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Predicting bioavailability of monoclonal antibodies after subcutaneous administration: Open innovation challenge

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ABSTRACT

Despite the increasing trend towards subcutaneous delivery of monoclonal antibodies, factors influencing the subcutaneous bioavailability of these molecules remain poorly understood. To address critical knowledge gaps and issues during development of subcutaneous dosage forms for monoclonal antibodies, the Subcutaneous Drug Delivery and Development Consortium was convened in 2018 as a pre-competitive collaboration of recognized industry experts. One of the Consortium's eight problem statements highlights the challenges of predicting human bioavailability of subcutaneously administered monoclonal antibodies due to a lack of reliable *in vitro* and preclinical *in vivo* predictive models. In this paper, we assess the current landscape in subcutaneous bioavailability prediction for monoclonal antibodies and discuss the gaps and opportunities associated with bioavailability models for biotherapeutics. We also issue an open challenge to industry and academia, encouraging the development of reliable models to enable subcutaneous bioavailability prediction of therapeutic large molecules in humans and improve translation from preclinical species.

Contents

Abbreviations: AUC, area under the curve concentration; BCS, Biopharmaceutics Classification System; BDDCS, Biopharmaceutics Drug Disposition Classification System; CDER, Center for Drug Evaluation and Research; C_{max}, maximal concentration; DCS, Developability Classification System; ECM, extracellular matrix; FcRn, neonatal fetal Fc (fragment crystallizable) receptor; FDA, US Food and Drug Administration; HA, hyaluronic acid; Ig, immunoglobulin; IV, intravenous; k_a, first-order absorption rate constant; mAb, monoclonal antibody; PBPK, physiologically based pharmacokinetic; PK, pharmacokinetic; pI, isoelectric point; rHuPH20, recombinant human hyaluronidase PH20; SC, subcutaneous; T_{max}, time to reach maximum concentration.

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1. Introduction

Biologic modalities have seen a consistent rise in approvals by the US Food and Drug Administration (FDA) Center for Drug Evaluation and Research (CDER) over the last decade, with a peak observed in 2018 with 17 biologics license application approvals and 10 approvals in 2019 (Fig. 1a). Reviewing the treated diseases and the therapeutic areas for these approved drugs provides an insightful reflection of the unmet medical needs and investments in the pharmaceutical industry across various therapeutic areas. Comparing the approvals in 2019 to those since 2014 as a function of therapeutic area, cancer remained the dominant therapeutic area in 2019, accounting for 27% of the approvals, and on par with the 5-year average of 29% (Fig. 1b). Approvals for neurological products and non-cancer hematology products increased from previous years, to 15% and 10% of approvals, respectively (Fig. 1b). Infectious disease product approvals were down, at 8% *versus* 12% in previous years, as were metabolism and endocrinology products, with no approvals (Fig. 1b). While these approvals in the aforementioned therapeutic areas reflect product research and development efforts initiated many years ago, they also set the stage for delivery opportunities in these therapeutic areas. The high-growth growth therapeutic areas set up two potential opportunities or problem statements for the future: (1) the need for product differentiation in a crowded marketplace and (2) the need for less invasive and more convenient methods of administration which enable improved patient access and compliance.

Subcutaneous (SC) injection is an important route of administration for biotherapeutics and responds well to these problem statements, providing an opportunity to differentiate and improve patient access and compliance. There has been an increasing trend towards SC delivery of monoclonal antibodies (mAbs) in recent years *versus* intravenous (IV) administration [1,2] (Fig. 1c). In a detailed analysis of mAb approvals by the FDA between 2008 and 2017, Viola et al. [2] estimated that approximately a third were administered subcutaneously, with increasing numbers in recent years. This finding is mirrored by our own analysis, which found that just over half of approvals for the 6-year period between 2014 and 2019 were for SC formulations [1] (Fig. 1c). Many mAbs approved so far, particularly for rheumatoid arthritis, have SC formulations, and SC dosing alternatives for cancer therapies (*e.g.*, trastuzumab, rituximab and daratumumab) are becoming more prevalent [3,4].

SC delivery is safe, effective, and valued by patients and healthcare providers alike [3]. It offers notable advantages over IV administration, including fixed dosing, lower hospital and clinical costs, reduced healthcare resource utilization and healthcare provider time, and increased patient throughput [3,5]. Patients may prefer SC over IV delivery because it reduces administration time and allows for self-administered or caregiver-supported dosing at home or in a setting other than an infusion center, thereby reducing treatment burden and improving quality of life [3,5,6]. Additionally, SC injection permits the treatment of patients with poor venous access and preserves venous capital in patients at risk of vascular exhaustion [7]. To enable SC administration, various formulation and device-based approaches have been evaluated. Commonly used devices for SC administration include prefilled syringes, injection pens/autoinjectors, and on-body injectors. There is significant ongoing research in developing these formulation/delivery approaches, however, that has been covered elsewhere.

Despite longstanding use and the myriad benefits of SC administration, many aspects of the bioavailability of SC delivered biologics are poorly understood. One such aspect is the marked interspecies difference in SC bioavailability [8]. It is also well recognized that SC bioavailability in the clinic has not been easily predicted based on preclinical evaluation as direct preclinical to human correlation is poor. Hence, the current SC drug development strategy includes an iterative preclinical and clinical evaluation which results in longer development timelines and increased uncertainty in bioperformance as development progresses. The lack of fundamental and mechanistic understanding around SC absorption and thus, lack of predictability, makes it challenging to determine *a priori* the molecule/formulation development strategy. Often it is not clear whether protein engineering should be considered for risk response or formulation modifications made to optimize stability and bioavailability.

With the aim of identifying and addressing development issues and critical knowledge gaps in the SC field, the SC Drug Delivery and Development Consortium was convened in 2018 as a pre-competitive collaboration of recognized industry experts in pharmaceutical drug delivery, device development, and commercialization. The Consortium has developed eight problem statements, which are described in detail in their recent publication [9]. One of these problem statements centers on the challenges of predicting human bioavailability of SC administered mAbs in development due to a lack of reliable *in vitro* and/or preclinical *in vivo* predictive models. A reliable predictive model for bioavailability would be a tremendously useful tool across the biopharmaceutical industry and help advance our understanding of how molecular properties, formulation parameters, and injection site physiology interact to affect absorption. Application of this knowledge may also accelerate drug development activities and reduce associated costs.

In this paper, we assess the current landscape in SC bioavailability prediction for mAbs and discuss the gaps and opportunities associated with bioavailability models for biotherapeutics. Additionally, we issue an open challenge to the scientific community to provide solutions.

