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Ongoing Challenges to Develop High Concentration Monoclonal Antibody-based Formulations for Subcutaneous Administration: Quo Vadis?

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ABSTRACT

Although many subcutaneously (s.c.) delivered, high-concentration antibody formulations (HCAF) have received regulatory approval and are widely used commercially, formulation scientists are still presented with many ongoing challenges during HCAF development with new mAb and mAb-based candidates. Depending on the specific physicochemical and biological properties of a particular mAb-based molecule, such challenges vary from pharmaceutical attributes e.g., stability, viscosity, manufacturability, to clinical performance e.g., bioavailability, immunogenicity, and finally to patient experience e.g., preference for s.c. vs. intravenous delivery and/or preferred interactions with health-care professionals. This commentary focuses on one key formulation obstacle encountered during HCAF development: how to maximize the dose of the drug? We examine methodologies for increasing the protein concentration, increasing the volume delivered, or combining both approaches together. We discuss commonly encountered hurdles, i.e., physical protein instability and solution volume limitations, and we provide recommendations to formulation scientists to facilitate their development of s.c. administered HCAF with new mAb-based product candidates.

Introduction

The desire to improve patient convenience and compliance with monoclonal antibodies (mAbs) and mAb-based biopharmaceutical treatments has led to a movement away from intravenous (i.v.) administration by health-care professionals to the more patient-friendly, self-administration by subcutaneous (s.c.) injections. The restricted dosing volume of conventional s.c. injections (< 2 mL) combined with the high dosing level requirements of many mAbs and mAb-related products (often > 100 mg/dose) are powerful drivers to require the development of highly concentrated antibody formulations (HCAF). Pharmaceutical development challenges for HCAF include high solution viscosity and/or physical instability, i.e., irreversible aggregation and/or limited solubility, leading to difficulties during large-scale manufacturing, long-term storage, and delivery/administration to patients.

There is a growing interest in answering scientific questions related to the development of HCAF. For example, a literature search with Scopus, starting with papers in 1992, shows a remarkable and steady increase in the number of published articles on HCAF, especially in the last decade (Fig. 1). Numerous original research papers and excellent reviews have appeared detailing various HCAF challenges including the fundamental aspects of protein-protein interactions (PPIs) at high mAb concentrations,[1,2] physical instability of mAbs at high concentrations,[3,4] high solution viscosity effects on drug product manufacturing,[5–7] device development for s.c. delivery using prefilled syringes with autoinjectors,[8–10] and in vivo performance during and after s.c. delivery including bioavailability, pain upon injection, and immunogenicity.[11–14] Moreover, two books have been published with relevant sections on HCAF: (1) “Current trends in monoclonal antibody development and manufacturing” edited by Shire et al.[15]; and (2) “Challenges in protein product development” edited by Warne and Mahler.[16] Another recent literature source

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[1] One of the initiators of this commentary was Prof Dr Wim Jiskoot. Sadly, we lost our colleague, an excellent scientist and friend, to cancer during the writing of this commentary. In his last days, Wim encouraged us to finalize the commentary, and expressed his wish to share our perspectives on high concentration monoclonal antibody-based formulations with the biopharmaceutical community.

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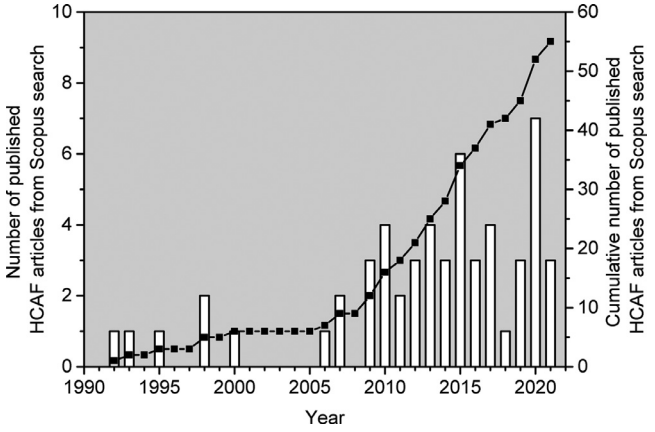

