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REVIEW ARTICLE:

**Mechanisms Influencing The Pharmacokinetics And Disposition Of Monoclonal Antibodies
And Peptides**

Amita Datta-Mannan

Department of Clinical Pharmacology, Lilly Research Laboratories, Lilly Corporate Center,
Indianapolis, Indiana.

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Running Title: **Pharmacokinetics And Disposition Of Biologics**

Corresponding Author: Amita Datta-Mannan
Lilly Research Laboratories
Eli Lilly & Company
Lilly Corporate Center
Indianapolis, IN 46285, USA
Email: datta_amita@lilly.com
Phone: (317) 651-3577
Fax: (317) 276-4218

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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 more than 90 mAb and peptide therapeutics on the United States market, with over 600 more in various clinical stages of development in a broad array of therapeutic areas, including diabetes, auto-immune disorders, oncology, neuroscience, cardiovascular and infectious diseases. Notwithstanding this potential, there is high rate 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 pharmacokinetic (PK) and disposition profiles leading to limited or diminished efficacy. Increased and thorough characterization efforts directed at disseminating mechanisms influencing the pharmacokinetics and disposition of mAbs and peptides can aid in improving the design for their intended pharmacological activity and thereby, their clinical success. The pharmacokinetics and disposition factors for mAbs and peptides are broadly influenced by target-mediated drug disposition and non-target-related clearance mechanisms related to the interplay between the relationship of the structure and physiochemical properties of mAbs and peptides with physiological processes. This review focuses on non-target related factors influencing the disposition and PK of mAbs and peptides. Contemporary considerations around the increasing in silico approaches to identify non-target related molecule limitations and enhancing the druggability of mAbs and peptides, including parenteral and non-parenteral delivery strategies that are geared towards improving patient experience and compliance, are also discussed.

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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 form 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 (Grilo and Mantalaris, 2019; Kaplon and Reichert, 2019; Recio et al., 2016). These two biological modalities combined also encompass greater than 30% of the number of molecules investigated in clinical trials (Grilo and Mantalaris, 2019; Mohs and Greig, 2017; Recio et al., 2016). 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 physiological 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 kDa to ~10 kDa and peptidic protein molecules that are non-mAb entities with larger molecular weights in the range of ~10 kDa to ~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, directed evolution strategies encompassing protein expression and purification, as well as, the realization of the unique target interaction specificity of biologics relative to non-protein based modalities. These advances have led the term biologics

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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 leads to additional structural diversity including fusion proteins (eg. Fc- or albumin-fusion constructs), conjugated molecules (eg. PEGylated, acylated and other time-extension technologies), domain based biologics (eg. Fabs, nanobodies, scFvs), bispecific antibodies and antibody-drug conjugates (ADCs). 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, auto-immune disorders, oncology, neuroscience, cardiovascular and infectious diseases (Grilo and Mantalaris, 2019; Recio et al., 2016). While exceptionally promising, there is a confounding rate of clinical attrition for protein-based therapeutics, estimated at 8% to 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 (eg. insufficient safety margins and strategic industry decisions), but frequently have been attributed to poor exposure-efficacy or pharmacokinetic/pharmacodynamics (PK/PD) profiles (Grilo and Mantalaris, 2019; Mohs and Greig, 2017; Recio et al., 2016).

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 towards 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

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pragmatic clinical success. Towards that end, there has been a recent surge of interrogations which have focused on dissecting and understanding the mechanisms and factors influencing their PK and disposition to advance improvements in this science. The PK and disposition properties of mAbs and peptides are influenced by two broadly categorized phenomenon: target-mediated drug disposition (TMDD) and non-target-related clearance mechanisms. This review focuses on non-target 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 non-target 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 physiological mechanisms influencing the peripheral clearance and tissue disposition of these biological modalities. Mechanistically, recent reports have highlighted that the intricate balance in the physiochemical properties of mAbs and peptides as an important aspect in determining these factors. Physiochemical attributes, such as the molecular weight, secondary and tertiary structure, charge and hydrophobicity related attributes, post-translational modifications (eg. glycosylation, deamidation, methylation), 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 (eg. PEGylation, acylation, polysialic acid) or fusion to larger domains (eg. Fc region of mAb or albumin moieties) that are intended to influence their PK and disposition. Thus, in addition to molecule-centric physiochemical properties, several studies have reported physiological-based protein interactions with circulating serum albumin and the neonatal Fc receptor (FcRn) as key pillars affecting PK and disposition for mAb and peptide biologics.