2. Current landscape in evaluating the bioavailability of mAbs

As mentioned in Section 1, direct translation from preclinical to human bioavailability of mAbs is challenging owing to a combination of intraspecies, interspecies, and individual factors. A survey of bioavailability data from marketed immunoglobulin (Ig)G (~150 kDa), IgG fusion proteins (100–250 kDa), and smaller biotherapeutics ranging in molecular weight from 4 to 60 kDa conducted by Richter et al. [8,10] reported marked interspecies variation in SC bioavailability of mAbs. For example, SC bioavailability for adalimumab in cynomolgus monkeys and humans were 96% and 64%, respectively, and 77% and 53%, respectively, for golimumab [8]. The lack of predictability is believed to stem from differences in hypodermis structure and physiology between humans and rodents or non-human primates [11], but species-specific susceptibility to presystemic catabolism may also be a factor. Although animal models of the minipig appear to be weakly predictive of human SC bioavailability, they do appear to be predictive of human linear clearance and show a correlation between SC bioavailability and systemic clearance [11]. Minipigs are not as widely utilized as rodents or non-human primates for preclinical pharmacokinetic (PK) studies [12]. More work needs to be done to better understand the mechanistic differences in absorption among species as this variation makes it challenging to predict bioavailability from rodent or non-human primate models and translate animal data to human. The animal model as a source of variation must be considered when constructing a predictive model for bioavailability from preclinical data.

This lack of translation favors the development of *in vitro* and/or *in silico* models. To our knowledge, there is currently no robust model or system available for the *a priori* prediction of bioavailability of subcutaneously injected mAbs. However, understanding of the factors and processes involved in SC absorption is undoubtedly improving. Key studies pertaining to the interaction of SC injection site physiology and molecular or formulation properties and their influence on absorption mechanisms are described below. While we highlight aspects that we consider to be important input factors and interactions when constructing a model to predict bioavailability, this paper is not intended to serve as a comprehensive review of the literature as such reviews have been previously published [3,13,14]. Our objective is to articulate the processes that may need to be further investigated and incorporated to develop a robust model. The factors described below may be used as an

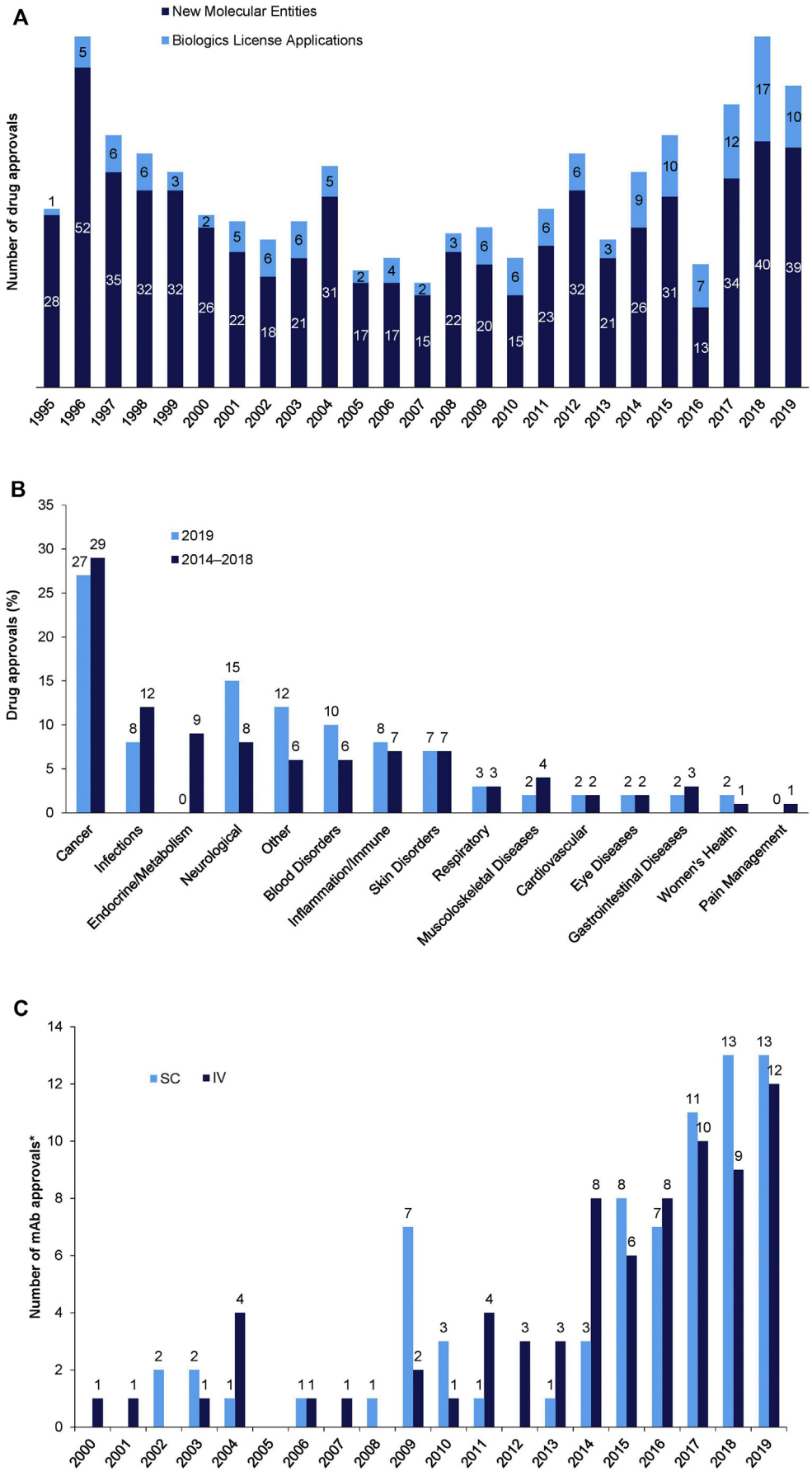

Fig. 1. (a) Annual numbers of new molecular entities and biologics license applications approved by the FDA Center for Drug Evaluation and Research (CDER) since 1995. (b) CDER approvals by selected therapeutic areas comparing 2014–2018 approvals *versus* 2019 trends. (c) Subcutaneous (SC) *versus* intravenous (IV) monoclonal antibody (mAb) approvals in the US from 2000 to 2019 [1]. *Figure classification counts different formulations and combination devices for a given mAb as separate product approvals.

initial framework; incorporation of additional factors may benefit the model.

It is important to understand the difference between bioavailability and absorption. In pharmacology, bioavailability (BA or F) is described as the fraction of an administered dose of a molecule that reaches the systemic circulation and is one of the principle PK properties of a drug. According to theory, the bioavailability of a medication administered intravenously is 100% and the bioavailability of any extravascularly administered formulation (including SC) is measured relative to this value, calculated as the ratio of dose-normalized area under the plasma concentration time curve. SC bioavailability of less than 100% is attributable to incomplete absorption due to local metabolism/degradation/precipitation at the injection site, and/or elimination (*e.g.*, by phagocytosis) during transit through the lymphatic system prior to eventual arrival in the systemic circulation (Fig. 2, Eq. 1). The fraction absorbed can be described as the fraction escaping local metabolism/degradation/precipitation at the injection site that gains access to either venous or lymphatic capillaries (Fig. 2, Eq. 2). As previously mentioned, the focus of this article is bioavailability prediction, but absorption will be discussed in the following sections as this is an important contributor to bioavailability.