Fig. 1. Published articles on high concentration antibody formulations (HCAF) over the past three decades. Number per year (bars) and cumulative number (line + squares) of journal articles on HCAF were obtained through a literature search using Scopus. The search performed on October 2, 2021, with the search terms "TITLE (high) AND TITLE (monoclonal) AND TITLE (concentration) AND TITLE (antibody)" returned in total 55 hits with the first article published in 1992.

relevant to this commentary is a comprehensive review of formulations of commercially available mAbs by Strickley and Lambert with a wealth of information on mAb drug product dosage forms and their related formulation development strategies administered by various routes of administration including s.c. delivery.[17] In addition, to facilitate the sharing of information and viewpoints from technical experts across various disciplines and organizations, industrial and academic scientists came together in 2018 to organize the SC Drug Delivery and Development Consortium including defining eight problem statements "to highlight key gaps, unmet needs and actionable issues".[9] In this commentary, we focus on illustrative examples from the literature we have selected to highlight our key points. To this end, our commentary is not a comprehensive review and the reader is referred to the references described above for this purpose.

The main subject of our commentary is to focus on one key issue commonly encountered by formulation scientists during development of HCAF dosage forms, namely, how to maximize the dose of the mAb drug. Considering the simple relationship of dose = concentration x injection volume, one can either increase the protein concentration, increase the injection volume, or do both to reach the highest dose level. These options and their associated formulation development challenges, as well as opportunities for overcoming them, to ensure a stable, well tolerated HCAF for s.c. administration are the focus of this commentary. In this context, formulation scientists need to tackle issues created by the non-ideal solution behavior of mAbs at high protein concentrations, especially as related to their reversible self-association due to concentration dependent PPIs. The practical challenges of such PPIs in highly concentrated mAb solutions include viscosity increases, and solubility limitations including gelation or phase separation, especially under refrigerated conditions. This in turn can lead to formulation process development issues with typical unit operations, including ultrafiltration-diafiltration, sterile filtration, and fill-finish steps, as well as syringeability and injectability problems during patient delivery. In addition, protein aggregation may cause clogging of filters during manufacturing and of syringe needles during administration of the product to the patient. Moreover, formation of aggregates may lead to diminishing the potency and enhancing the risk to induce anti-drug immune responses. Although we will focus on challenges with HCAF for s.c. delivery, these formulation challenges can become even greater when smaller dosing volumes are needed. For example, with intravitreal injections, with volumes ranging from 20 to 100μL (most common: 50 μL) using the narrow 30 G needles.

HCAF: What are We Talking About?

Current biopharmaceutical formulations with mAbs and mAb-based modalities widely differ in protein concentration, ranging between 12 μg/mL and 200 mg/mL[17] (Fig. 2). The first question is then: How does one define HCAF? There is no generally accepted definition in the literature. For instance, in the 2018 book "Challenges in protein product development" edited by Warne & Mahler, the authors do not define what they consider high concentration formulations of proteins.[16] On the "low end" of the high concentration range of approximately 10–20 mg/mL, mAbs typically deviate from ideal solution behavior due to PPIs. In other words, in dilute protein solutions (< ~20 mg/mL), the activity coefficient of a protein approaches unity, i.e., the effective concentration is similar the actual concentration. In contrast, in more concentrated solutions, PPIs between protein molecules can lead to large differences between the effective and actual protein concentrations (i.e., increases in the activity coefficient). For example, in the well-studied case of hemoglobin, the effective protein concentration is similar (~1.2-fold) to the actual concentration at 20 mg/mL, but is much higher, e.g. ~10-fold and ~20-fold, than the actual concentration at 200 and 250 mg/mL, respectively.[18] In fact, many biophysical techniques to characterize protein hydrodynamic size, e.g., dynamic light scattering and sedimentation velocity analytical ultracentrifugation, typically require mAb samples to be diluted to low protein concentrations (< ~20 mg/mL) to avoid artifacts due to such non-ideal solution behavior. This analytical limitation has led to evaluation of spectroscopic and newer analytical approaches, e.g., hydrogen exchange mass spectrometry (HX-MS), to directly characterize PPIs at high mAb concentrations.[19,20] On the "high end" of the high concentration range, it has been estimated, based on geometrical assumptions and packing models, that an upper limit of mAb concentrations is approximately 500 mg/mL.[21] Interestingly, as another benchmark, Nature's upper limit for soluble protein concentration in cells is ~300 mg/mL in the presence of other macromolecules and solutes,[22] and ~320 mg/mL in human tissue (within the eye lens).[23] Thus, as a starting point, the definition of HCAF is bracketed somewhere within a very wide protein concentration range of ~20 to ~500 mg/mL.

