaneous and intramuscular administration, along with depot-based delivery strategies geared toward minimizing pain to enhance the injection experience. In the case of peptides (particularly for chronic or subchronic therapeutic applications), while the majority are developed as subcutaneously self-administered modalities, there are considerable efforts being made toward enabling the delivery of peptides via other approaches. The delivery strategies include injectable sustained-release formulations requiring less frequent administration as well as noninvasive routes of delivery such as oral, transdermal, inhalation, and nasal routes. As a consequence of the pragmatic need to augment the druggability properties, it has become increasingly important to understand the factors/mechanisms affecting the delivery route-dependent absorption kinetics and the solubility and permeability properties of mAbs and peptides, which are thus discussed herein. There is also mention of content regarding the promising inscription in the development of computational-based approaches toward predicting the kinetic properties of mAbs and peptides as a means of designing molecules with enhanced druggability properties and informing in silico model-based biotherapeutic design.

Molecule-Centric Physicochemical Factors Influencing Disposition and Pharmacokinetics

Physicochemical factors such as molecular weight, metabolic stability, charge/hydrophobicity, glyc glysation, and chemical transformations (e.g., methylation, deamidation, oxidation, and isomerization) impact the absorption and disposition of biotherapeutics. The structural uniqueness of mAbs and peptidic proteins imparts some similarities and differences in the relative influence of these factors on the PK of these two biologic modalities. In this section, several physicochemical factors are reviewed, and their impact on disposition in relationship to the structure of mAb- and peptide-based therapeutic modalities is discussed.

Apparent Molecular Weight. Molecular weight (or apparent hydrodynamic size) is perhaps one of the most polarizing physicochemical differences between mAbs and peptidic proteins, affecting their PK and disposition in both the blood and tissue. The difference in molecular weight has led the biopharmaceutical industry to use distinct approaches in the design, engineering, and strategies for enhancing the PK properties of mAbs and peptides as medicinal agents. This section focuses on the influence of molecular weight on the peripheral blood elimination and tissue distribution of mAbs (molecular weight of ~150 kDa), peptides (molecular weight of ~1–10 kDa), and peptidic proteins (molecular weight of ~10–50 kDa) that are present in the blood circulation irrespective of route of administration. In addition, strategies leveraged to modify the native systemic clearance mechanisms due to the size of the molecules are discussed for peptides.

There is an inverse relationship between the rate of renal clearance (or elimination) of protein-based biologics and their size (or molecular weight). The general philosophy, based on a number of studies, suggests molecules that are less than 50 kDa are removed from systemic circulation by renal filtration through the renal glomeruli (Haraldsson et al., 2008). There are some known examples of renal filtration, which are influenced solely by the size of the molecules.
but instead are affected by the charge and interaction of the peptide with the FcRn (Sands, 2015). For example, in the case of albumin, reabsorption of large amounts of protein that pass through the glomeruli is mediated in renal tubules (Sands, 2015). Notwithstanding this phenomenon, given that the reabsorption of peptides designed as therapeutics is generally uncommon several approaches have been interrogated to reduce the renal filtration of peptides by increasing their apparent molecular weight. The goal of these efforts is to improve the pharmacokinetics and half-life of peptides in circulation in order to decrease both the amount of peptide required for therapeutic application as well as the dosing frequency to improve patience compliance/experience.

One strategy to increase the apparent molecular weight of peptides has been to fuse peptides to larger polymer-based entities. Polymers such as PEG, polysialic acid, and hydroxyethyl starch have been conjugated to peptides to slow glomerular filtration and are being evaluated as additional options to reduce the renal clearance of mAbs (Patel et al., 2014). There are a number of successful examples of slowing renal clearance via PEGylation. For example, PEGylation of forms of erythropoietin (Mircera; half-life ~135 hours in humans) and human growth hormone (Pegvisomant; half-life of ~144 hours in humans) increased the half-life of the molecules by ~14- and ~400-fold relative to their respective native peptides in humans, respectively (Turecek et al., 2016). Approaches to leverage polysialic acid and hydroxyethyl starch to improve the half-life of peptides show promising findings in exploratory studies in preclinical species and continue to be interrogated (Gregoriadis et al., 2000; Solá and Griebenow, 2010).

Another strategy to increase the apparent molecular weight of peptides has been to fuse these molecules to a larger entity such as plasma or serum proteins. There are several fusion approaches of peptides to circulating proteins, which involve a myriad of noncovalent and direct or covalent interactions with the peptide (Strohl, 2015). Increased binding of plasma proteins through modulation of noncovalent interactions of the peptide has been successfully used to slow the clearance of some peptide moieties (Strohl, 2015). For example, the slowed clearance of octreotide has been successfully used to slow the clearance of some peptide moieties (Patel et al., 2014).