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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 for improving patient experience and compliance. Optimizing the druggability of mAbs and peptide requires knowledge of the practical application to the therapeutic area and the patient population these modalities will be applied with regard to dose volume, route and frequency. mAbs (especially those developed for oncology) have historically been administered via the intravenous route, which can be potentially less convenient and more costly for patients, payers and providers. As mAbs are developed for indications which patients and providers want and need the flexibility for consistent self-administration, many non-intravenous delivery approaches are being interrogated for mAbs. Some of these approaches include other forms of parenteral delivery, such as subcutaneous and intramuscular administration, along with depot based delivery strategies geared towards minimizing pain to enhance the injection experience. In the case of peptides (particularly for chronic or sub-chronic therapeutic application) while the majority are developed as subcutaneously self-administered modalities, there are considerable efforts in 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 for augmenting the druggability properties, it has become increasingly important to understand the factors/mechanisms affecting the delivery route dependent absorption kinetics, solubility and permeability properties of mAbs and peptides and thus, are discussed herein. There is also mention of content regarding the promising insurgence in the development of computational based approaches towards predicting the kinetic properties of mAbs and peptides as a means of

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designing molecules with enhanced druggability properties and to inform *in silico* model-based biotherapeutic design.

MOLECULE-CENTRIC PHYSIOCHEMICAL FACTORS INFLUENCING DISPOSITION AND PHARMACOKINETICS

Physiochemical factors, such as molecular weight, metabolic stability, charge/hydrophobicity, glycosylation and chemical transformations (eg. 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 biological modalities. In this section, several physiochemical factors are reviewed, and their impact on disposition in relationship to the structure of mAbs and peptide-based therapeutic modalities.

Apparent Molecular Weight

Molecular weight (or apparent hydrodynamic size) is perhaps one of the most polarizing physiochemical differences between mAbs and peptidic proteins that affects their PK and disposition in both the blood and tissue. The difference in molecular weight has led the biopharmaceutical industry to use distinct approaches for 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 (~1- to ~10 kDa) peptidic proteins (~10- to ~50 kDa) that are present in the blood circulation irrespective of route of administration. In addition,

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strategies leveraged to modify the native systemic clearance mechanisms due to the size of the molecules is 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 that molecules that are less than 50 kDa are removed from systemic circulation by renal filtration through the renal glomeruli (Haraldsson et al., 2008; Sarin, 2010). The kidney glomeruli pore size is estimated at ~8 nm, thus proteins/peptides with a molecular weight of <2-50 kDa are liable to renal filtration through the glomeruli (Haraldsson et al., 2008). The large molecular weight of mAbs (~150 kDa) clearly preclude these molecules from renal clearance mechanisms; however, for peptides renal filtration can play a major role in the removal of these molecules from the systemic circulation. In the absence of renal insufficiency, many unmodified peptides have a short in vivo half-life (typically in range of minutes), making these modalities challenging to develop as therapeutics (Lau and Dunn, 2018). There are some known examples of renal reabsorption mechanisms for endogenous proteins in the tubules that also play a role in the rate, extent and magnitude of peptide renal filtration that 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 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 the reabsorption of peptides designed as therapeutics is generally uncommon, several approaches have been interrogated to reduce the renal filtration for peptides by increasing their ‘apparent’ molecular weight. The goal of these efforts is to improve the pharmacokinetics and half-life of peptides in circulation to decrease both the amount of peptide required for