During mAb drug product development, the term HCAF is often first used when changing from a first-in-human i.v. formulation

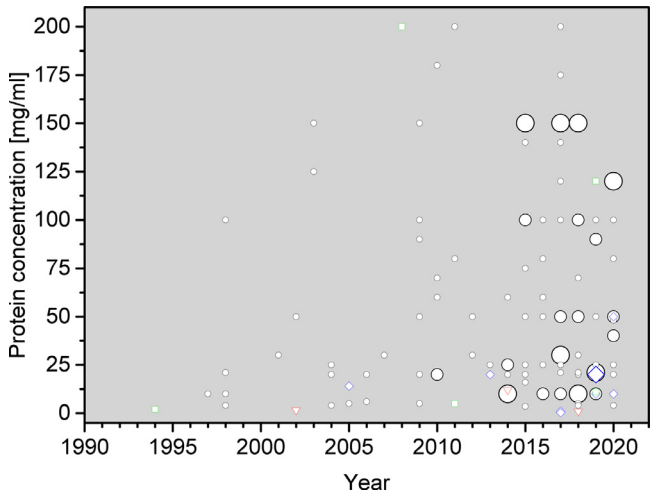

Fig. 2. Overview of the number of marketed mAb-based drug products approved each year, and their respective protein concentration, as reported by Strickley and Lambert17. mAbs (black circles), antibody-drug complexes, ADCs (blue diamonds), mAb fragments (green squares), and other variants such as BiTE® (bi-specific T-cell engagers; fusion proteins consisting of two single-chain variable fragments (scFvs) of different antibodies) and immunoconjugates (red triangles). The size of the symbols reflects the number of marketed products (° 1, o 2 and O 3 products).

(typically at 10–50 mg/mL) to a s.c. formulation. Consequently, high protein concentration for a s.c. formulation is defined as a concentration being higher than that of the i.v. formulation, but this value can obviously differ across mAb-based clinical candidates. Instead of using the protein concentration, Garidel et al. have suggested to use other properties of the formulation such as viscosity, aggregation, or phase separation to define HCAF.[21] This approach is in line with that used by Kovarcik, practically referring to HCAF as the point where viscosity increases become an issue for s.c. injection through G26+ needles.[24] Although such pragmatic definitions are tempting, they come with caveats and limitations: Would a HCAF falling under these definitions with, e.g., a high solution viscosity and/or aggregation issues, after addition of an effective viscosity reducing excipient or a stabilizing, non-ionic surfactant that mitigates aggregation, no longer be considered as a HCAF even though the protein concentration itself is as high as before? Moreover, unacceptable irreversible aggregation can occur readily even at very low protein concentrations (< 1 mg/mL), whereas in other cases, mAbs can be formulated at 100–200 mg/mL in a liquid formulation with decent stability, e.g., belimumab is available at 200 mg/mL.

One could argue that a pragmatic and arbitrary definition of "high concentration" during HCAF development may be a protein concentration of simply 100 mg/mL or higher. Notably, out of the 126 entries in the list of commercial mAb formulations from the review by Strickley and Lambert,[17] 23 have a mAb concentration of 100 mg/mL or more and would thus qualify as HCAF. Rodriguez and colleagues[25] recently used the term "ultra-high concentration" in the same way as suggested by Whitaker et al.[26] for mAb formulations > 150 mg/mL to differentiate those from mAb formulations at high (50–150 mg/mL) and low concentrations (< 50 mg/mL). However, with advances in formulation technologies, what is defined as a (ultra-)high concentration protein formulation today might no longer be considered such in a decade from now, given that a mAb concentration of 500 mg/mL is at least theoretically feasible. Consequently, in this commentary, we will further explore the challenges and solutions of administering HCAF subcutaneously independently of a particular underlying protein concentration, but rather with the overall goal to deliver the maximum dose.