Regardless of molecular weight, it is well established that the tissue distribution of any molecule is determined by movement of the entity across or between the endothelial cells that comprise the vascular network into the interstitial space between the vasculature and tissue. The size and generally solvent buried hydrophobic content of mAbs and peptide proteins preclude readily permissive passive diffusion across vascular endothelial cells. Instead, convection or diffusion between endothelia are conventionally considered to be the predominate mechanisms for mAbs and peptideic proteins (Jain and Gerlowski, 1986). In the case of molecules that are <15 kDa, the rate of diffusion is larger than the convection rate; in contrast, for the larger molecular weight mAbs (~150 kDa), the movement into the interstitial space is dependent on the pressure gradient in the blood vessels, and thus is driven by convection (Jain and Gerlowski, 1986). Irrespective of the diffusion- or convection-mediated transit of the molecules to the interstitial space, the quantity in and out of tissue is contingent on the amount of molecule within the interstitial space and the magnitude of the area (i.e., size) of tissue exposed (Sarin, 2010). In other words, highly vascularized tissues with discontinuous fenestrated endothelia (e.g., liver, bone marrow, and spleen) will have greater exposure to mAb- and peptideic protein–based molecules than tissues with continuous nonfenestrated endothelium (e.g., muscle, skin, central nervous system, and lung). Modeling data indicate the estimated interstitial fluid-to-blood ratio for mAbs to range from 0.3 to 1 at steady state, with organs having fenestrated or discontinuous endothelia being at the higher end of the scale (Cowell et al., 1986; Lobo et al., 2004). There are limited reports of interstitial fluid levels of proteins, but inferences from studies with albumin in skin suggest that as the protein size decreases the interstitial fluid-to-blood ratio would be expected to show increases (Aukland and Fadnes, 1973; Poulsen, 1974).

Additional studies dissecting the relationship between the size of the biologic on the partitioning and the interstitial space and tissue compartment present an opportunistic area for improving the development of biochemical modalities for enhanced tissue compartment targeting. Active transport via FcRn has also been reported to facilitate tissue distribution. The role of FcRn in the tissue distribution of mAbs and peptides is discussed subsequently.

The Neonatal Fc Receptor. The FcRn is a major histocompatibility complex class I–related receptor consisting of a α–FcRn chain and β2 microglobulin components (Brambell, 1966; Burmeister et al., 1994). FcRn is a crucial component with direct connectivity to the elimination half-life of mAbs (via the Fc region), Fc, or albumin fusion peptides and the fusion of a GLP-1 moiety to the Fc region derived from an IgG1 imparted a half-life of ~120 hours in humans compared with a GLP-1 molecule that had a half-life of minutes (Fala, 2015). In other cases, GLP-1 homologs such as the albumin-exendin-4 conjugate CJIC-1134-PC and albiglutide have been fused to albumin to increase their molecular weight and slow renal elimination, to the extent that the molecules have displayed half-lives of ~8 days and ~6 to 7 days, respectfully, in humans. Single domain antibodies such as nanobodies that bind to albumin have also been developed (Adams et al., 2016). For example, GSK2374697 is an albumin-binding domain antibody that binds to albumin and increased the serum persistence of a GLP-1 receptor agonist (O’Connor-Semmes et al., 2014). It is important to note that Fc regions and albumin also interact with FcRn. Thus, in addition to the increased molecular weight, the slowed clearance and prolonged half-life of peptides leveraging covalent or noncovalent fusion with an Fc region or albumin is also mediated via FcRn. Improved FcRn interaction increases the potential of the molecules to be salvaged from intracellular catabolism in vascular endothelial and epithelial cells. The role of FcRn in the clearance and disposition of mAbs and peptides is discussed further in more detail subsequently.
fatty acid conjugated (i.e., acylated) peptides. The role of FcRn in the clearance of mAbs has been studied extensively; however, there is a paucity of reports with regard to dissecting this mechanism for peptides. This section will provide an overview of the role of FcRn in the regulation of the systemic clearance and tissue distribution of mAbs and peptides.

The role of FcRn in mediating the long circulating half-life of endogenous IgG and albumin has been well established by both in vitro studies and in vivo murine knockout systems that show rapid elimination of endogenous IgG and albumin (Ghetie et al., 1996, 1997; Ghetie and Ward, 1997; Ward et al., 2003, 2005; Garg and Balthasar, 2007; Goebel et al., 2008; Andersen and Sandlie, 2009; Andersen et al., 2014). FcRn functions to salvage IgG and albumin taken into cells by means of a pH-dependent binding mechanism within endosomes. The high affinity of FcRn for IgGs (mediated through the Fc region) and albumin within the reduced pH environment of endosomes (pH < 7) facilitates binding, followed by recycling of the FcRn-IgG or albumin complex and release of bound species at the higher extracellular pH environment (pH ~ 7.4), where the FcRn affinity is markedly lower (Roopenian and Akilesch, 2007). A few studies have shown that IgG not bound to FcRn within endosomes undergoes proteolytic degradation in lysosomes (Ward et al., 2003, 2005). Thus, the dogma is that the proportion of IgG processed through the recycling versus degradative pathways is critical in determining the clearance and half-life of IgG and albumin molecules in the circulation. The increased emphasis on improving both the PK and disposition properties of mAbs and peptides made optimizing their interactions with FcRn through protein engineering a logical approach.