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therapeutic application as well as the dosing frequency to improve patient 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 (polyethylene glycol), polysialic acid (PSA) and hydroxyethyl starch (HES) have been conjugated to peptides to slow glomerular filtration and are being evaluated as additional options to reduce the renal clearance of peptide (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-fold and ~400-fold relative to their respective native peptides in humans, respectively (Turecek et al., 2016). Approaches to leverage PSA and HES 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; Sola 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 non-covalent and direct or covalent interactions with the peptide (Strohl, 2015). Increased binding of plasma proteins through modulation of non-covalent 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 is attributed to the molecule's ability to reversibly bind to circulating lipoproteins resulting in an ~100 min half-life in humans (Chanson et al., 1993). It is speculated that interaction of the peptide with lipoprotein may also facilitate increased albumin binding leading to its slowed glomerular filtration (Chanson et al., 1993). An additional effective technique that hijacks endogenous

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albumin binding to slow renal clearance involves direct conjugation of peptides to fatty acid moieties (Lim et al., 2013). The conjugation to fatty acid chains effectively makes the peptide a lipopeptide that can interact with serum albumin, prolonging the peptides effective circulation time due to an increased apparent molecular weight via binding albumin through non-covalent interactions (Lim et al., 2013). In the case of insulin detemir, linking an insulin analogue to myristic acid facilitates prolonged action compared with post prandial treatments (Poon and King, 2010). Similarly, conjugation of the GLP-1 receptor agonist analog, semaglutide, to a 18-carbon fatty acid chain, led to a half-life of ~168 hours in human and supported a once weekly dosing interval for the peptide compared to non-fatty acid containing GLP-1 receptor agonist molecules which have a daily dosing frequency due to their short half-lives (less than ~3- to 6- hours) (Guja and Danciulescu Miulescu, 2017).

The fusion of peptides to larger protein domains, such as a Fc region, other immunoglobulin fragments and albumin, or conjugation to polymers can also increase their molecular weight and apparent size leading to slowed renal filtration. Many of these strategies have been or are being applied to GLP-1 peptides to facilitate decreasing the frequency of administration. For example, in the case of Trulicity (or dulaglutide), 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 to a GLP-1 molecule which had a half-life of minutes (Fala, 2015). In other the cases, GLP-1 homologs, such as the albumin-exendin-4 conjugate CJC-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 a half-life of ~8 days and ~6-7 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

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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 non-covalent 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 in more detail further below.

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 peptidic protein molecules precludes 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 peptidic 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 area (ie size) of tissue exposed (Sarin, 2010). In other words, highly vascularized tissues with discontinuous fenestrated endothelia (eg. liver, bone marrow and spleen) will have greater exposure to mAb- and peptidic protein-based molecules than tissues with continuous, non-fenestrated endothelium (muscle, skin, CNS and lung). Modeling

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data indicates 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 (Covell 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 of the size of the biologic on the partitioning to interstitial space and tissue compartment is an opportunistic area for improving the development of biotherapeutic 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 below.

The Neonatal Fc Receptor (FcRn)

FcRn is a major histocompatibility complex class I-related receptor consisting of a α -FcRn chain and β 2 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 fatty acid conjugated (ie acylated) peptides. The role of FcRn in the clearance of mAbs has been studied extensively, however, there are 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 knock-out systems which show rapid elimination of endogenous IgG and albumin (Andersen et al., 2014; Andersen and Sandlie, 2009; Garg and Balthasar, 2007; Ghetie et al., 1996; Ghetie et al., 1997; Ghetie and Ward,

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1997; Goebel et al., 2008; Ward et al., 2005; Ward et al., 2003). 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 Fc region) and albumin within the reduced pH environment of endosomes (pH ~5-6) 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 Akilesh, 2007). A few studies have shown that IgG that is not bound to FcRn within endosomes undergoes proteolytic degradation in lysosomes (Ward et al., 2005; Ward et al., 2003). 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 the both 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 to albumin, and acylation (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), GIP-1 (Martin et al., 2013), insulin (Duttaroy et al., 2005), CD4 (Yeh et al., 1997), granulocyte colony stimulating factor (Halpern et al., 2002), α and β interferons (Bain et al., 2006; Subramanian et al., 2007; Sung et al., 2003), and growth hormone (Osborn et al., 2002). Successful clinical examples include etanercept (Enbrel, TNFR75-Fc), dulaglutide (Trulicity, GLP-1-Fc,) and albiglutide (Tanzeum/Eperzan, GLP-1-albumin) (Blair and Keating, 2015; Fala, 2015; Spencer-Green, 2000). Each of these have shown improved PK compared to unfused or non-acylated 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