Following SC injection *in vivo*, systemic absorption of mAbs proceeds primarily *via* the lymphatic system, the rate and extent of which is governed by myriad factors pertaining to the molecule, formulation, and injection site physiology (Fig. 3). Physiological factors such as temperature, local pH, neonatal fetal Fc receptor (FcRn) expression, interstitial fluid composition, pressure and flux, lymphatic capillary density, pore size, flow rate, and extracellular matrix (ECM) charge and tortuosity interact with molecular/formulation properties such as size, charge density, isoelectric point (pI), solubility, aggregation, and immunogenicity. FcRn and non-specific binding potentially play an important role in the extent and rate of transport from the injection site to the systemic circulation. Additionally, presystemic catabolism either locally or in the lymphatics may limit the amount of mAb ultimately reaching the central compartment. Fluid dynamics impacted by injection volume, tonicity, force, and viscosity may also play a role. Molecular/formulation factors are addressed below.

Owing to their *size*, direct absorption of mAbs into blood capillaries is likely precluded. The primary absorption pathways of mAbs are lymphatics [15] and potentially FcRn-mediated transcytosis into the systemic circulation [8]. The size of these molecules also impacts their diffusivity through the ECM, such that convective transport driven by fluid flow from capillaries to the lymphatic system likely dominates [16–18]. The potential for active transport mechanisms through endothelial cell surface proteins (*e.g.*, cadherins, catenins) and charge-based gating pathways has not been well studied [14].

In an *ex vivo* assay, positively *charged* humanized recombinant mAbs (mAb_1 pI = 9.1 and mAb_2 pI = 7.3) were shown to interact electrostatically with negatively charged components (*i.e.*, proteoglycans) in the SC rat tissue, with 50–60% unrecoverable and presumed bound to the tissue [19]. When extraneous hyaluronic acid (HA) was co-formulated with mAb_1, the recovery of soluble mAbs improved to about 70%, indicating that the HA was competing with the protein for binding sites. The effect was minimal with mAb_2 due to its overall neutral charge. The remaining 30% for both mAbs was retained within the tissue; this binding was attributed to unknown non-electrostatic interactions [19]. In another example, a fusion protein was engineered to remove highly basic regions in the sequence to produce variants with *pI* ranging from 8.8 to 9.4 [20]. The result was a marked improvement in maximal concentration (C_{max}) and area under the curve concentration (AUC), with each variant having a lower pI. Binding of the fusion protein variants to the ECM were decreased by an *in vitro* assay, suggesting that removal of positive regions in the protein mitigated the electrostatic interactions with the ECM.

Biologics with poor *aqueous solubility* may precipitate at the injection site and require redissolution in the extracellular fluid, if reversible, prior to absorption. This precipitation occurs through steric exclusion involving glycosaminoglycans and excipients [14]. The difference between the average human core body temperature (37 °C) and that in the SC tissue (34 °C) [21], should be considered when performing biorelevant physicochemical characterization.

SC injection is often perceived as more *immunogenic* than IV administration, possibly due to dendritic cell processing [2,22]. Although highly therapeutic-dependent, an increase in immunogenicity can be observed, as exemplified by the anti-drug antibody rates observed for IV (1%) and SC (8%) formulations of mepolizumab [23]. Protein self-association leading to soluble *aggregates* or precipitation into particulates is thought to increase immunogenicity because of the multiplicity of epitopes and conformational changes [24]. A recent study investigated the effect of protein precipitation at the injection site using an *in vitro* assay and *in vivo* imaging model [25]. Although retention of aggregated mAb was observed *in vivo* at the injection site, the authors

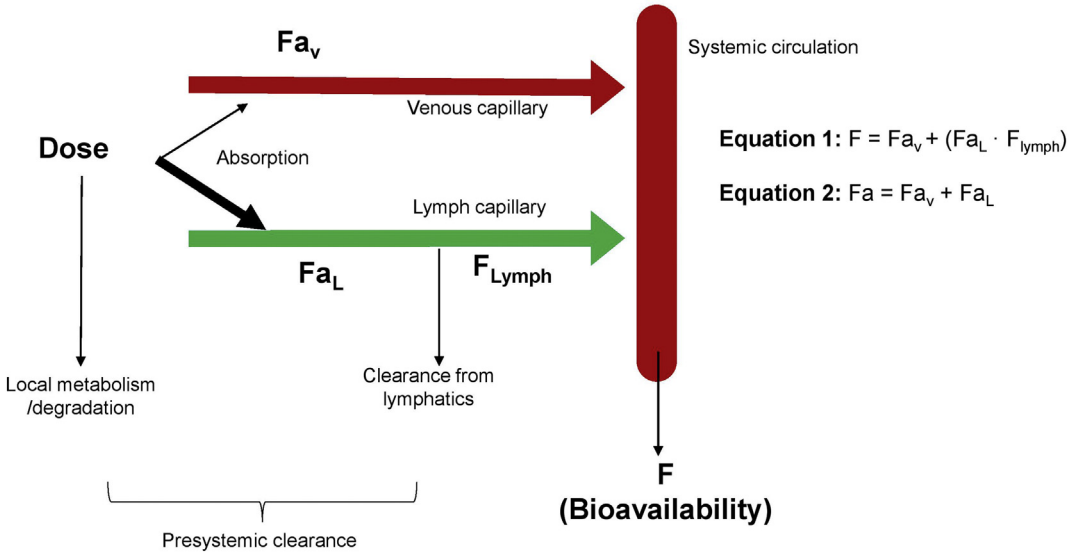

Fig. 2. Schematic representation of SC bioavailability. F, bioavailability; Fa, fraction absorbed; Fa_L, fraction absorbed *via* lymph; Fa_v, fraction absorbed *via* venous capillary; F_{Lymph}, fraction escaping lymphatic clearance.

