

Monoclonal antibody and protein therapeutic formulations for subcutaneous delivery: high-concentration, low-volume vs. low-concentration, high-volume

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ABSTRACT

Biologic drugs are used to treat a variety of cancers and chronic diseases. While most of these treatments are administered intravenously by trained healthcare professionals, a noticeable trend has emerged favoring subcutaneous (SC) administration. SC administration of biologics poses several challenges. Biologic drugs often require higher doses for optimal efficacy, surpassing the low volume capacity of traditional SC delivery methods like autoinjectors. Consequently, high concentrations of active ingredients are needed, creating time-consuming formulation obstacles. Alternatives to traditional SC delivery systems are therefore needed to support higher-volume biologic formulations and to reduce development time and other risks associated with high-concentration biologic formulations. Here, we outline key considerations for SC biologic drug formulations and delivery and explore a paradigm shift: the flexibility afforded by low-to-moderate-concentration drugs in high-volume formulations as an alternative to the traditionally difficult approach of high-concentration, low-volume SC formulation delivery.

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Introduction

Biologics are a rapidly growing class of innovative treatments for many serious diseases, including cancer, which is often treated in the clinic setting, and autoimmune diseases, where the site of care varies by the disease state. Biologic drugs are large, structurally complex therapeutic proteins that are usually produced in living cells and are highly sensitive to manufacturing processes. They are fundamentally different from small molecule drugs, which are chemically defined entities that can often be delivered orally in a tablet formulation. The molecular weight of a small molecule drug is typically less than 1 kDa (20–100 atoms), whereas the molecular weight of a biologic may exceed 1000 kDa (e.g., IgM antibodies).¹ Biologics are typically available as liquid formulations for intravenous (IV) infusion or subcutaneous (SC) injection. Unlike small molecule drugs, which are often stable after ingestion, biologic drugs are not generally suitable for oral administration because they are digested and deactivated before absorption into the blood stream.

The biologic drugs category includes recombinant proteins, enzymes, cytokines, and monoclonal antibodies (mAbs).² Antibodies and antibody derivatives are available in multiple formats, including canonical (full-length) antibodies, fragments (e.g., Fc, Fab, single-chain variable fragment [scFv], variable regions of camelid heavy-chain-only antibodies [VHH] fused with a human Fc fragment or another VHH), antibody-drug conjugates (ADC; e.g., mAb conjugate with cytotoxic small molecule drug payloads), bispecifics (e.g., heterodimeric bispecific antibody, scFv-

scFv bispecific antibody), and other formats (e.g., antibody-conjugate immunotoxins or immunocytokines, radiolabeled canonical antibodies or Fab fragments, Fc-fusion proteins).³ Some common targets for approved mAb products include cluster of differentiation (CD) 20, 38, and 152 (e.g., rituximab, isatuximab, ipilimumab); human epidermal growth factor receptor 2 (HER2) (e.g., pertuzumab, trastuzumab); programmed cell death protein 1 (PD-1) (e.g., pembrolizumab, nivolumab); and complement inhibitors C5 (e.g., eculizumab, ravulizumab).³ The annual number of biologic drug approvals was equivalent to the number of small molecule drug approvals for the first time in 2022; the major contributor to this biologic drug growth is the evolution beyond simple mAb formulations to newer modalities.⁴

Unlike small molecule drugs, administering effective doses of mAbs orally can be extremely challenging due to their physical instability, low permeability across the gastrointestinal membrane, and vulnerability to enzymatic activity.^{5,6} Most biologic products have traditionally been formulated at low concentrations (<30 mg/mL) convenient for IV infusion to maximize biologic stability,^{7,8} lower manufacturing costs during development by decreasing the amount of drug needed for the stability program, and reduce waste during clinical dose-escalation studies. Altogether, compared to high-concentration formulations, low-concentration biologic drug formulations are easier and less costly to develop and are more stable due to their lower concentrations.⁹ While IV infusion is appropriate for many biologics, SC administration is often preferable for cancer and chronic conditions such as metabolic

Table 1. Comparisons between IV and SC formulations.

Outcome	IV	SC
Bioavailability	100% bioavailability ¹¹	Less than 100% bioavailability ¹¹
Efficacy	Preferred for conditions that are acute or require a high C_{max} for therapeutic effect ¹²	Preferred for chronic conditions; suboptimal for conditions that require a high C_{max} for therapeutic effect ¹²
Safety	C_{max} -related adverse events are observed ¹²	SC dosing has demonstrated safety profile improvements with biologics ^{13,14} and fewer dosing errors due to fixed dosing with SC biologics. ¹⁵ Compared with IV dosing, SC administration decreases peak cytokine levels, which suggests a potential safety benefit for patients due to a subtler immune activation. ¹⁶
Formulation	Low concentration due to a high dose volume and high-volume capacity in the delivery method ^{5,7,8}	The common practice is to reformulate to a high-concentration formulation since a high dose volume is required and traditional combination products (PFS/autoinjector) have a low volume capacity. ¹⁷ This can be circumvented by using alternative SC delivery devices such as OBDS.
Drug container	Vial mixed into a fluid bag	PFS, autoinjector, or OBDS*
Time and motion	Set-up and administration take much longer and require healthcare provider oversight ^{18–20}	Substantial improvements in time and motion for treatments that have transitioned from IV to SC ^{19,20}
Patient preference	Chair time** Daratumumab: 238 minutes (3.97 hours) Rituximab: 180.9 minutes (3.02 hours) Trastuzumab: 75.5 minutes (1.26 hours) Not preferred by patients ^{21–23}	Chair time Daratumumab: 8.1 minutes (0.14 hours) Rituximab: 8.3 minutes (0.14 hours) Trastuzumab: 20.9 minutes (0.35 hours) Preferred by patients due to improved quality of life, self-administration, and a wider range of anatomical site options ^{21–23}
Site of care	Administered in a more costly site of care (hospital/infusion center/home infusion) ^{24,25}	Options in the site of care with potential for patient self-administration, which is the site of care with the lowest cost ^{24,25}
Provider preference	Not preferred by providers ^{26,27}	Preferred by providers ^{26,27} Improved patient adherence, feasible in patients with poor venous access, decreased burden on healthcare providers due to increased clinic throughput, and lower drug delivery-related costs and resource requirements ^{6,7,12,28,29}

*Volume capacity varies with autoinjectors having the lowest and OBDS the greatest capacity. **Lack of infusion chair capacity is a global challenge. Abbreviations: IV, intravenous; OBDS, on-body delivery system; PFS, prefilled syringe; SC, subcutaneous.

disorders and autoimmune diseases because it eliminates the need for costly visits to IV infusion centers. SC administration is an established, effective, and well-tolerated method of administering biologics, and is applicable across many therapeutic areas.¹⁰

As summarized in Table 1, SC administration has several benefits compared with IV administration. Some notable benefits of SC biologic administration include healthcare provider and patient preference, options for the site of care (in-clinic vs. self-administration), improvements in time and motion (infusion chair time reduction from hours to minutes), and in some cases, an improved safety profile, including decreased risk of infusion-related reactions.^{24,30} SC delivery offers substantial product differentiation when compared to IV alternatives. It is notable for allowing self-administration by patients, which removes the necessity for in-clinic billing.³¹ Payers particularly value features such as the option for treatments at home, the ease of use, the lack of additional charges like those associated with SC syringe pumps, and the inclusion of hidden needles.³¹ These aspects play a pivotal role in the decision-making process regarding the coverage for SC delivery devices. These benefits may also help to improve patient adherence.

The misconception that high-concentration liquid formulations are required for SC biologic drug development stems from the incorrect belief that only 2–3 mL can be administered SC without a permeation enhancer.^{5,28,32,33} The low volume capacity of traditional drug delivery devices (1–2.25 mL for autoinjectors and ≤15 mL for prefilled syringes [PFS] co-formulated with a permeation enhancer such as hyaluronidase) and the lower bioavailability of SC biologics contribute to this prevailing misconception. The push to create high-concentration formulations with strict volume limits is

costly and time-consuming because it requires careful formulation and testing to ensure that drugs are stable and effective at high concentrations at which protein-protein interactions (PPIs) are substantially increased. Creating a high-concentration biologic formulation, however, carries higher manufacturing costs and greater program risks. A parallel development of two formulation concentrations or a misstep in a formulation process can lead to delayed time to clinic, which leads to delayed time to market. Newer combination product-drug delivery devices such as on-body delivery systems (OBDSs) have higher volume limits (3.5–25 mL for a single device). These devices therefore offer new opportunities for SC biologic development as they allow for the use of low- to moderate-concentration, higher-volume formulations.

Here, we discuss key considerations for SC biologic formulations, including volume, concentration, viscosity, osmolality and tonicity, pH, and bioavailability; SC biologic product development considerations, including safety profile, development pathways, fixed and variable dosing, dosing intervals, and injection speed; and clinical considerations, including patient adherence and device preference. We then provide a thorough overview of the challenges associated with high-concentration (≥ 100 mg/mL) biologic formulations, including physical instability and immunogenicity, and the opportunities associated with higher-volume SC biologic development and higher-volume delivery methods as an alternative to traditional high-concentration, low-volume formulations. In the context of the challenges associated with high-concentration formulations, this review provides new insights about the potential value of high-volume SC biologics.