Strategies, including fusion to Fc domains or albumin and acylation (which facilitates interaction with albumin), clearly improve the elimination half-life relative to the native peptide. Some examples are hirudin (Syed et al., 1997), GLP-1 (Mehta et al., 2017), gastric inhibitory polypeptide 1 (Martin et al., 2013), insulin (Duttaroy et al., 2005), CD4 (Yeh et al., 1997), granulocyte colony stimulating factor (Halperrn et al., 2002), α and β interferons (Sung et al., 2003; Bain et al., 2006; Subramanian et al., 2007), and growth hormone (Osborn et al., 2002). Successful clinical examples include etanercept (Enbrel, TNFR75-Fc), dulaglutide (Trulicity, GLP-1-Fc), and albiglutide (Tanzanum/Eperzan, GLP-1-albumin) (Spencer-Green, 2000; Blair and Keating, 2015; Fala, 2015). Each of these has shown improved PK compared with unmodified or nonacylated counterparts. Studies with Fc-modified constructs and albumin variants indicate the importance of FcRn in slowing the clearance of modalities used as fusions for peptides. For example, an albumin variant (deemed K573P) with ~12-fold improved human FcRn affinity showed prolonged serum half-life in normal mice, mice transgenic for human FcRn, and cynomolgus monkeys (Andersen et al., 2006, 2012, 2014). Moreover, for the development of albiglutide, an engineered albumin variant with improved FcRn binding was used to enhance the kinetics (Bukrinski et al., 2017). Similarly, specific Fc variants with enhanced FcRn interactions (discussed subsequently) can result in ~2- to ~4-fold longer in vivo elimination phase half-life in monkeys (Dall’acqua et al., 2002, 2006; Hinton et al., 2004, 2006; Datta-Mannan et al., 2007a,b; Yeung et al., 2009, 2010; Deng et al., 2010). Along these lines, retrospective analyses of several humanized mAbs having similar human FcRn binding properties demonstrated that they had ~1.5- to ~2-fold differences in their elimination half-lives in humans, suggesting lack of direct quantitative correlation with FcRn binding (Suzuki et al., 2010). Similarly, our laboratory (Datta-Mannan et al., 2012), Gurbaxani et al. (2006), and Deng et al. (2010) were unable to directly correlate FcRn binding affinity to the pharmacokinetics of a number of IgGs in mice and/or monkeys. Varied FcRn binding formats, as well as the influence of other factors including target binding and nontarget-related mechanisms influencing the disposition of mAb (modifications, transformations, charge, and hydrophobicity discussed subsequently), have been implicated as potential reasons for the lack of a direct predictive relationship between any of the FcRn interaction parameters (k_on, k_off, and K_D) in vitro with antibody pharmacokinetics in vivo (Datta-Mannan and Wroblewski, 2014). In this regard, a few groups have reported promising utility in leveraging transgenic mice expressing human FcRn as a preclinical PK model to project human PK of mAbs and mitigate nonhuman primate usage (Tam et al., 2013; Proetzol and Roopenian, 2014; Avery et al., 2016). A recent report around the development of a double transgenic mouse model bearing both human FcRn and human serum albumin genes may also facilitate additional preclinical-to-clinical PK projections for mAbs, human serum albumin fusion molecules, and acylated constructs (Viuft et al., 2016). Irrespective of the preclinical model that best predicts human clearance, evidence of improving mAb clearance via enhancing FcRn interactions for mAbs has been demonstrated in humans. MEDI4893, a mAb that binds to α-toxin and contains a triple residue substitution (M252Y/S254T/T256E or YTE) within the Fc region that improves the FcRn interaction, was estimated to have an elimination half-life of ~80–112 days, which is ~4-fold longer than the systemic half-lives of other human IgGs (Yu et al., 2017). Similarly, motavizumab-YTE showed an extended half-life of 70–100 days in healthy adults, which is also ~4-fold longer than...
that of wild-type motavizumab (Robbie et al., 2013). The positive corroboration that a PK benefit can be manifested via enhanced FcRn interactions in humans for mAbs continues to leave a pillar of hope for enhancing the druggability of next-generation mAb therapies.

Given the expression of FcRn in multiple tissues (including those involved in clearance such as liver and kidneys), the receptor has the potential to have various roles in the tissue distribution of mAbs and peptides. A few studies have been conducted in which the expression of FcRn in various tissues and species including humanized transgenic mouse lines was examined (Garg and Balthasar, 2007; Chen et al., 2014; Yip et al., 2014; Fan et al., 2016; Latvala et al., 2017). A comprehensive comparative assessment of FcRn distribution in ~20 tissues from humans and nonclinical species (rat, mouse, cynomolgus monkeys, two humanized transgenic mouse lines, and the severe combined immunodeficient mouse) showed FcRn was expressed in endothelial cells and interstitial macrophages, Kupffer cells, alveolar macrophages, enterocytes, and choroid plexus epithelium (Latvala et al., 2017). In addition, in these studies the FcRn expression pattern was similar across each species with the exception of the human FcRn transgenic mouse Tg276 (Latvala et al., 2017). These findings may have implications in the selection of human FcRn transgenic lines for translational PK and disposition studies of mAbs, Fc fusion proteins, and other peptides/proteins leveraging FcRn-based interactions.