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~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., 2012; Andersen et al., 2014; Andersen et al., 2006). 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 below) can result in ~2- to ~4-fold longer *in vivo* elimination phase half-life in monkeys (Dall'acqua et al., 2002; Dall'acqua et al., 2006; Datta-Mannan et al., 2007a; Datta-Mannan et al., 2007b; Deng et al., 2010; Hinton et al., 2004; Hinton et al., 2006; Yeung et al., 2009; Yeung et al., 2010). The case of peptide acylation is unique (compared to Fc or albumin fusion strategies), in that the interaction with FcRn is indirect and mediated through binding to albumin. There are several reports of empirical-based PK studies examining the effect of fatty acid length, composition, and position of conjugation on peptide half-life with a variety of findings (Jensen et al., 2017; Lim et al., 2013). While improvements in peptide PK are generally observed following acylation, the disparity in the observations may be related to a number of additional contributing factors, such as peptide-specific charge, hydrophobicity and metabolic stability. Additionally, it has been postulated that binding of acylated peptides to albumin may affect FcRn interactions via conformational changes within the acylated peptide bound albumin (Barnett et al., 2013; Jovanovic et al., 2014). Schmidt et al. demonstrated that saturation of albumin with fatty acids interferes greatly with FcRn binding (Schmidt et al., 2013). In another approach, FcRn binding peptides were designed to interact directly with receptor as a means to improve the elimination half-life. When the FcRn binding peptides were fused to larger protein domains, improved clearance and half-life were observed for the domains relative to the native molecules (or non-FcRn binding peptide bearing moieties) (Datta-Mannan et al., 2018; Mezo et al., 2008; Sockolosky et al., 2012; Ying et al., 2015).

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Regardless of the method leveraged to improve half-life, there remains considerable opportunity in delineating the role of FcRn relative to other factors (eg. size, metabolism) in the improvements in PK observed for the fused and/or fatty acid-conjugated peptides. The information is important for both the design of novel peptide therapeutics, and has significant implications on understanding the FcRn-mediated disposition characteristics of various peptide biotherapeutic modalities.

In the case of mAbs, many studies have focused on mutating the Fc region to enhance the interaction with FcRn, with findings suggesting a somewhat controversial role for universally leveraging FcRn as a means to slow their systemic clearance. For example, a handful of specific Fc variants (T250Q/M428L, M428L, M252Y/S254T/T256E, M428L/N434S, N434A, N434H) have shown that improving the mAb affinity for FcRn at pH 6, with little to no influence on the pH 7.4 interactions molecule, can result in ~2- to ~4-fold longer *in vivo* elimination phase half-life in monkeys (Dall'acqua et al., 2002;Dall'acqua et al., 2006;Datta-Mannan et al., 2007a;Datta-Mannan et al., 2007b;Deng et al., 2010;Hinton et al., 2004;Hinton et al., 2006;Yeung et al., 2009;Yeung 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 a lack of a direct quantitative correlation with FcRn binding (Suzuki et al., 2010). Similarly, our laboratory, Gurbaxani *et al.* and Deng and coworkers were unable to directly correlate FcRn binding affinity to the pharmacokinetics of a number of IgGs in mice or monkeys (Gurbaxani et al., 2006) (Datta-Mannan et al., 2012;Deng et al., 2010). Varied FcRn binding formats, as well as the influence of other factors including target binding and non-target related mechanism influencing the disposition of mAb (modifications, transformations, charge and hydrophobicity discussed below), have been implicated a potential reasons for the lack of a direct predictive relationship between any of the