Subcutaneous formulation considerations

In this section, we review six key variables in drug formulation: volume, concentration, viscosity, osmolality and tonicity, pH, and bioavailability. These variables are interrelated: changes in one can affect the others. For example, higher concentration can lead to higher viscosity, which can make drug formulations more unstable and difficult to deliver. This challenge becomes even more conspicuous when considering the lower SC bioavailability typically observed with protein therapeutics. Higher doses have been used to compensate for reduced bioavailability, but this strategy is curtailed by the limited volume capacity of traditional delivery devices like autoinjectors and PFSs. An SC delivery method with greater volume capacity such as an OBDS may allow for an extended dosing interval since more drug product can be administered with each injection without requiring increased concentration.

Volume

SC formulations must provide a therapeutic protein dose that achieves efficacy equal to that of IV formulations, and bioavailability may be reduced with SC administration compared with IV administration.¹² Therefore, a SC drug product must be delivered at a higher volume or concentration, with multiple injections, or more frequently than a corresponding IV formulation.⁶ It is important to note that, while bioavailability < 100% indicates that the drug was not entirely absorbed into systemic circulation, this does not necessarily suggest that the drug does not reach its intended target. Despite several commercial examples before the introduction of hyaluronidase, there is still a common misconception that high-volume SC delivery is not possible without the inclusion of a permeation enhancer. Details for high-volume SC formulations, including those with and without permeation enhancers like hyaluronidase, are shown in Table 2. The successful SC delivery of protein therapeutics without permeation enhancers (e.g.,

evolocumab) proves that high-volume SC delivery has long been achievable without the use of permeation enhancers. Recently, two biologic drugs, pegcetacoplan and ravulizumab, were approved for delivery via an OBDS (Table 2). Notably, these drugs are not co-formulated with hyaluronidase.

Unlike IV infusion, SC injection volume with traditional devices such as autoinjectors is typically limited to 2.25 mL to reduce pain and drug leakage due to tissue backpressure.^{5,28,32} Because of this volume limitation, the use of traditional SC devices requires that formulations be highly concentrated or administered with multiple injections or at shorter dosing intervals. Device modalities for high-volume SC delivery are shown in Figure 1. OBDSs function as hybrids between SC syringe-driving pumps and autoinjectors because they have the volume capacity of the former and the ease of use of the latter. OBDSs are therefore a viable option for the administration of higher-dose formulations and avoid the necessity of high-concentration formulations or decreased dosing intervals due to volume limitations.

Concentration

Volume limits on SC formulations present challenges for the SC administration of biologic drugs, which sometimes require a dose of 500–600 mg per patient or up to 10 mg/kg.⁷ IV infusions typically have a total delivered volume of 100–250 mL and a concentration of <30 mg/mL; therefore, achieving a therapeutic dose of biologics in one SC injection with a volume of <2 mL requires a concentration of ≥100 mg/mL.^{7,33} IV solutions and reconstituted lyophilized (freeze-dried) solutions for IV administration typically have a mAb concentration of about 10 mg/mL, while SC solutions and lyophilized formulations for SC administration typically have a mAb concentration of <100 mg/mL.³⁴ Increasing the concentration of SC formulations would seem to solve this difficulty, but this approach has several significant challenges with regard to solubility, stability,

Table 2. High-volume mAb formulations for SC delivery.

Company	Brand Name	Drug Name	Approval Year	Delivery Method	Needle Gauge	Volume and Time	Concentration
CSL Behring	HIZENTRA®	Immune globulin infusion 20% (human)	2010	SC syringe pump	24 or lower	Volumes vary by disease and weight and may be up to 100 mL	200 mg/mL
CSL Behring	HYQVIA®	Immune globulin infusion 10% (human) with hyaluronidase	2014	Peristaltic pump or syringe pump	24	1–2 mL per minute for up to 3 hours (median 2 hours)	100 mg/mL
Amgen	Repatha®	Evolocumab	2016	OBDS	29	3.5 mL over 5 mins	120 mg/mL
Genentech	RITUXAN HYCELA®	Rituximab/hyaluronidase	2017	Syringe	25	11.7 mL over ~5 mins; 13.4 mL over ~7 mins	120 mg/mL
Genentech	Herceptin HYLECTA®	Trastuzumab/hyaluronidase	2019	Syringe	25	5 mL over 2–5 mins	120 mg/mL
Genentech	PHESGO®	Pertuzumab/trastuzumab/hyaluronidase	2020	Syringe	25	10 mL over 5 mins; 15 mL over 8 mins	120 mg/mL
Janssen	DARZALEX FASPRO®	Daratumumab/hyaluronidase	2020	Syringe	23	15 mL over 3–5 mins	120 mg/mL
Apellis	EMPAVELI®	Pegcetacoplan	2021	OBDS or SC syringe pump	29	20 mL over 20 mins	54 mg/mL
Alexion/ AstraZeneca	ULTOMIRIS® SC	Ravulizumab	2022	OBDS	29	7 mL over 10 mins	70 mg/mL
UCB	RYSTIGGO®	Rozanolixizumab	2023	SC syringe pump	26	3–6 mL – infusion pump over 9–18 minutes	140 mg/mL
Argenx	VYVGART HYTRULO®	Efgartigimod/hyaluronidase	2023	Syringe	25	5.6 mL over 1.5 minutes	180 mg/mL

Abbreviations: OBDS, on-body delivery system; PFS, prefilled syringe; SC, subcutaneous.

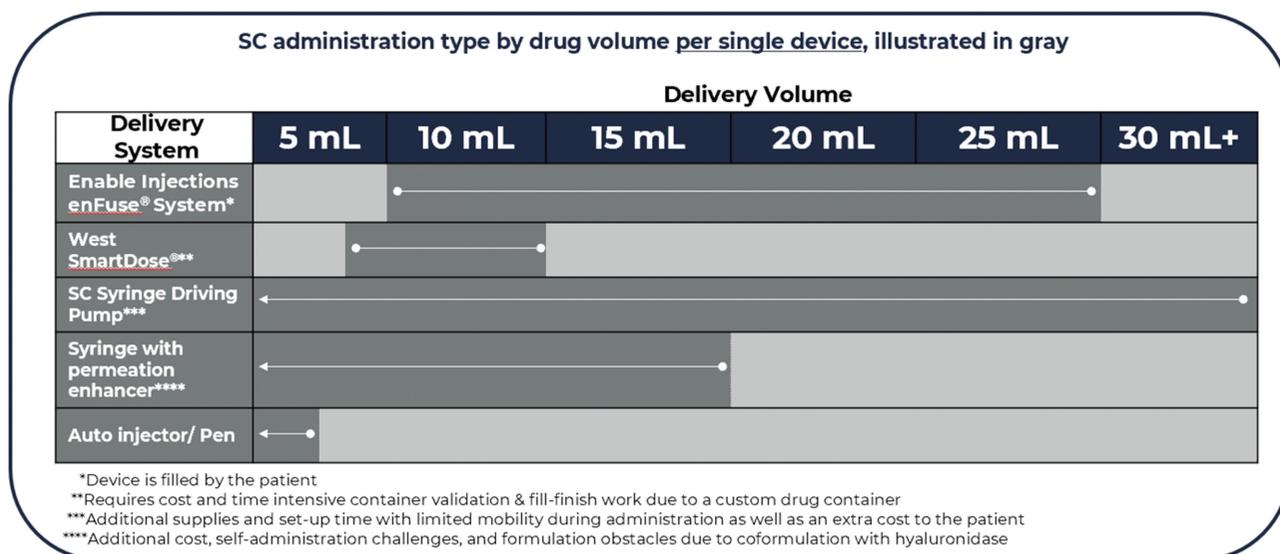


Figure 1. Drug volumes administered by SC delivery systems per single device.

viscosity, and manufacturability. Instability can lead to challenges during the validation, manufacturing, and delivery of a drug.³³ The increased viscosity of high-concentration formulations can pose additional challenges that are discussed in more detail below (Viscosity section). Increasing concentration can also increase the risk of aggregation, denaturation, and proteolysis. As the concentration of a protein increases, the molecules have a greater chance of coming in contact with each other and forming aggregates;³⁵ the proteins may unfold and lose their native structure, resulting in denatured proteins that are often inactive and may be harmful.

Solutions exist to change these behaviors of high-concentration biologic formulations, but there are trade-offs. Lyophilization may decrease aggregation risk and thereby improve the stability of high-concentration formulations, but can require fit-for-purpose lyophilization formulation and process development and long reconstitution times and potentially introduce more human error during the dose preparation and administration process. Currently approved excipients can only enhance biologic drug performance to a certain limit, and there are no excipients available that can be used with all mAbs.³⁶ Further optimization might require the development of novel excipients or the modification of existing ones. However, use of novel excipients introduces substantial risk into drug development, as there are then two new entities requiring regulatory approval. To achieve high drug doses for SC delivery, a low-risk and time-saving approach is to use traditional excipients within known limits while leveraging delivery methods with high volume capacities, such as OBDSs.