What Formulation Approaches are Available to Enhance the Concentration of mAbs in A 1–2 mL Injection Volume?

Excipient Selection for Liquid and Lyophilized Formulations

HCAF display a wide variety of solution viscosity values depending on the extent to which a particular mAb is prone to reversible self-association via its propensity to undergo PPIs. The mAb specific nature of this observation implies a key role for the complementarity-determining regions (CDRs) in the variable chains of the Fab region of a mAb. For example, it has been directly demonstrated with two different IgG1 mAbs using HX-MS[19,27] that both Fab-Fab interactions via the CDRs as well as Fab-Fc interactions via the CDRs and the C_H3 domain of the Fc region are the interaction sites of PPIs, resulting in elevated viscosity values for these two mAbs at high protein concentrations.

Strategies for excipient selection to reduce mAb solution viscosity are based on specific molecular mechanisms of PPI including excluded volume repulsions, electrostatic repulsions/attractions, and hydrophobic attractions.[28] For example, Johnston et al. created large reversible mAb clusters in water by minimizing the net protein charge with a buffer pH near the isoelectric point (pI) of the mAb, coupled with adding high concentrations of the co-solute trehalose as an extrinsic crowder, to provide strong depletion attraction. This created concentrated mAb-dispersions of equilibrium protein nanoclusters which reversibly dissociate into active monomers.[29] As another example, using the same HX-MS approach described above, the ability of series of charged excipients for one mAb, and a series of hydrophobic excipients with a second mAb, were demonstrated to interact with specific sites within the mAbs to disrupt specific PPI molecular interactions (i.e., charge-charge and hydrophobic attractions, respectively) and lower solution viscosity at high protein concentrations.[30,31]

Although viscosity reduction of aqueous HCAF solutions by excipient addition can often be achieved by changing the ionic strength, through pH optimization/buffer selection, or the use of specific additives, e.g., amino acids such as arginine,[15] a second key challenge is then to ensure adequate long-term storage stability of the mAb under these same HCAF conditions. Wang and co-authors provide an excellent overview of the physicochemical factors that play a role along with a "toolbox" to push the mAb concentrations up to a highest possible limit while maintaining physical stability.[32] As a general expectation, and as shown experimentally with various therapeutic proteins including for example rhIL-1ra, irreversible protein aggregation occurs more readily at high vs. low concentrations, consistent with elevated PPIs and higher activity coefficient values.[33] In terms of optimizing both viscosity and physical stability profiles simultaneously for HCAF, Whitaker et al. examined ~55 pharmaceutical excipients by empirical screening to identify additives that not only lowered the viscosity of two mAbs at > 150 mg/mL to levels allowing use in an autoinjector, but also preserved the storage stability profile of the mAbs.[26] Key excipients for viscosity reduction found in commercial s.c. mAb-based products are arginine, sodium chloride and, less frequently, other amino acids such as glycine, lysine and proline.[17] Novel excipients, i.e., not found in FDA/EMA approved products, for viscosity reduction are described in the literature. Yet to the best of our knowledge, they are not found in mAb-based drug products for clinical application.[34,35]

Although excipients used to improve mAb stability in HCAF during storage are considered inactive ingredients, they are not necessarily stable during storage. For example, non-ionic surfactants such as polysorbates are commonly added to minimize mAb aggregation. However, polysorbate degradation can be induced by enzymes among the residual host cell proteins (HCPs); various esterase enzymes can hydrolyze the ester bond in the surfactant molecule. Thus, polysorbate instability during storage is due to certain HCPs being co-purified with the mAb,[36,37] and this effect can be more pronounced at high mAb concentrations, e.g., as described by Labrenz et al. for polysorbate 80 degradation in formulations of 30, 60 and 90 mg/mL mAb.[38] As a result, formulation scientists may have to face loss of surfactant functionality in HCAF upon storage,[39,40] leading to the formation of free fatty acid-related particles, as well as the destabilization and aggregation of mAbs causing accumulation of proteinaceous particles.