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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 non-human primate usage (Avery et al., 2016; Proetzel and Roopenian, 2014; Tam et al., 2013). A recent report around the development of a double transgenic mouse model bearing both human FcRn and human serum albumin (HSA) genes may also facilitate additional preclinical-to-clinical PK projections for mAbs, HSA fusion molecules and acylated constructs (Viuff 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 which binds to alpha-toxin and contains a triple residue substitution (M252Y/S254T/T256E or YTE) within the Fc region that improves FcRn interaction was estimated to have an elimination half-life of ~80 to ~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 to 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 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. There are a few studies examining the expression of FcRn in various tissues and species including humanized transgenic mouse lines (Chen et al., 2014; Fan et al., 2016; Garg and Balthasar, 2007; Latvala et al., 2017; Yip et al., 2014). A comprehensive comparative

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assessment of FcRn distribution in ~20 tissues from humans and non-clinical 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 on 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 on mAb engineering and the selection of fusion/conjugation approaches for peptides is the role of FcRn in absorption of mAbs and peptides. Studies in FcRn knockout mice showed an ~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 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; Deng et al., 2012; Kagan and Mager, 2013). While these studies suggest FcRn plays a role in mediating mAb absorption, in a study conducted with five mAbs with enhanced FcRn binding a 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 leveraging FcRn to improve subcutaneous bioavailability may be challenging in isolation and involve the

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consideration of additional physiochemical properties of mAbs discussed below (Datta-Mannan et al., 2015b; Datta-Mannan et al., 2015c; Datta-Mannan et al., 2012; Hotzel et al., 2012; Igawa et al., 2011; Igawa et al., 2010). Oral delivery of mAbs and peptides remains a challenging space (mentioned below). 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 (Muzammil et al., 2016; Pridgen et al., 2013). For example, conjugation of nanoparticles with a Fc which bound FcRn at pH 6 compared to a non-binding 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 enhancer or other excipients which 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, 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 non-proteolysis-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

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and/or during 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 of these processes has been reviewed extensively elsewhere and the reader is directed to other reviews for more information (Bak et al., 2015; Laptos and Omersel, 2018). Additionally, it has been speculated by our lab and others that transformations or modifications can influence the clearance of biologics particularly through scavenger receptors (SRs) (Ashraf and Sahu, 2012; Datta-Mannan et al., 2016; PrabhuDas et al., 2017; Zani et al., 2015). The role of SRs 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 resurgence 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 (Jefferis, 2005; 2009; Sinclair and Elliott, 2005). One mechanism is via the liver-expressed asialoglycoprotein receptor which recognizes terminal galactosylation, binding these moieties and clearing the glycoprotein from systemic circulation. The second manner glycoproteins are removed from the circulation is through the mannose receptor which exclusively interacts with N-acetylglucosamine residues of N-glycans and is highly expressed on immune cells. As discussed above, the long half-life of IgGs is conferred by the interaction of their Fc region with FcRn (Datta-Mannan and Wroblewski, 2014). While this FcRn binding property is not influenced by natural glycoforms for endogenously circulating antibodies, some concern has been raised for recombinant mAbs produced in various cells (NS0, CHO or Sp2/0) (Harris, 2005;

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Liu, 2015). In the case of recombinant mAbs, there are typically minor amounts of non-natural glycoforms, especially high mannose glycoforms, 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 on cells expressing mannose receptor (most markedly expressed on immune cells), studies have shown mixed findings. In one study for a mAb by Millward and coworkers, 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 glycoforms (Millward et al., 2008). These data suggested that for mAbs derived from CHO cells Fc regions with high mannose-5 and mannose-6 glycoforms have no significant differences in kinetic properties (Millward et al., 2008). In contrast, for another mAb, kinetic studies in mice showed a slower clearance and longer half-life for degalactosylated IgGs compared to 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 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 lab 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; Goetze et al., 2011). In totality, the findings around the influence of glycosylation on the disposition of mAbs

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are heterogeneous and at best unclear or potentially mAb specific. It seems likely that the findings may need to be re-examined in the context of additional mechanisms influences the disposition of mAbs including (but not limited to) charge, hydrophobicity, stability and solubility.