Relatedly, an emerging area that has implications in mAb engineering and the selection of fusion/conjugation approaches for peptides is the role of FcRn in absorption of mAbs and peptides. Studies on FcRn knockout mice showed ~3-fold lower subcutaneous bioavailability for an IgG1 subtype mAb relative to wild-type mice, suggesting FcRn is important in mediating absorption from the subcutaneous space (Wang et al., 2008). It was speculated that the receptor may be mechanistically conferring increased transcytosis across the vasculature within the subcutaneous space. The hypothesis was further supported by the decreased subcutaneous bioavailability observed in a few studies with mAbs that showed increased FcRn affinity at pH 7.4 or blocked the interaction with the receptor, suggesting slowed/ablated transcytosis (Deng et al., 2010, 2012; Kagan and Mager, 2013). While these studies suggest FcRn plays a role in mediating mAb absorption, in a study conducted on five mAbs with enhanced FcRn binding at pH 6 and no receptor affinity at pH 7.4, no systematic increase in subcutaneous bioavailability in cynomolgus monkeys was observed for any of the evaluated mAbs (Datta-Mannan et al., 2012). These data along with other studies suggest that leveraging FcRn to improve subcutaneous bioavailability may be challenging in isolation and involve the consideration of additional physiochemical properties of mAbs, as will be discussed subsequently (Igawa et al., 2010, 2011; Datta-Mannan et al., 2012, 2015b,c; Hötzel et al., 2012). Oral delivery of mAbs and peptides remains a challenging space (mentioned subsequently). Recent studies with mAbs and Fc moieties suggest FcRn may also play a role in facilitating the absorption (via transcytosis) of mAbs and peptide in the gut following oral administration (Pridgen et al., 2013; Muzammil et al., 2016). For example, conjugation of nanoparticles with a Fc that bound FcRn at pH 6 compared with a nonbinding Fc improved the bioavailability by ~11-fold in mice following oral delivery (Pridgen et al., 2013). Moreover, in a cynomolgus monkey study, a mAb showed exposure following delivery to the small intestine (Muzammil et al., 2016). While the exposure was considered low (relative to parenteral delivery of mAbs), potentially due to proteolysis in the gastrointestinal tract, the proof of principle around the ability to absorb a mAb into the systemic circulation in the absence of permeation enhancers or other excipients that affect the integrity of the gut epithelial tight junctions was demonstrated (Muzammil et al., 2016). Additional exploration around the synergies between various disciplines (engineering, delivery technologies, and depot formulations) may lead to future alternatives for improving the druggability of mAbs and peptides.

**Modifications and Transformations.** Mabs and peptides are susceptible to various nonproteolysis-based transformation processes or modifications including glycosylation, deamidation, oxidation, and isomerization of certain residues. These mechanisms can cause structural perturbations in both mAb and peptide molecules and markedly influence their clearance and disposition. The genesis of the transformation and modification processes can be a myriad of possibilities during expression, purification, storage, and/or in vivo exposure. This section will cover a high-level overview of these mechanisms in the absence of the consideration of their origin; however, a substantial discussion on these processes has been reviewed extensively elsewhere and the reader is directed to other reviews for more information (Bak et al., 2015; Lapto and Omersel, 2018). Additionally, it has been speculated by our laboratory and others that transformations or modifications can influence the clearance of biologics, particularly through scavenger receptors (Ashraf and Sahu, 2012; Zani et al., 2015; Datta-Mannan et al., 2016; PrabhuDas et al., 2017). The role of scavenger receptors in the disposition of mAbs and peptides is an evolving space that is not presented herein.