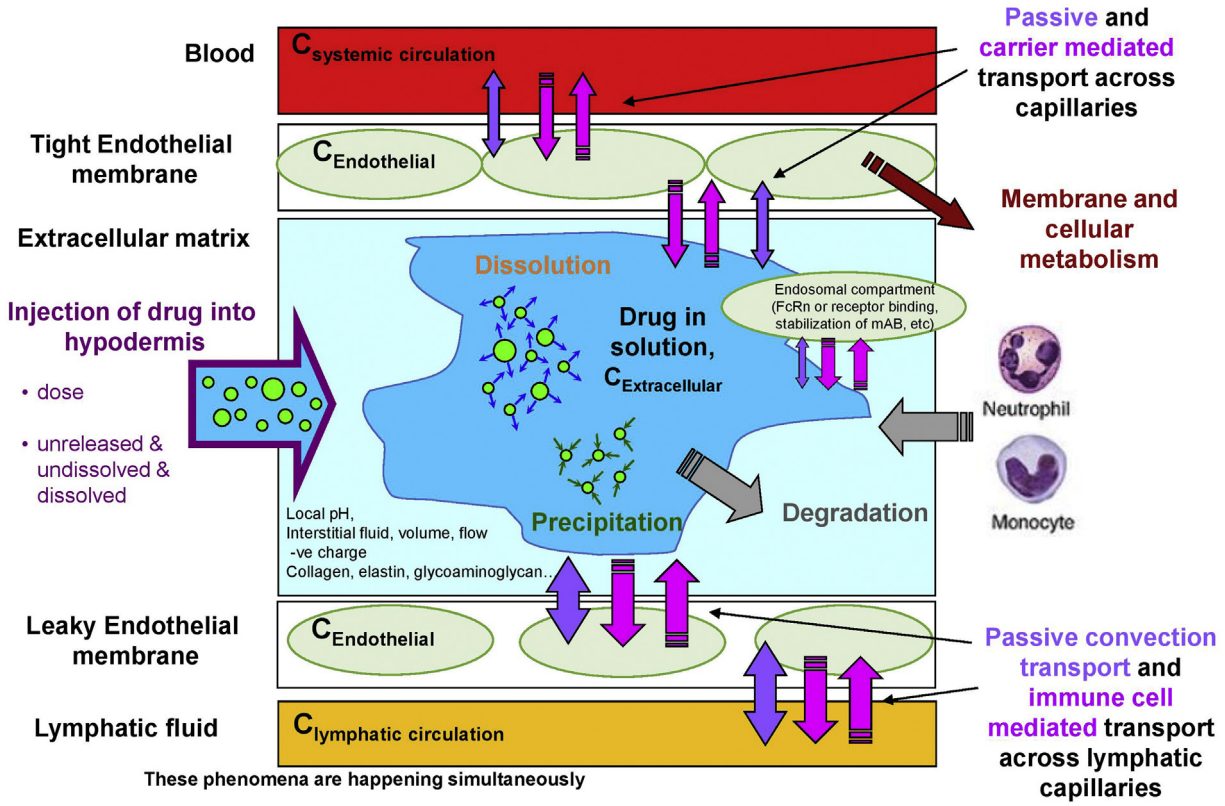

Fig. 3. Processes involved in SC absorption.

found that aggregation did not lead to an enhanced immunogenic response in terms of anti-drug antibody or cytokine responses. Although the impact on exposure was not found to be significant in this study, protein solubility and propensity to precipitate under physiological conditions may need to be factored into a predictive model for bioavailability. The immunogenicity risk of a molecule, as assessed by an *in vitro* assay or *in silico* prediction of immunogenic epitopes, should be considered when applying predictive modelling for bioavailability. For instance, molecules at high risk of an anti-drug antibody response may not be good candidates for a predictive bioavailability model.

Processes relating to *catabolism* have the potential to reduce bioavailability. Delayed absorption (*i.e.*, prolonged duration at the SC injection site) may increase susceptibility to local catabolism, thereby affecting bioavailability. Extracellular degradation, antibody endocytosis, and potential recycling through interaction with FcRn are among the processes that influence presystemic IgG catabolism [26]. Lymphatic absorption and the potential for proteolysis in the lymphatic trunk may also impact SC bioavailability [27]. Increasing the binding affinity of mAbs to FcRn at pH 6.0 while keeping a low binding affinity at pH 7.4 has been shown to improve the SC bioavailability of these molecules [28]. However, the opposite trend has also been reported where PK profiles of variant mAbs showed improved half-life or clearance following SC injection, but no clear effect was observed on the SC bioavailability [29].

In addition to physiological factors and the biophysical properties of the molecule, formulation composition can play a key role in absorption and bioavailability after SC delivery as it can impact the physical and biological stability at the injection site. Biologic formulations are typically liquid or lyophiles for reconstitution and may be of high concentration and in buffer solution with added excipients like sugars and surfactants to confer physical and chemical stability [24]. Formulation parameters such as mAb concentration, viscosity, formulation buffer species and ionic strength, and pH have all been demonstrated to affect diffusional rate and therefore bioavailability using an *in vitro* model [12]. Active transport processes were absent from the simulation experiments, but certain parameters that showed an effect on diffusion rate in the simulation can translate *in vivo*. For example, the bioavailability of rituximab was increased in mice when administered in a hypertonic buffer containing NaCl (54%) as compared to an isotonic buffer (29%), and was nearly 100% in osmolarity-matched buffers containing O-phospho-L-serine or mannitol. This finding was attributed to an increased fraction of dose trafficked *via* the lymphatics since hyper-osmolarity in the interstitium results in increased interstitial volume and lymphatic drainage [30]. A systematic understanding or classification of mAbs based on their likelihood of benefiting from formulation optimization approaches would be useful to focus early discovery and development efforts in the context of the multitude of factors that can impact bioavailability. These parameters should be components of any framework proposed for a predictive model [12,14].

There is a knowledge gap in the published literature regarding the relationship between bioavailability and the *fluid dynamics/biomechanics* within the SC interstitial space. Most published studies have focused on pain perception and injection site tolerability, which is governed by the injection volume, needle gauge, and flow rates [31,32]; or comparing bioavailability between a pre-filled syringe and an autoinjector device [33]. *Injection volume* can also potentially impact absorption rate and bioavailability. The volume of SC injections for commercial mAb products is typically 1–2 mL [34]; to achieve higher doses within this constrained volume, the formulation must have a high protein concentration (>100 mg/mL). Increasing the protein concentration in the formulation creates challenges with manufacturability due to high *viscosity* and limitations to shelf life stemming from an increased propensity towards aggregation or formation of subvisible particulates. The addition of recombinant human hyaluronidase PH20 (rHuPH20) as a SC dispersion enhancer has enabled SC dose volumes of 5 mL or larger in several commercial products [35,36]. rHuPH20 can be injected

prior to the active molecule or co-formulated with it. In either case, the temporary depolymerization of HA by hyaluronidase reduces the backpressure from the injection. This allows greater dispersion through the interstitial space, thereby increasing exposure to lymphatics and absorption [37]. The addition of rHuPH20 has been demonstrated to result in equivalent or increased AUC and C_{max} and reductions in the time to reach maximum concentration (T_{max}) compared with SC delivery without rHuPH20 for biologic drugs [35,38]. For example, co-administration of insulin analogs with rHuPH20 has been shown to reduce individual PK variability and result in more rapid onset and shorter duration of insulin action [39,40].

Fluid injected into the SC space may lead to an increase in interstitial pressure, with higher injection volumes presumably having the greatest impact. Increased interstitial fluid pressure above that in lymphatic capillaries may cause the intercellular junctions in the lymphatic capillaries to open up, resulting in an increase in lymph flow [41]. Illustratively, there is a substantial increase in interstitial pressure in the footpad and dorsal aspect of the rat foot as a result of an injection, due to their relative lack of adipose tissue. Conversely, there are minimal changes in pressure upon injection in the flank region where tissue is loose, and therefore less liposomal uptake into the lymphatics [41]. Recent draft guidance from the FDA underscores the importance of factors such as needle insertion depth and rate of infusion in influencing bioavailability [42]. Incorporation of the effects of fluid dynamics on absorption mechanisms should be considered within the context of species-specific SC physiology when constructing a model for bioavailability.

2.1. Current in vitro and in silico approaches to evaluating the bioavailability of mAbs

Predictive models are generally *in vivo*, *in vitro*, *in silico*, or a combination thereof. There are currently no validated biorelevant *in vitro* models for predicting SC bioavailability and *in silico* models are currently unable to predict SC bioavailability bottom-up.