In addition to the potential for glycosylation to influence PK for 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 non-fucosylated form of rituximab to FcγRIIIa enhanced the effector function activity by several orders of magnitude compared to 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 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 to their respective lower glycosylated counterparts (Darling et al., 2002; Datta-Mannan et al., 2015a; Shibata-Koyama et al., 2009; Webster et al., 2006). The glycans on peptides are often accessible to mannose or asialoglycoprotein receptors on cells which 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

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sialylation. The enhanced sialylation slows the clearance of Aranesp™, conferring ~3-fold longer half-life *in vivo* compared to 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 the 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 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; Laptos and Omersel, 2018). Due to bioanalytical challenges around detection in the complex environmental milieu of biological 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, deamidation 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 for biologics. In the case of mAbs, a few studies have clearly reported loss of

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antigen binding activity when deamidation occurred within the CDR, however the role of deamidation in mAb clearance and disposition is somewhat vague (Chelius et al., 2005; Harris et al., 2001; Huang et al., 2005; Yan et al., 2009). In a hallmark study by Liu and coworkers, 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 (Liu et al., 2009). These findings, generally led to a long time reasonable consensus in the field around the lack of a substantial influence of deamidation of asparagine residues conserved in the Fc region influencing PK. More recently, some labs have suggested revisiting this area, given data showing 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 (Dick et al., 2010; Wakankar et al., 2007). The phenomenon has also been noted to occur *in vivo* by Prueksaritanonti and Tang for a mAb, which resulted in complete loss of the molecule's target binding ability (Prueksaritanont and Tang, 2012). To the best of our knowledge, there are no published reports 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

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oxidation of methionine in the Fc region of mAbs reduces FcRn binding and consequently half-life in preclinical species (Folzer et al., 2015; Wang et al., 2011). 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 were not reported and remain unclear (Hensel et al., 2011; Wei et al., 2007). Taken together, it is clear that modification and transformation of mAbs and peptides are an important consideration 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 of clearance with protein structure, folding and dynamics may help facilitate more rationale and optimal design and engineering strategies for mAb and peptides.

Charge Heterogeneity and Hydrophobicity

Solvent exposed residues within mAbs and peptides are subject to interactions with *in vivo* matrices (ie. blood cells, other circulating proteins, vasculature and tissue components) which affect their non-target 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 (Boswell et al., 2010; Hong et al., 1999; Khawli et al., 2010). While the specific characterization of the influence of hydrophobicity has been noted in studies assessing mAb and peptide aggregation propensities, the direct connectivity with clearance is sparse (Boswell et al., 2010; Bumbaca et al., 2012; Datta-Mannan et al., 2015b; Datta-Mannan et al., 2015c; Dobson et al., 2016; Igawa et al., 2011). This is likely due to the early elimination during the drug discovery and

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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 related to charge and hydrophobic content-based physicochemical properties related to their residue composition, potential for metabolism (discussed below), chemical modification and transformations (Broom et al., 2017; Mathur et al., 2018; Sharma et al., 2014). Due to their more complicated large tertiary structures and dynamics, the role of hydrophobicity and charge on mAb clearance has been determined through more empirical based experimentation. For example, it was speculated the broad nonspecific tissue binding and sequestration observed for an anti-respiratory syncytial virus mAb A4b4, may have been due to hydrophobic interactions (Wu et al., 2007). Similarly, the rapid cynomolgus monkey clearance of an anti-human amyloid beta peptide mAb was connected to fibrinogen, which upon conversion to fibrin becomes increasingly hydrophobic (Vugmeyster et al., 2011). More recently, direct evidence of the disruption of the hydrophobicity-induced self-association interface of MEDI1912, an antibody targeting nerve growth factor, by three amino acid mutations enhanced was demonstrated to improve its serum persistence, whilst maintaining high affinity and potency (Dobson et al., 2016).