The study of mAb and peptide transformation and/or modification is a complex field that is highly intertwined with bioanalytical strategies to detect these processes. The insurgence of highly sensitive liquid chromatography approaches to separate various isoforms along with high-resolution mass spectrometry has spurred advancements in this evolving landscape (Wang et al., 2016). The glycosylation of mAbs and peptides is arguably the best characterized modification studied via these approaches. There are two well-established clearance pathways for glycosylated molecules (Jeffers, 2005, 2009; Sinclair and Elliott, 2005). One mechanism is via the liver-expressed asialoglycoprotein receptor that recognizes terminal galactosylation, binding these moieties and clearing the glycoprotein from systemic circulation. The second manner in which glycoproteins are removed from the circulation is through the mannose receptor, which exclusively interacts with N-acetylgalactosamine residues of N-glycans and is highly expressed in immune cells. As discussed previously, the long half-life of IgGs is conferred by the interaction of their Fc region with FcRn (Datta-Mannan and Wrblewski, 2014). While this FcRn binding property is not influenced by natural gyrocoforms for endogenously circulating antibodies, some concern has been raised for recombinant mAbs produced in various cells (NS0, CHO, or Sp2/0) (Harris, 2005; Liu, 2015). In the case of recombinant mAbs, there are typically minor amounts of non-natural gyrocoforms, especially high mannose gyrocoforms, such as mannose-5 and mannose-6, as well as variable amounts of glycosylation (Harris, 2005). While these have been speculated to potentially influence mAb PK and disposition due to the targeting potential of cells expressing mannose receptor (most markedly expressed in immune cells), studies have shown mixed findings. In one study on a mAb by Millward et al. (2008), it was demonstrated that a mAb with high mannose-enriched Fc showed no significant differences in PK examined in mice relative to the molecule with complex gyrocoforms. These data suggested that for mAbs derived from CHO cells Fc regions with high mannose-5 and mannose-6 gyrocoforms have no significant differences in kinetic properties (Millward et al., 2008). In contrast, for another mAb, kinetic studies of mice showed slower clearance and longer half-life for degalactosylated IgGs compared with the unmodified parent molecules (Newkirk et al., 1996). Similarly, a high mannose-5 glycan containing mAb was more rapidly eliminated than other forms in mice (Kanda et al., 2007). In terms of the impact of glycosylation of the mAb variable region on PK, there are also divergent findings.
There was a lack of pharmacokinetic differences observed in mice in the clearance of a mAb separated into fractions containing different amounts of fragment variable (Fv)-associated sialic acid, suggesting Fv-associated sialic acid differences are not a substantial clearance mechanism for mAbs (Huang et al., 2006). In contrast, another laboratory showed that version of a mAb that lacked galactosylation in the Fv had a more rapid clearance in rodents than those that were sialated (Coloma et al., 1999). Clinical studies also show mixed findings, suggesting both that enzymatic conversion of glycans in vivo as another option explaining the selective removal of distinct glycoforms, as well as more rapid clearance of a mAb with a high mannose Fc from serum (Chen et al., 2009; Goetzte et al., 2011). In totality, the findings around the influence of glycosylation on the disposition of mAbs are heterogeneous and at best unclear or potentially mAb specific. It seems likely that the findings may need to be reexamined in the context of additional mechanisms influencing the disposition of mAbs including (but not limited to) charge, hydrophobicity, stability, and solubility.

In addition to the potential of glycosylation to influence the PK of antibodies, it is worth noting that the core heptasaccharide (with variable outer arm sugar residues) within the CH2 region of the Fc is critical to the effector function as mediated through FcγR and C1q. Several studies have demonstrated altered effector mechanisms for aglycosylated antibodies, indicating glycosylation is essential for effector functionality (Liu, 2015). In the case of recombinant mAbs produced in various cells (NS0, CHO, or Sp2/0), the core is comprised of fucosylated entities that embody effector function, which can be modulated via engineering of the glycoform. For example, the increased binding of a nonfucosylated form of rituximab to FcγRIIIa enhanced the effector function activity by several orders of magnitude compared with the fucosylated form of rituxan (Shibata-Koyama et al., 2009). Additional studies in this area could inform engineering approaches around effector function and mAb PK/disposition.

In contrast to mAbs, for peptides it is much more apparent that the glycosylation profile can significantly influence the kinetics and disposition of molecules. In a few instances, hyperglycosylated forms of erythropoietin, neutrophil inhibitory factor, follistatin, and follicle-stimulating hormone showed slowed systemic clearance compared with their respective lower glycosylated counterparts (Darling et al., 2002; Webster et al., 2006; Shibata-Koyama et al., 2009; Datta-Mannan et al., 2015a). The glycans on peptides are often accessible to mannose or asialoglycoprotein receptors on cells that can interact with the glycans and mediate clearance of the peptides. For example, Aranesp is an engineered erythropoietin molecule that contains two additional N-glycosylation sites relative to recombinant human erythropoietin for enhanced sialylation. The enhanced sialylation slows the clearance of Aranesp, conferring ~3-fold longer half-life in vivo compared with recombinant human erythropoietin (Egrie et al., 2003). Additionally, in the case of an engineered version of follistatin fused to an IgG-derived Fc, glycosylation heterogeneity within the follistatin component led to the rapid clearance of various glycoforms by asialoglycoprotein receptor 1 (Datta-Mannan et al., 2015a). Reduced sialic acid content correlated with decreased serum half-life in mice and cynomolgus monkeys. Profiling of the glycan content led to glycan content specifications to limit the clearance of the follistatin-Fc fusion protein via this mechanism (Datta-Mannan et al., 2015a).