One such *in vitro* instrument is the Scissor model, which claims to mimic solubility, supersaturation, aggregation, diffusion, and pH within the SC space. Through the diffusion of mAbs from the Scissor system injection cartridge into a large volume physiological buffer, the model emulates mAb passive diffusion from the injection site into the systemic circulation [12,43]. In this model, parameters such as protein charge at neutral pH, pI, viscosity, and mAb concentration were found to influence mAb movement. While the Scissor system is not intended to reproduce the entirety or complexity of events that occur at the SC injection site, the data generated provided a reasonable prediction of human percent bioavailability for the test set of eight mAbs [12].

An excellent review by Kagan [44] provides a comprehensive overview of the various *in silico* PK prediction models that have been published and their evolution, up to the time of publication in 2014. These models generally fall into two categories: empirical models developed from studies measuring plasma/serum concentration, and mechanistic models which simultaneously describe the plasma PK profile and drug quantity in the lymphatic system. These models seek to predict absorption components in order to determine the PK profile in preclinical species and humans (*i.e.*, T_{max}, C_{max}, tissue/organ distribution concentration, AUC, *etc.*). SC absorption of therapeutic proteins is regulated by several factors which can be categorized into species, molecular, and formulation aspects. Mechanistic models have evolved to include both paracellular and lymphatic absorption components, target-mediated (or receptor-mediated drug disposition) binding of mAb to FcRn, and the role of this interaction in, among others, protecting mAbs from degradation and extending biological half-life.

Physiologically based biopharmaceutics models and physiologically based PK (PBPK) models are being applied mechanistically to determine whether variability or low SC bioavailability are dependent on molecular properties that may be influenced extrinsically *via* a formulation approach [44,45]. Bottom-up, mechanistic *in silico* models are in development by two leading commercial absorption software providers, Simulations Plus [46] and Simcyp [45]. In the Simcyp model, bioavailability must be empirically derived (*i.e.*, entered as user input), with the only drug-specific parameter being hydrodynamic radius. The model shows no apparent correlation between the accuracy of T_{max} predictions and the pI of therapeutic proteins. Although the data set used was limited, these findings suggest that factors in addition to pI and radius are likely implicated. A model specific to mAbs developed by Simulations Plus [46] is based on a hybrid PBPK mathematical model published by the FDA [47] that couples the physiologically based absorption process with a conventional compartment PK model. In this model, the subcutaneously administered mAb is first distributed in the interstitial space of the local SC tissue, which has three compartments (vascular, endosomal, and interstitial spaces) and includes a further three endosomal sub-compartments, with different pH values, coupled to a full PBPK model. The main mechanisms of absorption into systemic circulation are convective transport through the lymphatic endothelium and fluid-phase endocytosis. Specific to mAbs, the model simulates uptake into the endosomal space *via* fluid-phase endocytosis where unbound mAbs may be degraded. The protective effect of FcRn binding is factored in through the inclusion of terms such as pH-dependent binding of mAb to FcRn and competition between therapeutic mAb and endogenous IgG [46]. Additionally, loss mechanisms (*e.g.*, local proteolysis or clearance in lymphatics by dendritic cells or macrophages) must be accounted for through user input of a linear clearance component.

Of the mathematic models used to describe the PK of SC biologics, the simplest is a single pathway model with a first-order absorption rate constant (k_a) and a bioavailability term (F) [44], with or without Michaelis-Menten kinetics to describe saturable absorption [48,49]. The addition of a single transit compartment to explain a delay in absorption has been successfully employed [50]. Dose dependent absorption is accounted for by empirically varying k_a [51] or target-mediated disposition and saturable injection site-specific metabolism [50], although none is mechanistically supported. Several variations of empirical dual pathway models have been described, incorporating two parallel absorption processes, typically a zero-order process describing blood capillary absorption and a first-order process following an initial lag to represent lymphatic absorption. Single transit compartments can optionally be added to either of the pathways, if required, to fit further delays in absorption [44]. Mechanistic dual pathway models simulating the contribution of lymphatic absorption, parameterized from studies in lymph-cannulated animals, have been applied to various peptides/proteins [15,52–54]. Second generation models additionally account for the redistribution of systemically available protein to the lymphatic system, which has been shown to contribute significantly, potentially leading to overestimation of the contribution of lymphatic absorption in first generation models, a mechanism likely to be even more significant for larger proteins such as mAbs [44].

Due to their unique properties, additional factors must be considered in mechanistic mathematical modelling of SC absorption of mAbs. In addition to factors common to other molecules, binding to FcRn is potentially crucial, as it can protect against degradation at the injection site and provide an additional uptake mechanism in the form of FcRn-mediated transcytosis from the interstitium to the blood. Zhao et al. [47] were first to publish a PBPK approach to characterize mAb absorption post-SC administration; a different approach incorporating FcRn binding was later taken by Haraya et al. [55]. Kagan and Mager [56] captured dose dependent absorption of rituximab using a model incorporating saturable FcRn binding within three competing processes at the absorption site (degradation of free drug, absorption of free drug presumably through the lymphatics, and absorption of bound rituximab, presumably through FcRn-mediated transcytosis). A similar model using Michaelis-Menten kinetics in place of receptor mediated absorption mechanisms was used successfully by Dahlberg et al. [57]. In a more recent development, Varkhede et al. developed a minimal

PBPK model (*i.e.* a substantially lumped PBPK model) to test the hypothesis that interstitial proteolysis in lymph (represented as lymphatic trunk-lymph node clearance) is at least partially responsible for incomplete bioavailability of subcutaneously administered mAbs [27]. The model utilized physiological parameters related to the SC injection site (including FcRn binding and transfer) and the lymphatic system (lymph flows and compartment volumes) while the remainder of the body organs were represented using a two-compartment model. Sensitivity analysis indicated that the initial lymphatics are potentially rate determining for absorption of mAbs *via* the SC route and supported the conclusion that *in vitro* lymphatic proteolysis data may be used as model input data to enable bottom-up prediction of bioavailability.

Application of population PK models for SC absorption of mAbs and Fc fusion proteins was reviewed by Kagan [44] and revealed some interesting insights. First-order kinetics were selected to describe absorption in each of the 24 cases reported. Absorption was generally slow (k_a: 0.12–1.2/day), often with high intersubject variability. Covariate analysis for the absorption parameter was rarely reported, but in 5 of 24 cases, age was negatively correlated with absorption rate, which was postulated to result from a decrease in lymph flow with age. Only 13 of the 24 cases reported population mean bioavailability, with values ranging from 7 to 74% (mean 55%). Covariate analysis around the bioavailability parameter was generally not performed.

A major advantage of mechanistic models is that they can provide insights into the factors influencing absorption, which is essential for accurate assessments of bioavailability, optimization of delivery, and absorption prediction in humans, including the effects of population covariance. This was nicely illustrated in a publication by Gill et al. [45], describing a bottom-up whole-body physiologically based PK model used to predict absorption and PK profile of 12 therapeutic proteins (molecular weights 8–150 kDa). The model was able to predict plasma concentration profiles that were comparable to observed PK profiles, with T_{max} within 3-fold of observed values. A third of the predictions were within 0.8–1.25-fold of the observed values. There was no correlation found between the prediction accuracy of T_{max} and the therapeutic protein pI or molecular size. Approximately half of the therapeutic protein C_{max} simulated values were within 0.8–1.25 fold of the observed values. The authors rightly noted that this was unsurprising as C_{max} is not only dependent on absorption, but also on bioavailability, which was entered as a user input and not predicted.