Viscosity

Preferred solution viscosity for SC injections, especially when using PFSs, is < 10 centipoise (cP) in order to minimize injection force (also called glide force).³⁷ This can be a challenge for

high-concentration solutions.¹⁷ For reference, at room temperature, water, milk, and corn oil have viscosities of 1, 2, and 30 cP, respectively. It has been reported that increasing mAb concentration can increase solution viscosity exponentially due to reversible antibody self-association mediated by Fab-Fab interactions.^{38,39} Viscosity affects stability, processing, and reconstitution time if a formulation is freeze-dried.³⁴ High viscosity can also adversely affect ultrafiltration diafiltration unit operation speed and/or system pressure and the accuracy and fill rate of the filling operation during manufacturing.⁴⁰ Spray-drying and spray freeze-drying mAb formulations and then reconstituting them in suspension solutions such as benzyl benzoate or ethyl lactate to form ultra-high-concentration formulations appears to increase viscosity beyond the tolerable range for SC injection and may increase high and low molecular weight impurities.⁴¹

High viscosity also affects ease of administration. The force required to expel a highly viscous solution may be too high for certain populations, such as older patients or patients with rheumatoid arthritis.³⁴ Nurses who frequently administer high-viscosity formulations such as those co-formulated with hyaluronidase (e.g., daratumumab/hyaluronidase) have reported musculoskeletal problems due to the strain needed to apply sufficient pressure to depress syringe plungers with substantial backpressure for several patients daily.⁴² Hands-free administration via an OBDS is a possible solution to this problem as it enables the administration of drugs with minimal preparation and at the click of a button.

Higher solution viscosity can also lead to increased leakage at the injection site and can increase patient pain by necessitating the use of needles with a larger diameter.^{43–45} Viscous medications require needles with smaller gauges (i.e., larger-diameter needles), and the pressure required to depress a syringe plunger increases in correlation with both needle length and syringe size.⁴⁶ Although gauge choice may be limited by drug viscosity, higher-gauged (i.e., thinner) needles appear to decrease patient pain.⁴⁷ Adjustable, spring-loaded devices may also be considered for the

delivery of high-viscosity formulations.⁴⁸ Additional evidence regarding the role of formulation viscosity on patient pain is required; it is difficult to determine the factors that affect injection site pain due to a scarcity of clinical data, the inconsistent use of blinded and unblinded study designs, the influence of patient characteristics such as personality and previous pain experience, and variations in data collection and reporting that complicate comparisons across studies and biologics.⁴⁷

High-volume, low-concentration formulations provide an obvious alternative to the viscosity challenges of high-concentration formulations given the relationship between concentration and viscosity. The lower viscosities possible with high-volume formulations furthermore allow for easier withdrawal of a medication from its vial, lower force required to administer, use of smaller-diameter needles, and decreased pain. There are also several excipients that can be added to high-concentration mAb formulations to decrease their viscosity, including arginine, carnitine HCl, glycine, lysine, ornithine HCl, proline, and sodium chloride.^{40,49} Arginine is commonly included to decrease viscosity, inhibit aggregation, and act as a stabilizer during product storage and transportation.³⁴ The formation of nanoclusters or microparticles to limit the impact of viscosity on interparticle interactions by electrospraying or microglassification, or lyophilization of a mAb formulation with trehalose included as a crowding agent and reconstitution in a buffer close to the formulation's isoelectric point at a volume lower than pre-lyophilization,^{50,51} are also being explored. However, electrospraying and microglassification may necessitate long development times and the use of non-aqueous solvents for administration.⁴⁰ Additionally, there are device strategies designed to facilitate the injection of higher-viscosity solutions, such as tapered needles,⁵² needles with extremely thin walls to allow for a larger interior needle diameter without increasing the exterior diameter,⁵³ and devices with higher-pressure injectors.⁵

Osmolality and tonicity

Osmolality and tonicity are distinct but related measures: osmolality is defined as the millimoles of material dissolved per kilogram of solution, while tonicity is the effective osmotic pressure gradient between two solutions when divided by a semipermeable cell membrane.³⁴ When a solution is isotonic, it has the same effective solute and water concentration as a comparison solution (e.g., cytosol) and is in dynamic equilibrium, causing no net osmotic flow out of or into a cell and therefore no shrinking or swelling of a cell.⁵⁴ Hypertonic solutions have a higher solute and lower water concentration than a comparison solution and cause osmotic flow out of cells, resulting in shrinking cells, while hypotonic solutions have a lower solute and higher water concentration than a comparison solution and cause osmotic flow into cells, resulting in swollen cells.⁵⁴

Formulations should ideally be isotonic, with an osmolality of about 300 mOsm/kg (approximately that of saline or blood),⁵⁵ but increasing the concentration of a formulation while trying to reduce or maintain the total injection volume

may require hypertonicity. Hypertonicity can cause irritation, discomfort, and sensations of heat and pain; an upper limit of 600 mOsm/kg has been recommended to minimize these side effects.⁵⁶ Hypertonic solutions may also require a slower rate of SC injection for tolerability. The use of high-volume solutions may therefore minimize the resulting hypertonicity encountered with high-concentration mAb formulations and may improve tolerability and adherence. Tonicity may also be adjusted with the inclusion of an excipient such as sodium chloride or arginine, and osmolality can be adjusted with excipients such as maltose, mannitol, sorbitol, sucrose, or trehalose.³⁴ Although osmolality and tonicity are considerations in the development of SC formulations, they are less critical than some of the other factors reviewed here.

pH

The pH of commercially available mAb formulations ranges from 4.8 to 8.0 and plays a significant role in protein solubility and physical and chemical formulation stability.^{34,36} The overall charge of a molecule is determined by the relationship between the isoelectric point (pI; typical range 6–8) of the antibody and the pH of the solution.³⁶ mAbs have a low, approximately neutral charge when the formulation pH approaches the pI; this is associated with increased attractive hydrophobic or van der Waals interactions and aggregation, particularly in high-concentration formulations.³⁶ Deamidation and isomerization are most likely to occur when a formulation has a neutral or basic pH, but can also occur at lower pH levels, and the rate of these reactions is influenced by the steric effect.^{57,58} The impact of deamidation on mAb functionality varies by location; it exerts minimal effects if occurring in the Fc, but can reduce potency and binding affinity if occurring in the complementarity-determining region of Fab.⁵⁹ In contrast, mAbs experience repulsive interactions when the solution pH is further from the pI.³⁶ Lower pH ranges can provide improvements in disulfide shuffling and decrease deamidation rates.⁶⁰ Engineered protein therapeutics and proteins with weak disulfide bonds that are prone to disulfide shuffling can lead to decreased potency and increased immunogenicity.⁶⁰ However, higher solution pH is associated with decreased electrostatic interactions, increased mAb reversible self-associations, and greater solution viscosity, possibly due to decreased electrostatic repulsive interactions.⁶¹

Biologics have their own capacity to buffer a formulation and may be sufficient at higher concentrations without the addition of excipients.⁶² However, when the buffering capacity of a protein is insufficient, adding excipients such as salts to a formulation can decrease charge-mediated interactions and improve formulation stability.³⁶ Buffers or agents used to alter pH include acetate, adipic acid, aspartic acid, citrate, glutamate, histidine, lactic acid, 2-N-morpholino-ethane-sulfonic acid, phosphate, succinate, and tromethamine.³⁴ The concentration of buffers is typically limited to <50 mM to decrease aggregate formation and injection pain,³⁴ as buffer concentration, strength, and type have been associated with local pain. For example, including citrate appears to increase pain

compared with normal saline or phosphate.⁶³ Citrate is a strong buffer with many pIs between acidic pH and tissue pH, and a strong buffering effect due to a drug having to cross a buffer pI to reach tissue pH or a high buffer concentration can increase patient discomfort.⁶⁴

Many forms of chemical degradation, such as deamidation, are pH-dependent. However, high-volume, low-concentration formulations are not encumbered to the same extent as high-concentration formulations by viscosity or aggregation difficulties. As a result, they may be more easily optimized for the best pH range to maximize stability and minimize pain and irritation.