Along with glycosylation-based modifications, there are noteworthy concerns around the transformation of mAbs and peptides, which may result in perturbations to structure and function that influence PK and disposition. The transformation of mAbs and peptides via deamidation, oxidation, and isomerization of certain residues is commonly assessed during chemical stability testing in vitro as part of formulation development at relatively high protein concentrations (Bak et al., 2015; LaPorto and Omersel, 2018). Due to bioanalytical challenges around detection in the complex environmental milieu of biologic samples, progress in the characterization of transformation in vivo has been slow; however, there are a handful of studies examining the occurrence of these processes in vivo and the importance of their characterization on the clearance and disposition of mAbs and peptides. For example, deamination of asparagine and/or glutamine residues in peptides such as human growth hormone and epidermal growth factor have been speculated to alter clearance as a consequence of conformational changes due to alterations in surface charge distribution (Bischoff and Kolbe, 1994; Robinson, 2002). This overall increase in net negative charge may facilitate increased interaction with endothelial and epithelial cells, which has been noted in biologics. In the case of mAbs, a few studies have clearly reported loss of antigen binding activity when deamidation occurred within the complementarity-determining region (CDR); however, the role of deamidation in mAb clearance and disposition is somewhat vague (Harris et al., 2001; Chelius et al., 2005; Huang et al., 2005; Yan et al., 2009). In a hallmark study by Liu et al. (2009), deamidation of conserved asparagine residues with the Fc region of IgG1 and IgG2 subtypes was predicted to show no significant effect on their pharmacokinetics relative to the amidated species in humans. These findings, generally led to a long time reasonable consensus in the field about the lack of a substantial influence of deamidation of asparagine residues conserved in the Fc region influencing PK. More recently, some laboratories have suggested revisiting this area, given that data have shown changes in mAb charge distribution could alter clearance differentially based on the impact to local structural conformation, structural flexibility/dynamics, and solvent accessibility (Yan et al., 2018). Similar to deamidation, isomerization of aspartate to isoaspartic acid has been noted to play a significant role in alteration of protein structure and function. For instance, it has been speculated that isoaspartic acid is crucial for the aggregation of β-amyloid, and thereby facilitates the progression of neurodegenerative disorders like Alzheimer’s disease (Lashley et al., 2018). There are also examples of the isomerization of aspartate to isoaspartic acid in the CDRs of mAbs that decrease target binding and mAb efficacy (Wakankar et al., 2007; Dick et al., 2010). The phenomenon has also been noted to occur in vivo by Prueksaritanont and Tang (2012) for a mAb, which resulted in complete loss of the molecule’s target binding ability. To the best of our knowledge, there are no published reports showing that isomerization of aspartate to isoaspartic acid within mAbs or peptides affects their PK or disposition; however, it is entirely possible to envision that for molecules in which isomerization impacts structural integrity, charge, or target-mediated drug disposition, the clearance of the molecules may be influenced. In the case of oxidation, there are a number of reports demonstrating that oxidation of methionine in the Fc region of mAbs reduces FcRn binding, and consequently the half-life, in preclinical species (Wang et al., 2011; Folzer et al., 2015). Furthermore, oxidation of methionine or tryptophan in the CDRs of some mAbs induced a loss of antigen binding and decreased potency, although the influence of these transformations on clearance and disposition was not reported and remains unclear (Wei et al., 2007; Hensel et al., 2011). Taken together, it is clear that modification and transformation of mAbs and peptides are important considerations in the development of the modalities for enhanced disposition and PK/PD properties. Increased characterization of these processes in vivo and their connectivity to the relationship between clearance and protein structure, folding, and dynamics may help facilitate more rationale and optimal design and engineering strategies for mAbs and peptides.

**Charge Heterogeneity and Hydrophobicity.** Solvent-exposed residues within mAbs and peptides are subject to interactions with in vivo...
matrices (i.e., blood cells, other circulating proteins, vasculature, and tissue components) that affect their nontarget binding–mediated clearance. Depending on the nature of the molecule, the solvent-exposed residues may create a globalized or local/regionalized composition of charge and hydrophobic content heterogeneity within the mAb or peptide, which results in a net physiochemical distribution of these properties affecting the PK and disposition of molecules (Hong et al., 1999; Boswell et al., 2010; Khawli et al., 2010). While the specific characterization of the influence of hydrophobicity has been noted in studies assessing mAb and peptide aggregation propensities, evidence of direct connectivity with clearance is sparse (Boswell et al., 2010; Igawa et al., 2011; Bumbaca et al., 2012; Datta-Mannan et al., 2015b,c; Dobson et al., 2016). This is likely due to early elimination during the drug discovery and development process due to poor expression, purification, and/or solution solubility given the propensity of these molecules to aggregate. For peptides, a number of recent studies have predicted the elimination half-life as it relates to charge and hydrophobic content–based physiochemical properties related to their residue composition, potential for metabolism (discussed subsequently), chemical modification, and transformations (Sharma et al., 2014; Broom et al., 2017; Mathur et al., 2018). Due to their more complicated large tertiary structures and dynamics, the role of hydrophobicity and charge in mAb clearance has been determined through more empirical-based experimentation. For example, it was speculated that the broad nonspecific tissue binding and sequesterization observed in antirespiratory syncytial virus mAb (discussed subsequently), chemical modification, and transformations (Sharma et al., 2014; Broom et al., 2017; Mathur et al., 2018). Due to their more complicated large tertiary structures and dynamics, the role of hydrophobicity and charge in mAb clearance has been determined through more empirical-based experimentation. For example, it was speculated that the broad nonspecific tissue binding and sequesterization observed in antirespiratory syncytial virus mAb (discussed subsequently), chemical modification, and transformations (Sharma et al., 2014; Broom et al., 2017; Mathur et al., 2018).

In Vivo Stability.