In terms of charge, modifications (via glycosylation) and transformations (oxidation, isomerization and deamidation) cause changes to the overall net charge of mAbs and peptides, creating basic or acid moieties with altered PK and disposition as discussed above. A few studies have conclusively reported changes in the isoelectric point (pI) of a mAb or peptide of more than one pI unit can increase the systemic clearance and tissue retention affecting distribution likely due to increased charge-charge interactions with components of tissues that are agnostic to chemical modification and transformations or alterations in FcRn interactions (Boswell et al.,

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2010; Hong et al., 1999; Igawa et al., 2010; Khawli et al., 2010). For example, Igawa *et al.* showed that lowering the pI point from 9.2 to 7.2 by engineering the variable region (Fv) of a mAb reduced the rate of elimination in cynomolgus monkeys (Igawa et al., 2010). Along these lines, Li and coworkers demonstrated a mAb variant with a ~2.51 pI unit increase showed a more rapid clearance in rats (Li et al., 2014). There is some disparity between reports in the PK and disposition consequences as a result of changes in the ionic charge that show less than one pI unit difference for mAbs. For example, early studies by Boswell and coworkers and Khawli et al., for IgG1 molecules showed blood and tissue clearance were similar for mAbs with pI differences of less than approximately one pI unit (Boswell et al., 2010; Khawli et al., 2010). More recently, for mAbs, we and other have demonstrated local charge within the CDR and variable domain (Fv) of the framework that was not reported to impact the overall pI of the molecules can lead to charge imbalance and greatly impact the clearance of these molecules (Datta-Mannan et al., 2015c; Li et al., 2014; Sampei et al., 2013; Schoch et al., 2015). Taken together, the data suggest both global and local assessments of charge and hydrophobicity for mAbs and proteins are important for understanding the PK and disposition of these modalities. Indeed, retrospective correlation analyses of the *in vivo* PK and *in vitro* physicochemical properties of mAbs leveraging assays that measure charge and hydrophobicity show great promise and enabled the successful differentiation of mAbs at high risk for poor PK profiles from those with favorable clearance profiles (Avery et al., 2018; Bumbaca et al., 2012). The findings indicate leveraging physicochemical charge and hydrophobicity assays as preclinical *in vitro* tools to filter molecules prior to any *in vivo* PK assessments as a means to speed the selection and improve the probability of technical success for mAbs. The increased attention and research activity towards the development of *in silico* and other model-based drug design and selection tools to predict these phenomenon for improving

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druggability within the last few years also reinforces the importance of these factors in affecting non-specific clearance mechanisms (Mathur et al., 2018; Wolf Perez et al., 2018; Xu 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 biological modalities have unique stability considerations. The majority of mAb-based therapeutics are compositionally IgG1, IgG2 or IgG4 based. Compared with IgG1 and IgG2 molecules, IgG4s can have stability issues in which they swap *in vivo* with endogenous IgGs in a process deemed Fab arm exchange (Aalberse et al., 2009; Angal et al., 1993; Schuurman et al., 2001; Schuurman et al., 1999; Stubenrauch et al., 2010). Fab arm exchange involves half of the IgG4 molecule (heavy-light chain pair) exchanging and recombining with another IgG4 antibody. The process occurs under physiological conditions, thus therapeutic IgG4s are subject to exchange with endogenous IgG4 molecules in circulation thereby resulting in the therapeutic mAbs 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 and colleagues, by mutation of serine residue in position 228 within the hinge to a proline (Stubenrauch et al., 2010).

Given their more limited tertiary structure relative to mAbs, peptides are generally vulnerable to systemic proteolysis or catabolism by soluble or membrane-bound proteases such as endopeptidases, aminopeptidases and carboxypeptidases. Endopeptidases have an increased propensity to catabolize 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

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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 (Di, 2015; Ladner et al., 2004; Sato et al., 2006); (2) replacement of natural residues (L form) with non-natural residues (D-form) to decrease endoprotease catabolism (Chen et al., 2013; Tugyi et al., 2005); (3) modification of natural amino acids that mechanistically improve stability/protection of amide bonds via steric hindrance (Werle and Bernkop-Schnurch, 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 above (van Witteloostuijn 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 (ie amide bonds) within peptides require empirical *in vivo* testing to determine the consequences on elimination half-life.