If liquid HCAF are successfully designed to minimize solution viscosity, yet fail storage stability tests due to aggregation, then lyophilized dosage forms may be used as an alternative formulation approach. However, not only does this add complexity to the s.c. administration, e.g., need for a diluent and a reconstitution step, it also precludes the use of standard prefilled syringes and autoinjectors for self-administration by patients. This would thus require consideration of more complex dual-chamber syringes containing the freeze-dried product and the diluent in separate chambers. In addition, such freeze-dried HCAF can have very long reconstitution times of 30 min or more.[21,41] This can be due to the physical properties of the lyophilized cake, e.g., pore size and degree of crystallinity, which in turn can affect reconstitution times. Therefore, successful development of lyophilized HCAF is dependent not only on the formulation, i.e., mAb, excipients, but also the freeze-drying process parameters.[42] Interestingly, lyophilized HCAF also have the potential to be used with newer, alternate formulation technologies to facilitate even

higher concentrations of mAbs with HCAF as described in the next section.

Alternative Formulation Strategies with mAb Crystals or Protein Powders

Although still in development, alternative formulation approaches to obtain HCAF offer promise in terms of obtaining even higher mAb concentrations for s.c. delivery. One approach is to prepare solid protein powders and suspend them as dispersions in non-aqueous solvents for parenteral administration. This approach, as reviewed recently by Marschall et al.,[43] combines stabilization of high concentration mAbs as lyophilizates in a dosage form with low viscosity, yet without the need for reconstitution prior to administration to a patient. For example, lyophilized and milled protein powders have been prepared for this application.[44] Alternatively, Maa et al. have examined the use of spray-drying to prepare high concentration mAb powder suspensions.[45] In another study, spray-dried protein powders were prepared and suspended in ethyl acetate, benzyl benzoate or Miglyol 840 at > 300 mg/mL of the mAb. In addition, Bechthold-Peters et al. have discussed other protein drying techniques, e.g., supercritical fluid drying, that may be useful to prepare protein powders for this application.[46,47]

Alternatively, the formation of solid mAb crystals has been successfully used to develop highly concentrated mAb suspensions that have been tested in preclinical and early clinical development. A few (small) companies specialize in pushing this protein crystallization concept for HCAF. The first attempts were spearheaded by Altus Biologics.[48,49] S.c. administered mAb crystals may lead to sustained release effects. Considering the slow absorption of mAbs in solution from the s.c. injection site with a typical t_{max} of 5 days, and the long blood circulation times of mAbs with a typical half-life ~2–4 weeks, an extra sustained release effect may not be desired or required. Moreover, assuring constant mAb crystal quality may not be trivial[24] and little information is available on possible immunogenicity of these crystalline mAb formulations.

HCAF approaches using mAb-crystals or powders of freeze-dried mAb have yet to be approved by the FDA or EMA. Considering the long period of time these drying techniques and crystallization strategies have been studied in academic and industrial settings, the question may be raised: is this approach of creating dispersions of protein powders or crystals in non-aqueous solvents to develop high dose s.c. protein formulations a dead-end street for mAb-based products? Despite some promising formulation and stability data, there are certainly many technical challenges to scale-up this drying technology including how to reproducibly prepare protein powder dispersions/crystals, as well to perform fill-finish into primary containers under sterile conditions.

What if Formulation Approaches Fail to Provide a "Solution"?

Subcutaneous Injections: What is the Injection Volume Limit? And what About Injection Techniques? and Hyaluronidase?

In the literature, one consistently finds maximum injection volumes for conventional s.c. injections on the order of 1–2 mL. However, in a minireview, Mathaes et al. conclude that volumes larger than 2.5 mL may be considered, as the success of the larger volume s.c. injection depends on numerous multifaceted considerations including injection rate, injection site, injection volume, leakage/tissue back pressure, drug product formulation composition and individual patient characteristics including pain sensation sensitivity by the patient.[8] As examples of new precedents toward higher injection volumes for s.c. delivery, one can consider the recommended s.c. injection protocol for 600 mg of casirivimab and 600 mg of imdevimab injections for COVID-19 mAb therapy; it prescribes four 2.5-mL injections, at a mAb concentration of 120 mg/mL, with injections at four different sites.[50] Another recent example is the s.c. injection of 420 mg/3.5 mL of evolocumab through a wearable (on-body) device over a period of 5–9 min.[51]