Bioavailability

The bioavailability of IV-administered biologics is rarely equaled with SC administration.¹² While IV administration places a biotherapeutic directly into systemic circulation and therefore by definition results in 100% bioavailability, SC administration delivers a biologic to the extracellular space of SC tissue, where it must be absorbed into the lymphatic system and tissues and enter the circulatory system.¹⁰ As a result, bioavailability following SC administration can be similar to that of IV administration⁶⁵ but is often less than 100%.¹²

Bioavailability for an SC-administered biologic cannot yet be precisely predicted, but may be affected by the volume, pH, and viscosity of a formulation.¹⁰ Other drug-related factors, including pre-systemic catabolic activities, poor or slow absorption, interactions between SC tissue and drugs, surface hydrophobicity, and higher molecular weight, may also decrease bioavailability.^{66,67} Additionally, absorption patterns differ among individual peptide drugs and proteins, which can affect the efficacy, safety, and immunogenicity of a formulation.¹⁰ Individual patient factors such as weight, immunogenicity, injection site, and lymphatic flow rate may further affect bioavailability.⁶⁷

Biologics are typically cleared more rapidly when administered at higher doses, possibly due to aggregation or anti-drug antibodies,⁶⁸ which can negatively impact commercialization of the drug. For devices designed for home self-administration, decreased bioavailability necessitates higher doses and/or more frequent injections, and therefore increased costs.¹² If bioavailability is extremely low, varies too much within or between patients, or is low enough to necessitate an excessively high product volume and cannot be improved or stabilized, the development of a product may be prematurely terminated.¹⁰ Bioavailability of SC-administered biologics in humans is also difficult to predict for a variety of reasons, including a lack of correlation with preclinical bioavailability data in other species and a lack of validated preclinical models. However, some bioavailability modeling is becoming available, and if bioavailability is critical to a particular program, modeling can be used to select an ideal candidate. Bown et al.⁶⁹ reported successfully modeling the bioavailability of eight biologics following SC injection using an *in vitro* model, Lou and Hageman⁶⁶ used machine learning algorithms developed with 47 features of 36 mAbs in a training set to predict whether nine SC mAb formulations would have adequate ($\geq 70\%$) bioavailability, and Zou⁶⁷ used multivariate regression models based on an

observed inverse correlation between IV clearance and pI of a training set of 59 mAb formulations to predict the SC bioavailability of 27 mAb formulations. Modeling and molecular property selection like this may facilitate formulation property selection and the development of clinical dosing strategies. An informed SC bioavailability prediction coupled with the volume capacity of high-volume SC devices like an OBDS may help improve SC biologic drug development and delivery.

Subcutaneous delivery product development considerations

Safety profile

SC administration of biologics is as efficacious as IV administration and may sometimes confer an improved safety profile, perhaps due to a change in the pharmacokinetic profile of the drug. SC delivery not only has a delayed maximum concentration in the serum/blood, but it also has a lower peak concentration. If a particular biologic would cause adverse effects due to high peak serum levels, SC delivery would be even more beneficial for patients. For example, in a small case-control study of 25 patients with gastric mucosa-associated lymphoid tissue lymphoma, SC rituximab administration demonstrated no difference in complete remission or overall response rate, but a significant decrease in postinduction, grade 2, injection-related reactions and outpatient hospital length of stay compared with IV rituximab.⁷⁰ In a review of Hizentra® (an SC immunoglobulin) in seven open-label, Phase 3, prospective, multicenter studies of a total of 125 patients with primary immunodeficiency previously treated with the IV immunoglobulin Privigen®, it was reported that compared with Privigen®, Hizentra® was associated with higher infusion site reactions but with an approximately 10-fold lower incidence of systemic grade 2 adverse events, including fatigue, headache, vomiting, nausea, and pyrexia (≤ 0.22 events/infusion with Privigen® vs. ≤ 0.00915 events/infusion with Hizentra®).⁷¹ Treatment-emergent adverse events (TEAEs), particularly viral and non-viral upper respiratory tract infections, were also found to be higher with IV administration of CT-P13, an infliximab biosimilar, compared with SC administration in a randomized Phase 1/3 trial of 343 patients with active rheumatoid arthritis.⁷² Finally, SC amivantamab has been shown in a Phase 1b dose escalation study of patients with various advanced solid tumors to be well tolerated with decreased administration time and TEAEs, including infusion-related reactions, compared with IV amivantamab.¹³

In a meta-analysis of 6 retrospective studies and 3 randomized controlled trials of patients with multiple myeloma, Hu et al. found no statistical difference between bortezomib SC and IV in overall response rate, but found that SC administration was associated with a reduced incidence of peripheral neuropathy in both the retrospective studies and randomized trials and with a reduced incidence of thrombocytopenia and renal and urinary disorders in the retrospective studies compared with IV administration.⁷³ Similarly, SC daratumumab appears to be both non-inferior to IV daratumumab with respect to efficacy and pharmacokinetics and to have a better

safety profile in patients with relapsed or refractory multiple myeloma, with lower rates of infusion-related reactions and shorter administration time.^{14,74}

Development pathway

New SC formulations have traditionally been developed by changing an acceptable IV formulation into an acceptable SC formulation by increasing the drug concentration to 100–150 mg/mL and sometimes changing excipients. However, devices and/or permeation enhancers that allow for the easy delivery of higher volumes to patients have eliminated the need for high-concentration formulations.

In the development of a SC product, akin to the requisite testing of a drug at various concentrations and temperatures in IV infusion trials, a device or product compatibility study should be conducted before the product is introduced into clinical trials. A combination product compatibility trial rigorously tests the interoperability of drug and device, ensuring they perform effectively together without compromising safety or efficacy. Such a trial encompasses stability, pharmacokinetic, and performance assessments alongside regulatory compliance to validate that the integrated product is fit for clinical use. Additionally, a SC toxicology study should be performed in a relevant species to augment the systemic exposure studies that were completed during the testing of the corresponding IV product. Because full histology, blood chemistry, and pharmacokinetic studies are usually completed as part of IV toxicology studies, supplemental toxicology studies for SC products can often be abbreviated. For most biologics, SC administration should not be more toxic than IV administration at the same dose level. On the contrary, SC administration is likely to be less toxic than IV administration due to lower peak serum concentrations, as the drug must diffuse through the lymphatic system and tissues before detection in the circulatory system. One exception to this would be when a highly concentrated drug is active and toxic at the injection site. Therefore, SC toxicology studies should be designed to measure local reactions or injection site damage relative to a control such as saline.

Pharmacokinetics and bioavailability are likely to differ with SC administration. The blood concentration of the drug over time should be measured, with the peak concentration (C_{max}) generally expected to occur about two hours after injection in large animals and humans, unlike IV products, which are characterized by maximum concentration in the blood immediately after administration. Blood panels and blood chemistries should also be monitored throughout clinical trials, and punch biopsies at the end of a trial should be considered in order to ensure that tissue at the injection site remains healthy.

If a formulation change is required during clinical development (e.g., the protein concentration needs to be increased to fit traditional SC delivery devices) but is not expected to affect the function of a drug, analytical comparability may suffice, and additional SC toxicology studies may not be needed. Higher protein concentrations often do not affect the properties of biologics, and trial outcomes therefore remain predictable. Starting clinical development with an existing

formulation can save time and allow for earlier availability of SC clinical proof-of-concept results. This can in turn allow a faster time to market and facilitate decision-making about the final commercial drug formulation based on clinical data rather than assumptions. Transitioning from IV to SC drug development not only offers distinctive product differentiation, benefiting a broad spectrum of stakeholders, but also presents opportunities for intellectual property extension. For pharmaceutical companies, this intellectual property extension can prolong market exclusivity, allowing them to maintain a competitive advantage and higher pricing and protect market share from generic or biosimilar competition.

High-concentration biologic formulations are commonly developed as follow-on products, possibly due to the time- and cost-intensive nature of the development process. Given the higher volume capacities of newer SC delivery methods, IV formulations, which are already high-volume, can be minimally modified and taken through dose escalation studies for SC administration once bioavailability has been determined. Some examples of biologics that were originally available for IV administration and were later developed for SC administration include adalimumab, alemtuzumab, rituximab, tocilizumab, ravulizumab, efgartigimod, and trastuzumab. The developers of some of these drugs pursued SC programs while the IV version was not yet approved, while others pursued SC programs as part of a lifecycle strategy reacting to competition.

While developing a SC product from an existing IV product has several benefits, going straight to the development of a high-volume SC product without first developing a corresponding IV product can save substantial development time and costs. Golimumab (Simponi®), an anti-tumor necrosis factor mAb delivered in a 50 mg dose in an 0.5 mL solution via autoinjector or PFS for the treatment of moderate-to-severe rheumatoid or active psoriatic arthritis, active ankylosing spondylitis, or ulcerative colitis, is an example of a SC product that was developed directly without conversion from an existing IV formulation.⁷⁵ The decision to develop golimumab for SC delivery without first developing an IV formulation may have been made because existing treatments adalimumab (Humira®) and etanercept (Enbrel®) were already available in SC formulations. In this situation, it is unlikely that golimumab would have been able to compete in the market as an IV-administered drug unless it were shown to have a greater clinical benefit.

Device capabilities, dose volumes, dosing intervals, and fixed vs. variable dosing

When planning clinical dose escalation trials for a SC device, the drug concentration and device volume must be considered. If needed, a second formulation of a higher concentration could be developed once the recommended Phase 2 dose and commercial dose level are known. Table 3 shows a simplified dose-volume matrix of three different drug concentrations (10, 30, and 100 mg/mL) and the resulting volumes needed to treat a patient weighing 80 kg when dosing monthly at 1, 3, 10, and 30 mg/kg. Dose-volumes shown in green can be delivered using a single OBDS (assuming a maximum volume capacity of 25 mL per device),

Table 3. Dose-volume matrix.