The in vivo stability of mAbs and peptides in the systemic circulation is critical for them to become successful medicines. These two biologic modalities have unique stability considerations. The majority of mAb-based therapeutic drugs are compositionally IgG1, IgG2, or IgG4 based. Compared with IgG1 and IgG2 molecules, IgG4s have can stability issues in which they swap in vivo with endogenous IgGs in a process deemed antigen-binding fragment arm exchange (Angal et al., 1993; Schuurman et al., 1999, 2001; Aalberse et al., 2009; Stubenrauch et al., 2010). The antigen-binding fragment arm exchange involves one-half of the IgG4 molecule (heavy-light chain pair) exchanging and recombining with another IgG4 antibody. The process occurs under physiologic conditions; therefore, therapeutic IgG4s are subject to exchange with endogenous IgG4 molecules in circulation, thereby resulting in the therapeutic mAbs being functionally monovalent with potentially reduced efficacy (Margni and Binaghi, 1988; Silva et al., 2015). The instability in IgG4 subtype therapeutic mAbs can be mitigated, as reported by Stubenrauch et al. (2010), by mutation of serine residue in position 228 within the hinge to a proline.

Given their more limited tertiary structure relative to mAbs, peptides are generally vulnerable to systemic proteolysis or catalysis by soluble or membrane-bound proteases such as endopeptidases, aminopeptidases, and carboxypeptidases. Endopeptidases have increased propensity to catalyze peptides with higher serine, threonine, proline, or glutamine residues, whereas aminopeptidases tend to degrade peptides with N-termini containing glycine, alanine, threonine, valine, methionine, or serine. Programs to predict peptide stability have been developed and are leveraged during the discovery/development of peptides to substitute residues to enhance peptide PK while maintaining efficacy. Mitigation of these catabolic sites is also approached by a variety of other means including 1) modification of the N- and C-termini via acetylation and amidation, respectively, to reduce exoprotease liabilities (Ladner et al., 2004; Sato et al., 2006; Di, 2015); 2) replacement of natural residues (L form) with non-natural residues (D-form) to decrease endoprotease catalysis (Tugyi et al., 2005; Chen et al., 2013); 3) modification of natural amino acids that mechanistically improve stability/protection of amide bonds via steric hindrance (Werle and Bernkop-Schnürch, 2006); 4) cyclization of peptides to integrate conformational constraint that decreases recognition by proteases (Di, 2015); and 5) fusion to larger domains (albumin or Fc) or chemical conjugation (PEGylation, acylation) as discussed previously (van Wittenloostijn et al., 2016; Zorzi et al., 2017). These peptide stabilization approaches can have a myriad of varied effects on PK; often times, due to the number of degrees of freedom.
Peptide- and mAb-based biologics can vary significantly in their potency depending on their mechanism of action (i.e., agonist vs. antagonist). Many of the mAbs that are available as medicines or are in clinical testing are antagonists, which require high-dose administration. In contrast, the majority of peptides (e.g., incretins, insulins, and enzyme replacement therapies) are agonist hormones, which compared with mAbs can be administered at very low doses. These broad differences in mechanism of action have led to similar and diverging considerations for improving the druggability of mAbs and peptides.

Historically, many mAbs and peptides were delivered intravenously (Skalko-Basnet, 2014; Bittner et al., 2018). As the field of biologics as a class of medicines has increased (especially over the last 2 to 3 decades), there has been a concomitant rise in efforts to improve patient compliance and experience associated with the administration and delivery of these modalities. Due to the lower dosage and self-administration needs (especially in the treatment of chronic diseases such as diabetes) peptides have logically led the way for subcutaneous delivery (Skalko-Basnet, 2014; Bittner et al., 2018). Currently, advancements in the development of peptides is well connected to subcutaneous delivery and device considerations, including the use of autoinjector and/or pump systems and formulations that minimize any injection site pain or reactions (Skalko-Basnet, 2014; Bittner et al., 2018). Despite the general dogma that continuous stridess in parenteral administration of peptides improves patient experience, the rich history of nonparenterally delivered small molecule drugs creates a highly debatable vision for the potential administration of peptide-based modalities by means other than subcutaneous injection. Nonparenteral delivery of peptides by transdermal, oral, and inhalation methods has been interrogated for several decades with mixed success (Patel et al., 2014). For example, Exubera, a form of inhalable hexameric insulin that showed comparable efficacy to injected short acting insulin, was approved in the United States and United Kingdom in 2006. The failure to gain acceptance by payers, providers, and patients due to a lack of pharmacological benefit relative to subcutaneous injection and its increased cost resulted in Exubera being removed from the market (Santos Cavaiola and Edelman, 2014). More recently, a report on another inhalable insulin drug, Afrezza, showed a pharmacological benefit in adults with type I diabetes compared with insulin aspart (Mohanty and Das, 2017). Additional time is required to analyze whether these observed benefits align with patient and provider needs. The generally low solubility, stability, and permeability of most peptides in the gastrointestinal system have limited their oral bioavailability (typically, <1% relative to subcutaneous injection), also making it challenging for the biopharmaceutical industry to readily develop orally delivered peptides. However, this is an active area of research for peptides, especially for the treatment of type II diabetes. For example, the announcement to pursue the development of oral semaglutide, which showed an oral bioavailability of ~0.5%–1% in humans (compared with subcutaneous injection), demonstrates the interest in this space to enhance peptide druggability (Davies et al., 2017).