Other insights can be obtained by conducting sensitivity analyses where the model input for a specific variable is changed from 0.1 to 10-fold while the other parameters remain constant, enabling assessment of the variable's impact on PK outputs, absorption, or bioavailability. Table 1 summarizes the key findings of a sensitivity analysis conducted from multiple publications. Caution should be exercised in interpreting the table as not all the sensitivity analysis results reported in the table may apply to all therapeutic proteins.

Based on our review of the available models, while there are promising advances in modelling capability, particularly in relation to rate of absorption, none are yet capable of mechanistic bottom-up bioavailability prediction.

2.2. Potential directions for models moving forward

SC modelling is acknowledged as complex due to multiple, interrelated nonlinear pathways and poses a unique challenge to the biopharmaceutics modelling community. A concerted effort must be made to control known variables in experimental study design and improve collection of metadata to facilitate the development and validation of models. There is a paucity of data around some of the key factors known to influence SC absorption in preclinical species and humans, and a determined research effort to address these gaps is required. Examples, by no means exhaustive, are discussed below. Highlighted gaps are based on recent review publications and authors' experience:

- Precipitation is often postulated as a mechanism for apparent saturable absorption of proteins with increasing dose levels. Further characterization of precipitation risk and redissolution rate, tissue response, and immunogenicity of precipitate or insoluble aggregates is needed; enhancements in simulated *in vitro* models may be helpful for such characterization.
- Regarding temporal spread of injected formulation (including impact of injection force, volume, vehicle/active pharmaceutical ingredient properties) and impact on absorption rate and tissue response, imaging tends to be of *ex vivo* samples and the link to PK is yet to be established.
- Development of techniques to characterize diffusivity/convective flow through biorelevant ECM and impact of size/pI could add value and is underway at various academic institutions, including the Swedish Drug Delivery Forum at Uppsala University. Reddy et al. [16] describe an *in vivo* model for quantifying interstitial convective transport of injected macromolecules.
- Development of assays to determine FcRn binding for relevant mAbs or other binding in relevant species (and corresponding relative expression levels in those species), and *in vitro* measurement of FcRn-dependent transcytosis [59].
- Susceptibility to first pass catabolism, including degradation at the injection site or lymphatic elimination (proteolysis, incubations in lymph fluid).
- Characterization of injection site physiology: consensus views on volumes and fluid fluxes (interstitial fluid, blood, and lymph), composition, exchange surfaces, and distances at common sites in key species. Imaging of lymphatic duct density (*e.g.*, by lymphoscintigraphy) may be valuable [14].
- Characterization of target expression levels/target-binding affinity in SC tissues.
- Approaches for interspecies scaling of the above parameters (*e.g.*, allometric exponents for body weight-based scaling of skin blood flow and lymph flow in the thoracic duct have been reported) [44].
- Systematic studies on the impact of formulation and device or syringe factors and, by inference, development of strategies to enhance bioavailability or tailor exposure profile (*e.g.*, block charge-based interactions with ECM, enhancing transport in SC space). Furthermore, understanding how injection flow rates due to volume, speed of injection, needle dimensions, *etc.* when combined with the SC physiology influence the local maximum concentration that in turn may impact precipitation, bind saturation, *etc.*
- Advances in experimental design to include more measurements (*e.g.*, fraction remaining at injection site) than just systemic PK for deconvolution of absorption processes, in addition to sufficient PK sampling to adequately characterize the absorption phase. Akin to the utilization of bile duct cannulation for the identification of limitations in oral bioavailability (deconvolutes absorption issues from first pass hepatic clearance or gut wall metabolism), lymph cannulation models should be more frequently leveraged to understand absorption path/kinetics in SC bioavailability.
- The mechanistic basis for impact of pathology *e.g.* obesity (adiposity, SC blood flow), diabetes (SC perfusion).

Successful predictive models of the future will likely combine multiple *in vitro* characterizations (with minimal *in vivo* experimentation) to predict rate and extent of SC absorption of a therapeutic protein. Over time, it would be beneficial to develop smarter processes (through refinement of the characterization process) to focus on the aspects most likely to be rate-limiting for a therapeutic protein or drug class.

3. Opportunities

It is clear from the foregoing discussion that SC administration of biologics is complex, and our understanding is incomplete. Yet,

Table 1
Review of a mechanistic modelling sensitivity analysis of various inputs and a corresponding impact on absorption and bioavailability predictions.

Model parameter	Molecule	Output parameter evaluated	Sensitivity analysis conclusion	Reference
MW	Various	% absorbed *via* lymphatic mechanism	S shaped curve observed showing that as MW increased >5 kDa, the % absorbed *via* lymphatic mechanism increased. Correlated with *in vivo* animal data	[45]
Hydrodynamic radius	Various	Ci relative to Cp	Prediction correlated with animal data showing Ci/Cp decreased with increase in hydrodynamic radius according to theory	[45]
Lymphatic flow rate, elimination rate during lymphatic transport	Omalizumab	T_{max}	T_{max} is predicted to be highly sensitive to lymphatic flow rate and not sensitive to the other physiological parameters evaluated, including elimination rate during lymphatic transport	[47]
Various physiological parameters (*e.g.*, lymphatic flow, transit time of drug from lymph system, endosomal uptake)	Omalizumab	SC bioavailability	$K_{lymph} = \tau >$ lymphatic flow rate $> >$ endosomal uptake rate of antibody = FcRN concentration = endosomal return rate of mAb. Bioavailability will increase as K_{lymph} or τ decreases, or when lymphatic flow rate increases at its low range.	[47]
pI	Various	C_{max} and T_{max}	No correlation with pI. This could be due to limited range of pI for therapeutic proteins investigated (5.2–8.8, except for one with pI 11.2)	[45]
Lymphatic recirculation	Trastuzumab	Bioavailability	Simulations using the model indicated that on average each trastuzumab molecule recirculated 4–5 times through the lymphatic systems before being eliminated and explained the overestimation of SC bioavailability relative to IV	[57]
FcRn expression and binding affinity	Rituximab	Bioavailability	10-fold difference in binding affinity or the receptor expression level had a predicted significant effect on bioavailability *e.g.* reduction in bioavailability from 69% to ~20% when binding affinity is reduced 10-fold (*i.e.*, K_D is increased from 1 to 10)	[44,58]

Ci, concentration in lymphatics; C_{max}, maximal concentration; Cp, concentration in plasma; FcRn, neonatal fetal Fc receptor; IV, intravenous; K_D, equilibrium dissociation constant; K_{lymph}, drug elimination rate during lymphatic transport; MW, molecular weight; pI, isoelectric point; SC, subcutaneous; τ, transit time for drug from lymph system to systemic circulation; T_{max}, time to reach maximum concentration.