Drug Concentration (mg/kg)	Monthly Dose (mg/kg)				Weekly Dose (mg/kg)			
	1	3	10	30	0.25	0.75	2.5	7.5
10	8.0	24.0	80.0	240.0	2.0	6.0	20.0	60.0
30	2.7	8.0	26.7	80.0	0.7	2.0	6.7	20.0
100	0.8	2.4	8.0	24.0	0.2	0.6	2.0	6.0

Matrixed dose-volumes are shown in mL of formulation needed and are based on a patient weighing 80 kg and a subcutaneous on-body delivery system that can deliver a volume of 5–25 mL. Green: one device is sufficient for delivery of the dose-volume; yellow: a dilution is required for SC delivery of the dose-volume; orange and red: at least two SC devices are required for delivery of the dose-volume.

those shown in yellow can be delivered with traditional SC devices or diluted for delivery with an OBDS, and those shown in orange and red must be delivered using multiple devices.

Within the framework of planning a SC development strategy, the prospect of adopting a weekly dosing regimen as opposed to the traditional monthly or biweekly dosing schedules emerges as a viable consideration, particularly in response to safety challenges. Table 3 also shows a volume-dose matrix for the same 1, 3, 10, and 30 mg/kg monthly equivalent doses, but for a drug administered weekly, with the dose approximately quartered for each administration. Either or both dosing frequencies can be considered for initial dose-escalation clinical trials.

Use of high-volume, low-concentration formulations in tandem with a high-volume capacity SC delivery device such as an OBDS can represent a favorable convergence. This approach circumvents the hurdles inherent in creating a high-concentration SC formulation while mitigating the potential inconvenience and lower patient adherence associated with shorter dose intervals. Dosing intervals can be extended if using high-volume SC devices rather than traditional devices like autoinjectors, which have low volume capacity and may therefore limit the weekly dose. However, it is important to note that extended dosing intervals rely on appropriate safety and pharmacokinetics. More frequent dosing could further mitigate undesirable side effects that are caused by transiently high serum concentrations when drugs are administered monthly rather than weekly.

The development of SC ravulizumab (ULTOMIRIS® SC) underscores the significance of device volume capacity and the impact it can have on formulation development. According to publicly available preclinical data, ravulizumab (ULTOMIRIS®) was initially developed for SC administration without the previous development of an IV formulation.⁷⁶ A concentration higher than 100 mg/mL may have been difficult to achieve at a volume low enough for traditional, low-volume SC delivery methods. This may have been why the sponsor moved to Phase 3 development of an IV formulation rather than directly pursuing SC administration as originally intended. The 100 mg/mL IV formulation was approved by the Food and Drug Administration (FDA) in 2018 and was administered every 8 weeks at a dose of 3,300 milligrams (33 mL) for the treatment of paroxysmal nocturnal hemoglobinuria. Pegcetacoplan (EMPAVELI®), a competitor, was introduced in 2021 and was available as a twice-weekly, 20 mL (54 mg/mL) infusion administered via a SC syringe pump.⁷⁷ In July 2022, the FDA approved ULTOMIRIS® SC in the form

of two 245 mg/3.5 mL OBDSs (West SmartDose®) and a once-weekly, lower-concentration formulation (70 mg/mL). The manufacturer reduced the concentration from 100 mg/mL to 70 mg/mL in the SC formulation and greatly increased the dosing interval from every 8 weeks IV to weekly SC.⁷⁸ ULTOMIRIS® SC was administered via an OBDS with a capacity of 3.5 mL, requiring the use of two devices for a total dose-volume of 7 mL. Roughly 2 years after the approval of pegcetacoplan (EMPAVELI®), it was approved for administration via an OBDS that has a 5–25 mL capacity (Enable Injections enFuse®) delivering 20 mL with a single OBDS.⁷⁹ Using an OBDS with a large volume capacity allows the use of a single device for the same dosing interval and could have permitted an extended dosing interval and a lower drug concentration.

While mAb IV infusions were customarily developed with a variable dosing model, with dose adjusted for body weight or surface area to correct for interpatient differences in drug distribution and elimination,¹⁵ SC alternatives to these established IV treatments can benefit from changing to a fixed dose or the use of dose bands for different weight categories.¹² Benefits of fixed dosing include decreased costs, less complicated management, and elimination of dose calculations, thereby decreasing the risk of dosing inaccuracies, particularly when a formulation is intended for home use.^{12,15}

Although pharmacokinetic variability has been modeled for some mAbs,⁸⁰ mAb distribution occurs solely within blood plasma and extracellular fluids, and mAbs are eliminated via proteolytic catabolism and intracellular degradation after target binding.¹⁵ Unlike the hepatic and/or renal clearance of small molecule drugs, whose volume of distribution is determined by muscular, adipose, and connective tissue, these processes are not directly proportional to body weight or size but to the volume of blood plasma and extracellular fluids.¹⁵ A fixed dosing approach may therefore be feasible for monoclonal antibodies; several have already been approved as fixed dose therapies, including catumaxomab, nivolumab, obinutuzumab, ofatumumab, and pertuzumab.¹⁵

Fixed dosing approaches also reduce healthcare costs by decreasing the risk of spillage, as prepared infusions can be used for other patients if treatments are canceled and the entire content of vials can be used to prepare infusions, and by avoiding overdosing with body weight-based dose calculations.¹⁵ Hendriks et al. estimated a savings of more than €3 million at population level between August 2014 and November 2016 at their comprehensive cancer center in the Netherlands when they switched to fixed dosing for ipilimumab, nivolumab, and pembrolizumab.¹⁵

Injection rate

The extent to which injection speed affects pain remains unclear. A study using a viscous placebo to assess the impact of both volume and injection time found that the lowest pain score was associated with the highest volume (3.5 mL) administered over 10 minutes ($p = .004$), while the highest pain score occurred with administration over 1 minute ($p = .004$).⁸¹ Another study comparing 2 mL of a mAb given over 5 seconds versus 15 seconds also reported lower pain scores with slower injections, although the differences did not reach statistical significance.⁸² In a recent clinical feasibility study with 32 healthy volunteers, no clinically (≥ 10 mm on a visual analog scale) or statistically significant difference in injection pain was found between 5 mL, 4.1-minute and 10 mL, 8.2-minute injections at a constant rate of 20 $\mu\text{L/s}$.⁸³ Higher flow rate and injection speed may be more important considerations for traditional, hand-administered SC devices such as autoinjectors or stationary infusion pumps than for OBDSs, which do not require the patient to hold the device while the drug is delivered and allow for light-to-moderate activities during treatment administration.

Improving SC delivery can be achieved by reducing injection time, the number of steps required, and the complexity of each step. While reduced injection time provides economic value, this value decreases when direct administration by a health care professional (HCP) is required (e.g., drugs formulated with hyaluronidase that are not administered with a SC syringe pump). Hands-free administration with an OBDS frees HCPs to perform other clinical activities, which may provide more economic value despite a longer injection time.^{43–45}

Injection-related pain, device characteristics, and patient adherence

As reviewed here, SC treatment has a wide range of benefits for patients compared with IV treatment, including increased convenience and flexibility and decreased cost.⁸⁴ Although patients broadly prefer SC to IV treatment, adherence to long-term treatment can be as low as 50% in developed countries and even lower in developing countries.⁹

Pain with SC injection should be avoided and can adversely affect patient adherence. Injection-related pain may be influenced by several factors, including injection site and technique, needle characteristics, volume and speed of injection, formulation viscosity, osmolality, buffer, pH, and excipients (e.g., preservatives).²⁹ Injection-related pain can further result in pre-administration anxiety and unwillingness or inability to self-administer a medication.⁸⁵ As previously reviewed here, certain formulation characteristics such as the inclusion of hyaluronidase can necessitate larger needle diameters, which increase patient pain.^{43–45} In a recent open-label randomized crossover study, only one patient reported injection site pain with an OBDS compared with three patients reporting injection site pain with a SC syringe pump.⁸⁶ In this study, the OBDS used a much smaller needle diameter than that of the SC syringe pump.