DELIVERY STRATEGIES FOR IMPROVED DRUGGABILITY: INJECTABLES AND BEYOND

Peptide- and mAb-based biologics can vary significantly in their potency depending on their mechanism of action (ie agonists versus antagonists). 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 (eg incretins, insulins, enzyme replacement therapies) are agonist hormones, which compared to 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.

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Historically, many mAbs and peptides were delivered intravenously (IV) (Bittner et al., 2018; Skalko-Basnet, 2014). As the field of biologics as a class of medicines has increased (especially over the last 2-3 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 (SC) delivery (Bittner et al., 2018; Skalko-Basnet, 2014). Currently, advancements in the development of peptides is well-connected with SC delivery and device considerations, including the use of autoinjector and/or pump systems and formulations that minimize any injection site pain or reactions (Bittner et al., 2018; Skalko-Basnet, 2014). Despite the general dogma that continued strides in parenteral administration of peptides improves patient experience, the rich history of non-parenterally delivered small molecule drugs creates a highly debatable vision for the potential administration of peptide-based modalities by means other than SC injection. Non-parenteral delivery of peptides by transdermal, oral and inhalation methods have been interrogated for several decades with mixed success (Patel et al., 2014). For example, Exubera, a form of inhalable hexameric insulin which 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 SC injection and its increased cost, resulted in Exubera being removed from the market (Santos Cavaiola and Edelman, 2014). More recently, a report from another inhalable insulin, Afrezza, showed a pharmacological benefit in adults with Type I diabetes compared to 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

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peptides in the gastrointestinal system have limited their oral bioavailability (typically <1% relative to SC injection) also making it challenging for the biopharmaceutical industry to readily develop orally-delivered peptides. This is, however, 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 to SC injection), demonstrates the interest in this space to enhance peptide druggability (Davies et al., 2017).

The SC delivery of mAbs to improve drugability has also progressed with a number of molecules for osteoporosis, autoimmune, oncology and neurological disorders developed or in clinical trials using SC administration. Relative to peptides, SC administration has been more challenging for mAbs due to the industry generally relying on the pre-filled 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 pre-filled syringe systems. The pre-filled syringe systems can become challenging due to the associated constraints including limited practical volume of SC 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 a slower rates of SC bolus injection, but this requires alternative delivery approaches and systems. For example, the co-formulation of mAbs with recombinant human hyaluronidase has been shown to facilitate increasing injection volumes up to 5 mL in pre-filled syringes in the absence of adverse injection site effects (Wasserman, 2014). From the device delivery perspective, on-body SC delivery systems such as intradermal and patchable pumps are being developed to manage delivery of ~5-10 mL volumes (Berteau et al., 2015; Viola et al., 2018).

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There are reports of expanding the technologies to even higher volumes (~30 mL) to enable SC self-delivery especially of mAb based oncology therapies within the next decade (Viola et al., 2018). Whether high-volume, autonomous SC mAb delivery is mediated by formulation and/or device, several reports indicate an important pillar for the success of these approach 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 SC administration (ISO standard series 11608). Alignment of the needs around high volume mAb delivery, patient experience and delineating mechanisms that influence SC adsorption and disposition continue to be an opportunistic area for improving mAb druggability.

SUMMARY AND FUTURE PERSPECTIVES

Tremendous strides have been made within the last two decades on the discovery and development of mAbs and peptides as medicines. As the field has progressed, there is concerted awareness on 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 non-linear PK and/or species-centric clearance mechanisms (Betts et al., 2018; Sharma et al., 2014). 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

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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 complementary determining regions 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, hydrophobic regions) are increasingly leveraged for the design of mAbs. The release of an open-source computational tool called the Therapeutic Antibody Profiler (TAP) that highlights mAbs with uncharacteristic developability attributes compared to 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 non-essential 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 make 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 drugability of mAbs and peptides should also continue to emerge and grow. The application of information around the mechanisms

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influencing the disposition and PK of mAbs and peptides therapies is uniquely positioned to facilitate the rapid progression of these modalities as medicines and progress towards the increased availability of novel biological modalities.

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