The maximum s.c. injection volume can be further dramatically increased by use of recombinant hyaluronidase PH20 (rHuPH20), an enzyme that degrades the local hyaluron matrix. rHuPH20 itself (Hylenex™) is used for rehydration therapy, increasing absorption of other s.c. injected drugs and with imaging agents.[52] In addition, rHuPH20 is co-formulated with four anti-cancer mAb-drugs and is sequentially dosed in human immunoglobulin replacement treatment with a maximum allowed s.c. injection volume of up to 600 mL (Hyqvia™).[8,17] These four s.c. administered, co-formulated rHuPH20-mAb anti-cancer products demonstrate that s.c. injections are not just developed for self-administration by the patient. S.c. injection can also be beneficial within a hospital setting as the time spent per patient is significantly reduced when switching from i.v. to s.c. delivery. Interestingly, in the case of immunoglobulin replacement therapy, a lifelong intervention for patients with primary immunodeficiency disease, pump driven s.c. infusion volumes without hyaluronidase up to 50 mL per injection site have been part of standard administration protocols for several years.[11,53]

Strategies to Inject Viscous Media: from High Pressures to Needle Shapes to Lubricating Oils

Preferred solution viscosity values for subcutaneous injections, especially when the liquid formulation is filled into prefilled syringes, are below 10 cP.[45] The options to select delivery devices for viscous parenteral solutions beyond that level are numerous and multifaceted.[54] The existing arsenal ranges from using tapered needles, i.e., they have a larger diameter at their proximal end than at their distal end, to needles with ultra-thin walls while maintaining the same outer diameter,[55,56] to high pressure injectors with various pressure profiles, which are available in various types. Some are designed to inject solutions with viscosity values up to 1000 cP through 25 G needles such as the Bespak and Arc-Bios systems.[9] Of course, these technologies typically result in longer development times and come at an extra cost. Novel, still untested, ideas encompass syringes with internal lubrication phases at the barrel and needle wall.[57] Even if it is possible to inject highly viscous formulations, one still needs to overcome the manufacturing issues for a highly viscous drug product.

In conclusion, taking the time and making the effort to go through various s.c. injection strategies may be time well-spent, especially for HCAF with high-viscosity values that contain mAbs that are not stable in the presence of viscosity lowering excipients.

Avoiding the Problem from the Start: mAb Lead Optimization or alternative mAb Formats

If all the above options fail and no acceptable administration strategy for a highly concentrated therapeutic mAb product can be found: What then? One can go back to the drawing board and reengineer the mAbs with improved developability properties, consider using mAb-fragments, or generate more potent mAb molecules leading to lower dose ranges.

Protein Engineering of mAbs

The constant part of the four IgG isotypes is highly conserved with over 95% sequence homology.[58] The variable parts of the IgG molecules show much more variation and are the sites of the CDRs that dictate antigen binding specificity. For example, for the IgG1 isotype family, the predominant isotype of the therapeutic mAbs, the overall amino acid sequences are overall very similar, with only the twelve CDRs in the variable regions being highly variable; i.e., around 120

−180 amino acids in a 1350+ amino acid IgG1 molecule are non-conserved. The question is then, can mAb-engineering improve the developability of the molecule, both for viscosity reduction and preventing aggregation, without affecting its antigen binding or Fc pharmacological activities?[59] Reviews describing different approaches and successful attempts have been recently published and several examples follow below with more details.[60,61]

Wu and colleagues engineered their IgG1 molecule by increasing its pI by mutations in the variable region, by altering the hydrophobicity of the CH3-H region, and by introducing an N-glycosylation site in the CH2-H region. They reported for the last approach a solubility increase of at least eight times with preserved binding affinity.[59] Kant and co-workers identified aggregation-prone regions (APRs) in mAbs. The only exposed APRs were found in the CDR-3 heavy chain region as described by the authors: 'Although aggregation-prone regions are thought to occur in the antigen binding region to drive hydrophobic binding with antigen, we were able to rationally design variants that display a marked decrease in aggregation propensity while retaining antigen binding through the introduction of artificial aggregation gatekeeper residues'.[62] These groups have focused their mAb engineering on aggregation reduction. Protein engineering approaches achieving both aggregation and/or viscosity reduction with mAbs have been published as well,[63–66] but the fundamentals of altering amino acid sequences to reduce PPIs between mAb molecules, while maintaining the biological antigen binding activity, are still not fully understood.