In order to improve adherence, clinical development programs for new SC therapies and devices must include consideration of the needs of specific populations, such as children and older patients, patients with hepatic or renal impairment, and patients with cognitive, physical, or visual impairments, as these patients may have different safety, efficacy, and pharmacokinetic outcomes and may experience increased difficulty self-administering SC treatments.⁸⁴ Device characteristics such as smaller or concealed needles, which have been associated with dramatically increased adherence, should also be considered in efforts to improve patient adherence.⁸⁷

Device preference

mAb formulations for SC self-administration are available in multiple formats, including vial and syringe, autoinjectors, PFSs, and high-volume OBDSs.^{34,84} The vial and syringe combination is typically the first commercially available presentation for high-volume SC mAb products, with drugs provided either in liquid form ready for injection or lyophilized for reconstitution before injection.⁹ PFSs are disposable (single-dose) or reusable (repeat-dose) cartridge-based devices originally introduced in the 1980s for the administration of insulin and now commonly used for biologics; they usually have a volume-per-injection limit of < 5 mL.^{34,88} High-volume OBDSs can be attached to the body with adhesive for injection of formulations with volumes > 3.5 mL over a specified time period and then removed and discarded when administration is complete.^{5,9} OBDS delivery mechanisms can be either electromechanical or mechanical (elastomeric), their delivery rate can be pre-determined or variable to increase patient comfort, and their volume limit can be as high as 100 mL using multiple devices.⁵

Autoinjectors may be preferred to PFSs among patients with a fear of needles, as they allow self-administration without needle visibility, or among patients with chronic conditions that can impair dexterity, such as rheumatoid arthritis.^{9,89} However, evidence of a distinct patient preference between these methods remains equivocal. In one recent Phase 2 open-label study comparing administration of an adalimumab biosimilar with a PFS or an autoinjector in 48 adults with rheumatoid arthritis, there were no significant differences in mean injection site pain either immediately or at 15–30 minutes after injection, or in overall impression of the two devices; however, patients indicated an overall preference for the autoinjector over the PFS.⁹⁰ Two less recent randomized trials with a total of 640 adults with rheumatoid arthritis found greater patient satisfaction with use of a prefilled pen compared with a PFS for etanercept administration.⁹¹ In an open-label randomized crossover trial comparing administration of an etanercept biosimilar in 51 healthy male volunteers, the autoinjector had equivalent dosing and tolerability to the PFS.⁹² In a larger open-label randomized trial of 421 adults with psoriasis, significantly greater satisfaction was reported with administration of etanercept using a prefilled pen compared with a PFS, with greater satisfaction reported among older patients and those who had had psoriasis for longer.⁹³

Table 4. Bioavailability and other aspects of SC mAb formulations with hyaluronidase compared with IV administration.

SC mAb Formulation	Bioavailability Compared with IV Formulation	SC Bioavailability Improved with Hyaluronidase Co-formulation	Other Comparisons with IV Administration
HYQVIA® (immune globulin infusion 10% [human] with hyaluronidase)	93.3% ⁹⁴	Yes	Hyaluronidase allows for the full monthly dose of IgG (≤600 mL) to be delivered with a single injection site every 3–4 weeks rather than multiple times weekly ⁹⁵ with similar bioavailability and a lower peak concentration compared with IV IgG. ⁹⁴ Co-formulation with hyaluronidase was reported to improve bioavailability by approximately 20% compared to SC IgG without hyaluronidase in 31 patients with PID in a prospective open-label study evaluating co-formulation who had previously participated in a prospective open-label study of 49 patients with PID evaluating SC IgG without co-formulation. ⁹⁴
Amivantamab with hyaluronidase	Unavailable	Unknown	An ongoing Phase 1b dose-escalation study with 81 patients with various advanced solid tumors found that SC amivantamab 1600 mg (or 2240 mg in patients weighing ≥80 kg) administered every other week had similar exposure to the approved IV dose (1050 mg every other week, or 1400 mg in patients weighing ≥80 kg); resulted in lower C _{max} and equal or higher C _{trough} and AUC _{0–336 hr} , and was associated with a lower incidence of infusion-related reactions and related symptoms. ¹³
Crenezumab with hyaluronidase	66% ⁹⁶	No	A Phase 1 study with 72 adult participants receiving SC crenezumab at different combinations of dose (1,700–6,800 mg), flow rate (2–4 mL/minute), and infusion volume (10–40 mL) and with and without co-formulation with hyaluronidase found that hyaluronidase decreased infusion site swelling incidence but was associated with larger areas of infusion site erythema, and co-formulation with hyaluronidase did not significantly affect patient pain, dose proportionality, bioavailability, drug half-life, or systemic exposure. ⁹⁶
VYVGART HYTRULO® (efgartigimod with hyaluronidase)	~50% ⁹⁷	Unknown	VYVGART HYTRULO® has comparable half-life to that of IV administration but is associated with more injection site reactions (reported in 21 of 55 patients, or 38%), including rash, skin redness, itching, bruising, pain, and hives. ⁹⁷
Herceptin HYLECTA® (trastuzumab/hyaluronidase)	77% ⁹⁸	Unknown	C _{trough} and AUC _{0–21} are slightly higher in patients receiving SC trastuzumab and differences were larger at the extremes of body weight. ⁹⁹ Bioavailability of the SC regimen is estimated to be 77%. ¹⁰⁰ Within-patient pharmacokinetic comparison of SC and IV data were not obtained, but estimated bioavailability was comparable to that seen a Phase I study with four patients receiving both SC and IV trastuzumab. ¹⁰⁰ When administered SC, trastuzumab has an absorption half-life of 2.5 days. ¹⁰¹
RITUXAN HYCELA® (rituximab/hyaluronidase)	64.6% for follicular lymphoma, 63.4% for chronic lymphocytic leukemia ¹⁰²	Unknown	C _{trough} and AUC for rituximab SC are non-inferior to rituximab IV. ⁹⁵
PHESGO® (trastuzumab/pertuzumab/hyaluronidase)	70% for pertuzumab, 80% for trastuzumab ¹⁰³	Unknown	In a randomized, open-label, Phase 3 study, PHESGO® produced similar serum C _{trough} , safety, and efficacy compared with IV administration. ¹⁰⁴
DARZALEX FASPRO® (daratumumab/hyaluronidase)	70% ¹⁰⁵	Unknown	Efficacy and pharmacokinetics with DARZALEX FASPRO® SC are non-inferior to IV. ⁷⁴
Tocilizumab/hyaluronidase	79.5% ¹⁰⁶	Yes	In a study in healthy volunteers, tocilizumab in combination with hyaluronidase slightly improved tocilizumab exposure compared with tocilizumab alone; PD markers were similar. ¹⁰⁷

Abbreviations: AUC, area under the curve; AUC_{0–21}, area under the curve for 21 hours of exposure; AUC_{0–336 hr}, area under the curve for 336 hours of exposure; C_{max}, peak concentration; C_{trough}, trough concentration; IgG, immunoglobulin G; IV, intravenous; mAbs, monoclonal antibodies; OBDS, on-body delivery system; PID, pharmacodynamic; PFS, prefilled syringe; PID, primary immunodeficiency disease; SC, subcutaneous.

As shown in Table 4, formulations that include hyaluronidase and are administered with a syringe use larger-diameter needles, likely due to the high viscosity of hyaluronidase. It is well documented in recent studies that subcutaneous administration is more painful when using a lower-gauge needle (i.e., a larger needle diameter) than when using a higher-gauge needle (i.e., a smaller needle diameter). For example, when comparing a thinner (29-gauge), sharper (5-bevel) needle with a thicker (27-gauge), less sharp (3-bevel) needle, two double-blind randomized trials with a total of 241 healthy volunteers found that the thinner, sharper needle decreased perceived pain by 40% and improved skin penetration by 69%, while five surveys of a total of 368 patients with multiple sclerosis

found that the thinner, sharper needle was associated with less pain, better ease of insertion, and decreased injection site reactions, bruising, burning, and stinging.⁴⁴ Similarly, a randomized trial of 36 healthy volunteers receiving three abdominal SC injections of 3 mL of 1% lidocaine with three differently gauged needles found that injections administered with the thinnest (27-gauge) needle were less painful than those administered with the thicker (23- or 21-gauge) needles.⁴⁵ And finally, a study using an automated needle injection system found that pain was reported in 63% of insertions with a 23-gauge needle, 53% of insertions with a 27-gauge needle, and 31% of insertions with the thinnest, 32-gauge needle ($p < .0001$).⁴³ The thickest needle also caused

more bleeding, and bleeding insertions were reported to be 1.3 times more painful ($p = .004$).⁴³ Thinner needles are associated with decreased patient pain and may therefore improve adherence.

Many patients may prefer high-volume OBDSs, including patients with needle phobia; in a recent survey study of a general adult population, 63.2% of 1,325 participants reported needle phobia and 91.1% identified smaller needles as helpful in alleviating this fear.⁸⁷ OBDSs with hidden needles would be expected to alleviate the barrier of needle phobia. OBDSs also permit patients to pause injections, and single-use devices do not require cleaning or other maintenance. A recent cross-sectional online survey with 191 participants reported that patients would prefer OBDSs to autoinjectors if injection frequency was reduced from biweekly to monthly or quarterly and if injection duration was shortened from 33 to 8 minutes.¹⁰⁸ OBDSs can deliver treatment at a constant flow rate or low pressure.⁸⁶ A recent open-label randomized trial of 23 patients with primary immunodeficiency found similar immunoglobulin G exposure with a constant flow rate SC syringe pump (reservoir volume ≤ 50 mL, flow rate ≤ 50 mL/hour) and a low-pressure OBDS (reservoir volume 10–20 mL, flow rate ≤ 25 mL/hour) and a numerically lower rate of TEAEs and device-related adverse events with the low-pressure OBDS.⁸⁶ Ease of use, mobility during infusion, and decreased setup time and injection site pain were the most common reasons patients cited in a recent survey study for preferring an OBDS over a SC pump.⁸⁶ There is a notable scarcity of high-quality evidence surrounding the tolerability and discomfort associated with SC infusion of high volumes at varying rates, especially with different formulation viscosities. The subjective experiences of these devices may significantly differ, influenced by personal characteristics, alternative treatments available, and the nature, duration, and severity of the disease in question.¹⁰ This situation decidedly underscores the pressing need for more targeted research in this area.¹⁰

Devices for self-administration of SC injections are quick and convenient to use and have many other potential benefits. Patients can receive treatments weekly rather than monthly without increasing healthcare system burdens, which would limit peak and trough serum levels, minimize toxicity risks, and reduce volume or concentration requirements for each dose. Alternatively, in instances where the delivery device possesses a substantial volume capacity, the feasibility of extended dosing intervals emerges, allowing for the administration of a more substantial dose, contingent upon the safety and pharmacokinetic characteristics of the drug in question. Monthly administration has also been shown to increase patient adherence compared with daily or weekly treatment.¹⁰⁹ Constant-pressure devices such as OBDSs also reduce backpressure, discomfort, and bruising, and the lack of a strong spring can reduce the risk of unintended intramuscular injections, while constant-flow devices such as SC infusion pumps allow for a fixed injection time but limit mobility.