The subcutaneous delivery of mAbs to improve druggability has also progressed with a number of molecules for osteoporosis, autoimmune, oncology, and neurologic disorders developed or in clinical trials using subcutaneous administration. Relative to peptides, subcutaneous administration has been more challenging for mAbs due to the industry generally relying on prefilled syringe systems as the container (Li and Easton, 2018). While these are readily available, for mAbs the solubility and viscosity of individual mAbs in the resulting formulation can greatly impact manufacturability using prefilled syringe systems. Prefilled syringe systems can become challenging due to the associated constraints including limited practical volume of subcutaneous delivery (~1–1.5 ml), which requires high mAb concentration (typically, at least ~200 mg/ml) using isotonic solutions for solution stability (Li and Easton, 2018). The delivery of larger volumes (up to 10 ml) of mAbs is possible with slower rates of subcutaneous bolus injection, but this requires alternative delivery approaches and systems. For example, the coformulation of mAbs with recombinant human hyaluronidase has been shown to facilitate increasing injection volumes up to 5 ml in prefilled syringes in the absence of adverse injection site effects (Wasserman, 2014). From the device delivery perspective, on-body subcutaneous delivery systems such as intradermal and patchable pumps are being developed to manage delivery of ~5–10 ml volumes (Bertea et al., 2015; Viola et al., 2018). There are reports of expanding the technologies to even higher volumes (~30 ml) to enable subcutaneous self-delivery, especially of mAb-based oncology therapies, within the next decade (Viola et al., 2018). Whether high-volume autonomous subcutaneous mAb delivery is mediated by formulation and/or device, several reports indicate an important pillar for the success of these approaches is mitigating/managing pain during the injection event (Viola et al., 2018). The United States and European Union regulatory agencies have begun to establish guidelines around patient experience with subcutaneous administration (International Organization for Standardization standard series 11608). Alignment of the needs around high-volume mAb delivery, patient experience, and delineating mechanisms that influence subcutaneous adsorption and disposition continues to be an opportunistic area for improving mAb druggability.

Summary and Future Perspectives

Tremendous strides have been made within the last two decades in the discovery and development of mAbs and peptides as medicines. As the field has progressed, there is concerted awareness of the importance of applying the understanding of mechanisms influencing the disposition and PK of mAbs and peptides to the selection of molecules for clinical interrogation. Indeed, predictions of mAb and peptide human PK from preclinical species is routinely leveraged for the design of human dose selection and frequency. Allometry, with empirically derived exponents for mAbs and peptides and population PK modeling have been successfully used to adequately predict human mAb and peptide PK from preclinical animal exposure data in the absence of nonlinear PK and/or species-centric clearance mechanisms (Sharma et al., 2014; Betts et al., 2018). Albeit while generally successful at predicting human PK, the current approaches require input from empirical animal PK studies following generation (expression and purification) of molecules. The progress made in understanding this space opens up the potential for increasingly extending into and leveraging in silico approaches for the design and selection of mAbs and peptides with the desired human PK properties prior to the generation of molecules and extensive in vivo preclinical PK studies. Promising findings in a study of 61 IgG1 subtype mAbs showed rapid and normal cynomolgus monkey clearance was correctly predicted for 86% and 75%, respectively, of the molecules based on their sequence-derived predicted hydrophobicity with the CDRs and net charge (Sharma et al., 2014). More recently, the development of designing computational tools that can accurately predict aggregation tendencies linked to physiochemical attributes (charge patches and hydrophobic regions) is increasingly being leveraged for the design of mAbs. The release of an open-source computational tool called
the Therapeutic Antibody Profiller, which highlights mAbs with uncharacterizable developability attributes compared with known therapeutics, demonstrates the value of building in silico approaches that will readily predict in vivo PK and disposition to improve the efficiency and reduce the number of nonessential preclinical studies. Increased emphasis on understanding the interplay and relative weighting of the mechanisms influencing the disposition and PK of mAbs and peptides within these in silico tools will facilitate the design of the next wave of medicines with increased target and tissue binding selectivity and specificity for enhanced therapeutic benefit. In addition to these advances, there remains significant opportunity to optimize mAb and peptide druggability to enhance patient experience, and thus strategies to improve their PK and disposition. The limited permeability of mAbs and peptides currently makes these modalities predominantly viable as parenterally delivered. As advancements at the interfaces of multiple disciplines including drug design, engineering, delivery technologies, digital health, and clinical practices progresses, a natural evolution of strategies for enhancing the druggability of mAbs and peptides should also continue to emerge and grow. The application of information around the mechanisms influencing the disposition and PK of mAb and peptide therapies is uniquely positioned to facilitate the rapid progression of these modalities as medicines and progress toward the increased availability of novel biologic modalities.

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