Design of New mAb-based Formats Including Conjugates and Fragments

Alternative strategies to increase the potency of the mAb used in oncolytic treatments include covalently attaching a highly active pharmacological unit to the protein-chains. Ten of these antibody-drug-conjugates (ADC) are presently approved and commercially available. All are antitumor agents, stored in lyophilized form except one; they are injected intravenously, not subcutaneously, with ADC concentrations far below 100 mg/mL.[17] So far, the ADC route of pharmacologically empowering the mAbs has not required development of HCAF.

Another approach is to design antibody fragments instead of using the entire mAb molecule. Several commercially available mAb fragments (Fab) are available including abciximab and ranibizumab. In another case, the basic mAb-molecular structure can be redesigned. Certolizumab pegol is a combination of only a Fab fragment of an anti-TNF-alfa mAb and polyethylene glycol (40 kDa). Its total molecular weight is 91 kDa. Certolizumab pegol is available as a 200-mg/mL solution for s.c. injection and its pharmacokinetic behavior (t_{max} and $t_{1/2}$) is comparable to full-length mAbs. Other examples are nanobodies, or single domain antibodies, containing a single monomeric variable antibody domain derived from camelid heavy chains with a molecular weight of 12−15 kDa. The only approved nanobody formulation is a bivalent single domain antibody, caplacizumab, with a molecular weight of 28 kDa and a relatively short in vivo half-life leading to a s.c. injection interval of one day.[67]

Conclusions and Recommendations

The drive to develop HCAF comes from the increased "popularity" of the s.c. route of administration which offers improved patient convenience and compliance. HCAF pose many challenges during the development phase of these dosage forms, e.g., unacceptable solution viscosity increases and physical instability of the protein, e.g., aggregation, limited solubility and phase separations. This commentary points out that formulation scientists involved in the development of high concentration mAb-product candidates are not empty handed in terms of approaches and solutions to these challenges.

First, s.c. administration of mAb-based products is a multidisciplinary topic and a team of experts across various departments and organizations is needed to choose the most promising from these different pathways. Early-stage interactions with the mAb-protein molecular biologists might help in selecting mAbs candidates, not only on the basis of target affinity or pharmacological action such as binding affinity, but also to minimize aggregation and PPIs through high throughput screening and in silico screening of mAb-libraries. Such developability assessments to improve the "drugability" of a mAb helps to circumvent HCAF issues upfront allowing for a more streamlined development pathway.[68,69] Often a combination of technical hurdles and timeline considerations require selection of biologically promising mAb therapeutic candidates with a high viscosity profile at high protein concentrations. Thus, defining viscosity limits and syringeability behavior -with and without an autoinjector- should be in the target product profile to set clear development goals for all departments on the team including clinical, regulatory, manufacturing and device experts. In addition, an immunologist and pharmacokineticist should be included to shine light on expected potential issues with the immunogenicity and bioavailability upon s.c. administration.

Second, as shown in Table 1, there are different approaches that can lead to an acceptable mAb drug product dosage form to be administered at the required high dose by the s.c. route. The most common approach is development of standard liquid or lyophilized HCAFs. In terms of excipient selection, the formulation scientist can consider a rational design approach, an empirical approach, or a combination of these two options. The rational approach includes elucidation of the sites of PPIs and the nature of such interactions followed by selection of excipients that will disrupt such undesired interactions at high concentrations.[30,31] The empirical approach includes screening different classes of additives from a list of excipients already found in approved parenteral drug products.[26] Often, the former approach is scientifically satisfying yet time-consuming and lacks certainty of success, while the latter approach is labor intensive and difficult to justify in terms of why such additives work. Thus, a combination of screening studies to identify excipient hits, followed by or in parallel with more mechanistic preformulation characterization studies to understand the nature of PPIs within a given mAb and the role excipient hits play to disrupt such interactions, can be considered the optimal approach. Either way, the effect of viscosity lowering excipients on the long-term storage stability and aggregation propensity remains a key topic for consideration as part of liquid and lyophilized HCAF development.