Delivery methods with a hands-free, low-pressure mechanism that responds to changes in SC backpressure may be ideal for administering viscous SC formulations. Most high-volume SC delivery methods use constant-flow

mechanisms that often require manual administration by an HCP and push volume into the SC space regardless of changes in SC backpressure. However, to date there is only one high-volume OBDS (enFuse®) that uses a low-pressure delivery mechanism and allows administration without a manual push by an HCP.

Development benefits of low-concentration, high-volume formulations

High volume as an alternative to high concentration

High-concentration biologic formulations have been considered desirable because biologics often require high doses and formulation volume has been limited by traditional SC delivery methods. ‘High-concentration’ biologic formulations are variably defined. Current biologic formulations range in protein concentration from 12 $\mu\text{g/mL}$ to 200 mg/mL ³⁴ and “high” concentrations typically fall within 50 to 150 mg/mL but range widely from 20 to 200 mg/mL .^{7,110} A cutoff of ≥ 100 mg/mL is commonly used.¹¹⁰ These high-concentration biologics account for about one-third of FDA-approved drugs.¹¹⁰

High-volume (>3 mL), low-concentration SC formulations are an alternative to high-concentration, low-volume formulations. Administration of high-volume formulations can be facilitated by injection at multiple sites, more frequent injections, or injections over longer periods of time (e.g., 5–20 minutes).^{5,28,81} Primary immunodeficiency is one example of a condition most often treated with high-volume immunoglobulin formulations of >10 mL; SC treatment can be administered with a syringe-driving pump weekly to monthly.¹² In a recent early clinical feasibility study, SC injections of volumes up to 10 mL (8.2 minutes) in the abdomen and 5 mL (4.1 minutes) in the thigh with viscosities up to 20 cP with a constant rate (20 $\mu\text{L/s}$) syringe pump system were found to be feasible and tolerable in 32 healthy volunteers.⁸³ In two recent Phase 1 studies with healthy volunteers, SC administration of crenezumab at volumes ranging from 4 mL (600 mg) to 40 mL (7200 mg) were well tolerated with or without co-formulation with a permeation enhancer (hyaluronidase), and volume, dose, concentration, flow rate (≤ 4 mL/min), or the presence or absence of hyaluronidase did not appear to affect pain or the type or incidence of TEAEs.⁹⁶ Similarly, acceptable tolerability and safety were demonstrated in a study with healthy volunteers using a viscous placebo buffer characteristic of a high-concentration mAb formulation at volumes of 3.5 mL in 3 injections over 1, 4, or 10 minutes in different abdominal quadrants.⁸¹

Side effects of high-volume infusion pump-facilitated SC mAb administration can include pain, itching, erythema, and induration; doses are therefore often split across multiple more frequent injections either once weekly or every two weeks.¹² While doses of >2 mL can be split across multiple injection sites or given in smaller doses over multiple infusions, this decreases patient convenience and adherence and makes serum levels less reproducible.^{12,81} OBDSs can facilitate at-home SC administration of high-volume formulations.⁵ Autoinjector design solutions for managing the increased

force required to administer higher volumes include the use of a linear spring to provide extrusion force, expansion of compressed gas to replace spring force, and control of force with torsion springs and regulators.⁵ Primary containers have also been developed to increase tolerance of the force required for high-volume injection, but these devices can be limited in volume and more difficult and costly to produce than traditional autoinjectors.⁵

Co-formulation with a permeability enhancer

Hyaluronidase (recombinant human hyaluronidase, or rHuPH20) is an effective permeability enhancer that enzymatically alters the SC space. Hyaluronan (also called hyaluronic acid) is a chain of sugar molecules in the extracellular matrix that holds a large amount of water, providing hydration and contributing to structural support. Hyaluronidase breaks down hyaluronan, resulting in a decrease in the viscosity and organization of the extracellular matrix, which leads to enhanced spreading and diffusion of injected substances.¹² A study evaluating the effect of SC co-administration of hyaluronidase in rats found that it increased absolute bioavailability of cetuximab from 67% to 80% and increased cetuximab recovery in the lymphatics from 35.8% to 49.4%, but did not significantly affect the bioavailability of trastuzumab, possibly because its absolute bioavailability is already high (~77–99%).¹¹¹ While hyaluronidase increases absorption, the impact on bioavailability is inconclusive (Table 4). It is well evidenced that high-volume SC delivery is possible without a permeation enhancer; the most compelling examples are the numerous high-volume SC drug launches which occurred before and after the launch of hyaluronidase co-formulations, including Repatha®, ULTOMIRIS® SC, EMPAVELI®, RYSTIGGO®, and Hizentra® SC (Table 2).

Permeation enhancers such as hyaluronidase may also be used to facilitate SC-injected fluid dispersion for higher-volume formulations (Table 2).^{6,12} However, hyaluronidase is incompatible with certain drugs, including benzodiazepines, furosemide, and phenytoin;⁸¹ may cause physical or chemical formulation stability difficulties; and may result in a requirement for multiple injections, as with HYQVIA® (Table 4).¹⁷ The inclusion of hyaluronidase in a co-formulation also requires additional development time and costs, may increase risk of infusion site erythema,⁹⁶ and requires development with another combination product such as an autoinjector, PFS, or OBDS. When administered with a PFS, a drug co-formulated with hyaluronidase (Table 4) can require a significant amount of force to manually push at the constant flow rate despite the use of larger needle diameters for these viscous formulations. As reviewed here, repeated administration can lead to musculoskeletal injuries among nurses, requiring the introduction of SC syringe pumps, which further increase administration expenses.⁴² Furthermore, the poor ease of use with the syringe and needle format limits patient self-administration, which is often a critical parameter for SC launches.

Physical instability

The development of high-concentration biologic formulations can be challenging due to their physical instability at higher

concentrations, including limited solubility, phase separation, opalescence, irreversible aggregation,^{33,112} high solution viscosity during manufacturing,^{113,114} intermolecular PPIs,^{115,116} and clinical performance issues such as bioavailability and immunogenicity.^{12,29,117} As with other protein-based biotherapeutics, biologics are subject to physical instability throughout development, including during processing, formulation, storage, and distribution.⁶⁸ These challenges can cause significant difficulties with manufacturing, storage, and administration by adversely impacting filtration, filter- and needle-clogging, shelf life, and potency.^{17,118}

Physical instability most commonly occurs through aggregation, although higher temperatures and mechanical stress (e.g., shaking, pumping) increase the chance of PPIs and protein unfolding.⁶⁸ These reversible self-associations involve the formation by monomeric mAb units of multimers (e.g., dimers, trimers) that dissociate when the formulation is diluted.^{38,39,68} According to Lumry-Eyring theory, monomers first form an irreversible nucleus (nucleation) and then grow to soluble aggregates.¹¹⁹ Aggregates are classified by size into small subvisible particles (also called soluble or submicron particles; <1 µm), subvisible particles (1–100 µm), and visible particles (>100 µm), with subvisible and visible particles considered to be insoluble.¹²⁰ Although subvisible particles are too small to be seen by the human eye unassisted, they contain thousands to millions of protein molecules.¹²¹ Subvisible particles can mimic immunogenic viruses and bacteria in their size and in exposed surface presence of normally unexposed repetitive epitopes.¹²² Immune responses to subvisible particle aggregates can cause a range of adverse effects, including antibody formation and loss of activity, which may require additional treatment to reverse immunity and can decrease efficacy.¹²¹

Aggregation can occur through covalent interaction, such as dityrosine or mixed-disulfide linkage, or via noncovalent interaction, such as hydrophobic or van der Waals interaction, electrostatic forces, or hydrogen bonding.^{123,124} Aggregation can also be increased by formulation characteristics such as concentration, pH, and ionic strength.⁶⁸ Reversible self-associations of mAbs can result in irreversible, insoluble aggregate formation¹²⁵ and affect a solution's viscoelastic properties, viscosity, turbidity, opalescence, and phase transitions,^{38,39,126,127} especially when refrigerated.¹⁷ This can cause manufacturing difficulties such as membrane clogging, high backpressure, and increased shear stress during pumping,^{33,40} as well as difficulties with fill-finish unit operations such as nozzle clogging and narrowing and an increased risk of fill rejects.