smaller focused efforts published in the literature have shown some success in correlating SC bioavailability with various factors. This research may be at a tipping point and, with the assistance of an organized framework to manage the data, it could accelerate the transition. A very successful framework that could be used as a model is the Biopharmaceutics Classification System (BCS) for orally delivered small molecules. The theory behind the classification system was first published by Amidon et al. in 1995 [60], with further expansions by Yu et al. in 2002 [61] and Rosenberger et al. in 2018 [62]. In all its iterations, it has generally remained the same four quadrant model with either high or low solubility and high or low intestinal permeability. The most recent version, called the Developability Classification System (DCS), seeks to bridge the gap between the original system, which was designed to guide regulatory decisions, and the need for early evaluation of the suitability of drug candidates for oral delivery [62]. For example, it assessed the compensatory nature of permeability and solubility during oral absorption and provided a way to estimate the dose at which solubility becomes rate-limiting to absorption. A refined DCS also evaluated the formulation effort needed to overcome either poor solubility or dissolution rate-limited absorption, with more intense efforts likely to require enabling formulations for the solubility-limited molecules [62].

Another variation that incorporated metabolic aspects was the Biopharmaceutics Drug Disposition Classification System (BDDCS) introduced by Wu and Benet in 2005 [63] and further refined by Benet et al. in 2011 [64]. The basis for this work was a very strong correlation between the intestinal permeability rate and the extent of metabolism. A simple framework like the BCS, translated to SC bioavailability of biologics, could be a catalyst to guide early drug discovery and developability scientists to select better biologics to help patients in need and could also inform appropriate formulation selection prior to clinical entry. The ideal scenario would be an efficient and simple tool that could be implemented on a variety of compounds as they are being developed. An *in silico*, *in vitro*, or a mixed approach would fit that criteria.

The concept of an open data library to enable researchers to focus on building models rather than finding the raw data would be extremely beneficial. PharmaCircle™ has a repository of SC bioavailability data for 124 pharmaceutical compounds, 87% of which are biologics [1]. Data for all the mAbs were organized and plotted against the various parameters available in the repository; however, none clearly showed a correlation or trend with SC bioavailability. Some of the parameters commonly stated to correlate with bioavailability, such as pI, did not show clear trends (Fig. 4).

Given this finding that general trends, such as pI *versus* bioavailability, are difficult to validate across large data sets, a different approach could be pursued. An engineering approach similar to the original BCS where the focus is on the rate-limiting steps to arrival in systemic circulation may produce a better framework. Using this approach, there are two major events to focus on: (1) molecular transport encompassing the movement of the mAb from the site of injection to the systemic circulation and (2) the extent of catabolism that occurs during that transit.

There are several factors to consider when focusing on movement of biologics from the SC space into the systemic system, including formulation viscosity, physical stability, charge, diffusivity, lymphatic transport, and convection. As described in Section 2, these parameters have been shown to play a role in molecular transport through the interstitial space in different studies with different methodologies [12,16–19]. It is unclear at this point how to weigh each factor or what the key rate-limiting step is during this molecular transport event. Although it is known that the transport pathway is typically *via* the lymphatics if the molecular weight is greater than 20 kDa, as is the case for mAbs, a common tool to determine extent of catabolism/degradation does not exist. Predictive tools could be developed to determine catabolism at the injection site and in the lymphatic system. While the true rate-limiting factors are yet to be determined, we could use the broad categories of molecular transport and catabolism extent to create a simple classification as shown in Fig. 5.

By means of the classification system concept presented in Fig. 5, one may be able to develop a unique model for each class of mAbs, with more predictive power as an ensemble of models than a single model. The purpose of sharing this concept is to stimulate researchers' thought process on the most relevant rate-limiting steps, the additional data that may be needed to successfully develop a new classification system (*e.g.*,

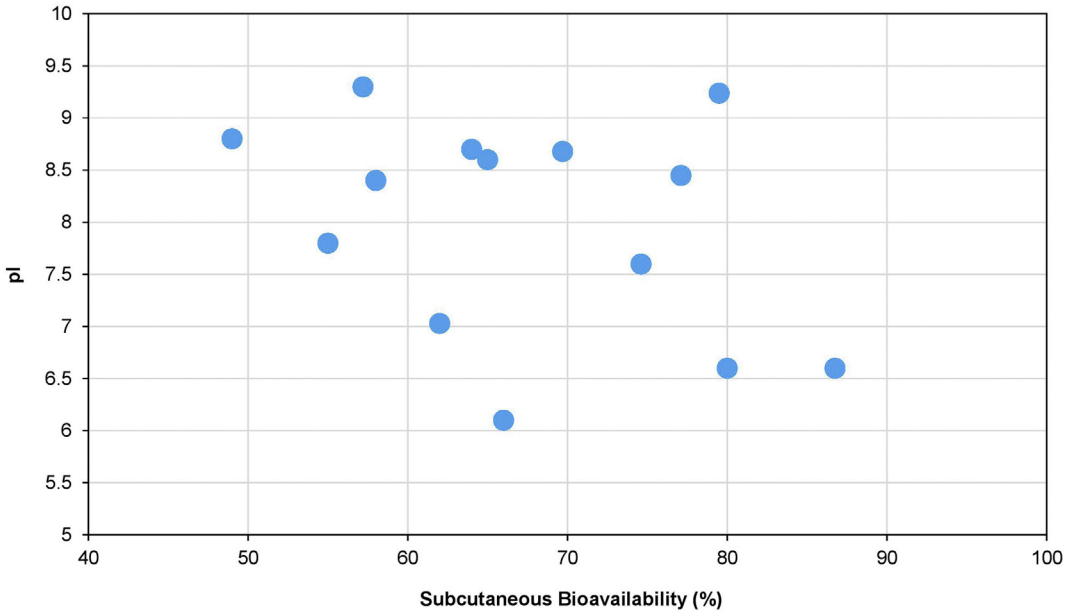

Fig. 4. Plot of isoelectric point (pI) *versus* subcutaneous bioavailability of mAbs [1]*. *Figure is limited to the 14 mAbs for which the pI information is publicly available.

lymph volume, injection site depot shape, *etc.*), and the data standards or procedural guidelines required to ensure the comparability of data across research laboratories. There is also a need to leverage advanced techniques to extract key patterns in data that may not be apparent at first glance. Techniques like principle component analysis and technologies such as artificial intelligence may reveal key factors requiring further study and facilitate better differentiation of mAbs into approach classes.

4. Conclusion and open innovation challenge

Given the trends in therapeutic area approvals and a growth in biologics portfolio, SC delivery of mAbs is anticipated to see continued growth. SC administration of mAbs is expected to improve access and compliance for patients while being cost effective for the caregiver and the healthcare system. Improving the capabilities to better predict bioavailability would allow the discovery and development of mAbs intended for SC administration to be more efficient, thus getting medicines to patients faster.