Table 1 also provides alternative approaches to HCAF development vs. standard liquid or lyophilized dosage forms. Newer formulation technologies such as preparation of crystalline mAbs or amorphous protein powders suspended in (non-)aqueous media are being studied, but such technologies have yet to be established beyond proof-of-concept studies. A more practical approach is to push the injection volume of s.c. delivery to higher limits by using multiple injection sites, formulation with recombinant hyaluronidase, or use of new delivery devices. Such increases in injection volumes have demonstrated success, but can add complexity and cost.

Finally, when the aim to develop a patient-friendly s.c. administration via HCAF fails, two remaining options include either molecule redesign or alternative routes of administration. The redesign of the candidate molecule includes altering the mAb sequence with improved developability, utilizing fragments of the mAb, or designing more potent molecules. Obviously, this approach sends the mAb drug candidate back to basic research with major delays in product development. In contrast, there are plenty of options to improve patient's convenience and experience with i.v. administrations, e.g., reducing the residence time for patients receiving the infusion by administering the same dose in a smaller volume. In the case of anti-TNF-alpha

Table 1
Summary of Different Formulation and Delivery Approaches to Achieve the Required mAb Dose for Subcutaneous Administration.

Approach	Advantages	Disadvantages
Liquid formulation optimization	• Straightforward, if successful either through an empirical screening or rational design approach • Feasibility demonstrated for several marketed products • Standard packaging/devices can be used	• Limited number of approved excipients • Optimal conditions for low viscosity conditions and physical stability may require different formulation compositions • Prediction of viscosity and/or aggregation at high concentrations, based on biophysical parameters obtained at low protein concentrations, may be possible but is still challenging
Freeze-dried formulation optimization	• Feasibility demonstrated for several marketed products and lyophilization is an established technology. • Concentration may be increased by reconstituting with a lower reconstitution volume than starting volume • Many proteins display high stability as lyophilized drug products	• Additional manufacturing step required with associated increased costs • Additional reconstitution step required for patient administration • Long reconstitution times for high concentration formulations
Crystalline protein suspensions in (non-)aqueous solution	• Low viscosity values can typically be achieved • Very high protein concentrations can be achieved	• Complex manufacturing • Potential colloidal instability of suspension • No marketed products to date
Amorphous, micronized protein/excipient powders in non-aqueous solvents	• Low viscosity values can typically be achieved Very high protein concentrations can be achieved	• Complex manufacturing • Potential colloidal instability of suspension • No marketed products to date
Push injection volume to the limit	Depending on the dose and highest achievable concentration, one can select from different options: • Increase volume per injection • Split one dose into several s.c. injections • On-body injectors- s.c. infusions for prolonged injection times and higher volumes	• May cause discomfort to patients • Devices come with extra cost and complexity
Formulation with recombinant hyaluronidase	• Higher volumes can be injected s.c., • Higher dose at same protein concentration, or same dose at several-fold lower protein concentration may then be feasible • Commercial products are available	• Coformulation with hyaluronidase may cause stability problems • Analytical challenges in case of co-formulation • Consecutive injections may be required • Hyaluronidase may cause tissue damage
Design devices, including needles for highly viscous formulations	• Higher protein concentrations can be administered as the maximum viscosity limit is increased	• Problems with unit operations during manufacturing for viscous formulations (e.g., concentration, filtration, filling) • Viscosity may be linked to other physical instabilities (solubility limits including gel formation, phase separation) • Additional development costs and time due to device aspects • Higher regulatory effort with delivery devices

mAb-based drugs, both i.v. and s.c. versions are widely available often with similar efficacy and safety profiles depending on the target patient population and disease.[70] For example, s.c. administered adalimumab (an anti-TNF-alpha mAb) remains the top selling mAb drug (2019 sales of $19.6 billion in the United States), yet the i.v. administered anti-TNF-alpha mAb infliximab remains the number eight selling mAb drug with sales in the United States of $5.3 billion in 2019.[71] Thus, patient preference plays a key role in selection of HCAF dosage forms. Although focusing on ways to provide s.c. administered dosage forms is a priority in many cases, a backup strategy of making i.v. delivery experience more convenient and patient friendly has the potential to be well-received by many patient populations being treated for different disease states.

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