Because protein concentration and aggregation are inversely related, aggregation risk can be decreased by decreasing concentration and increasing volume.^{128,129} Kizuki et al. recently reported that higher fill volume (2 mL vs. 1 mL) decreased the threshold and acceleration of micron-sized aggregate generation in three protein solutions in vials exposed to tri-axial vibration to mimic low g-force stresses.¹³⁰

Aggregation risk can also be decreased by excipients such as sugars, salts, surfactants, or amino acids, which balance attraction and repulsion forces or adjust solution pH, or by lyophilization.⁸⁴ Sugars such as sucrose or trehalose stabilize

mAbs by inducing protein hydration and retaining folded protein structure, decreasing aggregation and denaturation in both powder and liquid formulations, and acting as cryoprotectants to improve the long-term stability of frozen formulations.^{84,131} Salts such as kosmotropic sulfate and phosphate anions increase protein structural stability.¹³¹ Non-ionic surfactants such as polysorbate 20 or 80 or poloxamer 188 are added to most high-concentration mAb formulations to reduce aggregation at the interface,¹¹⁰ but are not always chemically stable during storage, may be subject to instability, and have been associated with several adverse reactions, including injection site pain, acute hypersensitivity, and systemic immunostimulation reactions.^{17,47,84} Polysorbate 80 is included in a concentration range of 0.1–2 mg/mL (0.01–0.2%), polysorbate 20 in a concentration range of 0.1–4 mg/mL (0.01–0.4%), and poloxamer 188 is currently included at a concentration of 0.5 mg/mL in two high-concentration products.¹¹⁰ Amino acids such as arginine, reviewed here as a method of decreasing viscosity, can be included to adjust ionic strength and decrease aggregation, and can be used in both liquid and lyophilized formulations.¹¹⁰ Lyophilization of high-concentration formulations is another strategy to reduce risk of aggregation. Advantages of this approach include ease of aseptic handling and improved stability, while disadvantages include the need for a sterile diluent, potentially long reconstitution times, and equipment costs and complexity.⁸⁴ Difficulties with manufacturing and fill-finish operations may also be alleviated by lyophilization strategies such as partial reconstitution. Finally, using high-volume SC devices with low-to-moderate-concentration formulations may help mitigate aggregation issues.

Immunogenicity

Aggregation due to physical instability can increase the risk of immunogenicity, which involves an unintended immune response to administered antibodies and/or endogenous proteins.^{36,68,110} Anti-drug antibody responses (ADA) can occur following a single dose or with repeated administration of a drug, and can cause injection site and acute hypersensitivity reactions, increase clearance, and decrease efficacy and response duration.^{117,132} ADA are also associated with increased risk of adverse events like anaphylaxis and cytokine storms.³⁶ Less commonly, ADA during replacement therapy can cause adverse immunological events, such as increased relapse rate during recombinant interferon- β treatment in patients with relapsing-remitting multiple sclerosis,¹³³ pure red-cell aplasia in patients taking recombinant erythropoietin,¹³⁴ and thrombocytopenia in patients taking recombinant thrombopoietin.¹³⁵ SC administration has been shown to be more immunogenic than IV administration in mice for total antibody development, but may not have the same effect on inhibitory titers.¹³⁶ When converting an IV mAb product for SC administration, it is typically necessary to evaluate immunogenicity in all anticipated clinical use scenarios, such as monotherapy, restarting treatment after a temporary interruption, and switching from IV to SC treatment.⁷⁵

Immunogenicity is influenced by many factors, including patient-related factors such as age, sex, autoimmune condition, immune status, and human leukocyte antigen haplotype; product-related factors such as dosage form and protein sequence variation, structure, stability, cellular expression system, post-translational modifications, and immunodominant epitopes; and treatment-related factors such as route of administration, dose, duration, and frequency.^{36,137–139} The clinical consequences of immunogenicity can vary widely and are therefore difficult to predict.³⁶ SC injections can sometimes trigger a more successful and efficient immune response than IV infusions, probably due to variations in antigen presentation mechanisms and repeated immune system exposure via dynamic skin antigen-presenting cells, and may be more immunogenic than IV infusions.^{36,140} First-pass interactions with SC-administered proteins can occur at the injection site with skin-resident and monocyte-derived dendritic cells as well as immune cells brought to the skin during an immune response, or in the lymphatic system with dynamic skin-derived antigen-presenting cells during transport to systemic circulation.¹¹⁷ High-concentration mAb formulations may be more immunogenic due to their increased aggregation risks.³⁶

Rates of immunogenicity vary considerably by product and there is no definitive evidence that SC administration results in more ADA than IV administration. In a prospective cohort study of 272 patients with rheumatoid arthritis, SC administration of adalimumab resulted in immunogenicity in 28% of patients, reducing adalimumab serum concentration and likelihood of minimal disease activity or clinical remission; this study did not provide comparative data for IV administration.¹⁴¹ A small cross-sectional study of 51 women with non-metastatic, HER2-positive breast cancer taking trastuzumab (HERCEPTIN®) either in a fixed-dose regimen of 600 mg SC every 3 weeks or a variable-dose regimen of 6 mg/kg IV after a loading dose of 8 mg/kg IV found no evidence of anti-trastuzumab antibodies in patients who received trastuzumab either IV or SC.¹⁴² In a randomized trial evaluating mepolizumab in 576 patients with recurrent asthma exacerbations and eosinophilic inflammation despite high-dose inhaled glucocorticoids, anti-mepolizumab antibodies were found in 4% following 75 mg IV administration compared with 5% following 100 mg SC administration and 2% with placebo.¹⁴³ Similarly, a Phase 3b randomized trial of abatacept in 1,457 patients with rheumatoid arthritis unresponsive to methotrexate found abatacept-induced antibodies in 1.1% with SC administration and 2.3% with IV administration.¹⁴⁴ ADA rates were also similar between IV and SC administration in a randomized trial of vedolizumab in 216 patients with moderate to severe ulcerative colitis¹⁴⁵ and in a randomized trial of tocilizumab in 1,262 patients with rheumatoid arthritis inadequately responsive to previous treatment.¹⁴⁶ No ADAs were reported in either the SC or IV group in a Phase 1 trial of inebilizumab in 28 patients with relapsing multiple sclerosis¹⁴⁷ or in two randomized trials of tezepelumab in 64 healthy volunteers and 12 patients with atopic dermatitis.¹⁴⁸

There are several strategies for immunogenic response prevention, including immunosuppressants such as azathioprine, mercaptopurine, or methotrexate; co-administration of

inhibitors or function-blocking antibodies targeting chemokine receptors; reduction of product-related risk factors like protein aggregates and impurities such as residual host cell proteins or endotoxins; protein modification and re-engineering; and tolerance induction to therapeutic proteins.^{117,132}

Conclusion

Biologics are used to treat a wide variety of conditions. Because many of these conditions are chronic, reliance on IV formulations decreases patient quality of life and constitutes a heavy healthcare burden both in direct and indirect costs. The development of SC formulations therefore confers enormous benefits for patients and healthcare systems, most notably the capacity to self-administer treatment at home in far less time and at a lower cost than is required for IV infusion in a hospital, clinic, or office setting. In this review, we outlined key challenges faced in the development of SC biologics, including volume, concentration, viscosity, osmolality and tonicity, pH, and bioavailability, as well as tactics commonly used to adjust these formulation properties. We also reviewed SC biologic formulation development considerations, including safety profile, development pathways, injection rate, and clinical considerations such as patient adherence and device preference. High-concentration mAb formulations facilitate the administration of the higher mAb doses required for therapeutic efficacy with SC formulations, but require overcoming several key barriers, most notably physical instability. Due to innovations in high-volume SC delivery mechanisms such as OBDSs, high-volume, low-concentration SC formulations without permeation enhancers are able to circumvent these barriers and are therefore a valuable alternative to high-concentration, low-volume formulations. In the context of the challenges associated with high-concentration formulations, this review provides new insights about the potential value of high-volume SC biologics.

Abbreviations

ADA	Anti-drug antibody responses
ADC	Antibody-drug conjugate
AUC	Area under the curve
CD	Cluster of differentiation
C _{max}	Peak concentration
cp	Centipoise
C _{trough}	Trough concentration
FDA	Food and Drug Administration
HCP	Health care professional
HER2	Human epidermal growth factor receptor 2
IgG	Immunoglobulin G
IV	Intravenous
mAb	Monoclonal antibodies
OBDS	On-body delivery system
PD	Pharmacodynamics
PD-1	Programmed cell death protein 1
PFS	Prefilled syringe

pI	Isoelectric point
PID	Primary immunodeficiency disease
PPI	Protein-protein interaction
rhuPH20	Recombinant human hyaluronidase
SC	Subcutaneous
TEAE	Treatment-emergent adverse events

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