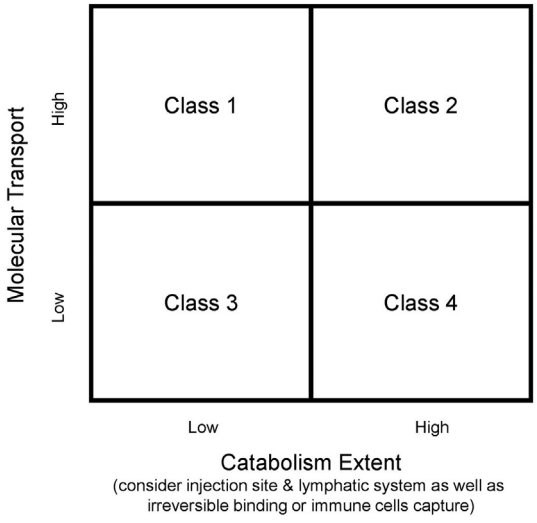

Fig. 5. Classification system concept for mAbs: molecular transport *versus* catabolism extent.

The prior sections of this manuscript discussed approaches currently in use to assess bioavailability of mAbs, challenges with regards to predictability of available *in silico*, *in vitro*, and *in vivo* models, future directions and opportunities with such models through fundamental understanding of rate-limiting variables to absorption and bioavailability, and finally a need for a classification framework to streamline discovery and development candidate advancement. Collectively, this highlights an unaddressed scientific need. To address this need, both fundamental/mechanistic and empirical research needs to be pursued with systematic data collection across a broad range of mAbs with varying properties. Furthermore, enabling a forum/network to allow data sharing and ideation can profoundly accelerate the pace of research in this space. The authors and members of the SC Consortium challenge scientists in academia and industry to address the following pre-competitive needs and use the Consortium as a platform to share information with the similarly interested members of the scientific community:

1. *In silico*, *in vitro*, and simulation models that enable the prediction of SC bioavailability of therapeutic large molecules in humans and improve translation from preclinical species.
   - *In silico*, *in vitro*, and simulation models that enable scientists to establish whether SC bioavailability can be impacted *via* formulation approaches, akin to the SC biopharmaceutics classification described in Section 3.
   - Fundamental characterization-driven exploration of new hypotheses that may help identification of attributes/variables that advance the prediction of SC bioavailability.
   - Imaging technologies and devices that enable scientists to explore the impact of lymphatic flow, immune response, formulation, *in vivo* precipitation risk, and other parameters that impact both SC bioavailability and absorption.

2. Preclinical *in vivo* models that enable the prediction of SC bioavailability of therapeutic large molecules in humans.
   - *In vivo* models that simulate the primary rate-limiting variables for SC bioavailability such as local catabolism and subcutis structure/transport.
   - Models that allow clinically relevant dose and dose volumes.

3. Provide the authors with missing data in the Excel document described below and share PK human and preclinical animal SC and IV raw data on mAbs in a format that can be used by the community to conduct batch analysis or detailed studies.
   - To encourage these investigations, the authors have complied three sets of data from the literature and public domain that the scientific community may wish to use to validate their hypotheses:
     i. Table 2, which captures SC bioavailability data in human and corresponding preclinical species for a range of marketed mAbs.
     ii. Excel document (Supplementary Data in Appendix A) containing information on 26 marketed mAbs, including molecular formula, molecular weight, Chemical Abstracts Service registry number, antibody class, lowest and highest SC bioavailability, water solubility, clearance, elimination half-life, and volume of distribution.
     iii. The authors encourage others to establish if *in silico* or *in vitro* bioavailability input data can be used to accurately predict SC bioavailability for the 12 therapeutic proteins described in the publication by Gill et al. [45]. This publication contains useful references to human PK data, SC physiological inputs, and other information that could be a useful starting point or comparison for future models. An Excel document containing the PK data is supplied by the authors (Supplementary Data in Appendix B). We encourage model generation using the development data sets and model validation using the validation data sets included.

The Consortium, when possible, will assist the community in collecting data through controlled and well-designed experiments that generate meaningful data towards building the SC classification system. During the 2 years from the time of publication of this article, the Consortium will assist, where possible, by:

1. On a case-by-case basis, helping to provide mAbs from member companies for investigations relevant to the objectives of this publication.
2. On a case-by-case basis, providing letters of support for government research grants.
3. Connecting researchers to other collaborators with complementary interests and capabilities that may be of mutual benefit.
4. Compiling any research findings on the challenge set and developing a publication after the 2 years to provide an update on advances.

Disclosures

Manuel Sánchez-Félix is an employee and stockholder of Novartis and a stockholder of Eli Lilly and Company.

Matt Burke is an employee and stockholder of Radius Health, Inc.

Hunter Chen is an employee and stockholder of Regeneron Pharmaceuticals, Inc.

Claire Patterson is an employee of Seda Pharmaceutical Development Services, Ltd. and was formerly employed by AstraZeneca.

Table 2
SC bioavailability in human and corresponding preclinical species data for a range of marketed mAb and Fc-fusion proteins.

Molecule	Tradename	MW (kDa)	SC bioavailability	References	Other information
Adalimumab	Humira®	148	Human: 52–82% (64%) Monkey: 94–100% (96%)	[65–67]	Human PK study: [68,69]
Alirocumab	Praluent®	146	Human: 85% Monkey: 73–77% Rat: 44–97%	[2,70]	T_{max}: Human: 3–7 days Monkey: 3–4 days Rat: 2–3 days
Canakinumab	Ilaris®	145	Human: 63–67% Monkey: 60%	[67]	IV bolus mice, rat, and cynomolgus monkey PK data: [71] Human PK data: [72]
Certolizumab pegol	Cimzia®	91	Human: 76–88% Rat: 24–34%	[67]	Fab conjugated to 40 kDa PEG [73]
Etanercept	Enbrel®	150	Human: 76% Monkey: 73% Mice: 58%	[67]	Fusion protein with IgG1 Fc
Golimumab	Simponi®	150	Human: 53% Monkey: 77%	[67,69]	Study in humans evaluating impact of SC injection site on bioavailability (includes IV data): [74]
Omalizumab	Xolair®	149	Human: 53–71% (62%) Monkey: 64–104% (84%) Mice: 90%	[2,65,67,75]	
Bevacizumab	Avastin®	149	Monkey: 98% Rat: 69% Mice: >100%	[76,77]	
Rilonacept	Arcalyst®	251	Human: 43% Monkey: 70% Rat: 60% Mice: 78%	[67]	Fusion protein with IgG1 Fc [78]
Rituximab	Mabthera®	145	Human: 71% Minipig: 71% Mice: 63%	[2,79]	T_{max}: Human: 3 days Minipig: 1 day Mice: 2 h
Sarilumab	Kevzara®	150	Human: 80% Monkey: 78%	[2,80]	T_{max}: Human: 2–4 days Monkey: 2–5 days
Trastuzumab	Herceptin®	148	Human: 82% Minipig: 82% Mice: 83%	[2,81]	T_{max}: Human: 4 days Minipig: 1 day Mice: 7 h

IV, intravenous; Fab, fragment antigen-binding; Fc, fragment crystallizable; MW, molecular weight; PEG, polyethylene glycol; PK, pharmacokinetic; SC, subcutaneous; T_{max}, time to reach maximum concentration.

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Appendix A. Supplementary data

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