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Review Article

US FDA-approved therapeutic antibodies with high-concentration formulation: summaries and perspectives

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ABSTRACT

Thirty four (34) of the total US FDA approved 103 therapeutic antibody drugs, accounts for one third of the total approved mAbs, are formulated with high protein concentration (100 mg/mL or above) which are the focus of this article. The highest protein concentration of these approved mAbs is 200 mg/mL. The dominant administration route is subcutaneous (76%). Our analysis indicates that it may be rational to implement a platform formulation containing polysorbate, histidine and sucrose to accelerate high concentration formulation development for antibody drugs. Since 2015, the FDA approval numbers are significantly increased which account for 76% of the total approval numbers, i.e., 26 out of 34 highly concentrated antibodies. Thus, we believe that the high concentration formulations of antibody drugs will be the future trend of therapeutic antibody formulation development, regardless of the challenges of highly concentrated protein formulations.

Statement of Significance Thirty four therapeutic antibodies approved by the US FDA with high-protein concentration formulation (100 mg/mL or above) are analyzed.

KEYWORDS: therapeutic antibodies; high concentration; formulation; US FDA; subcutaneous; approval; dosage; administration route; formulation composition; pre-filled syringe; lyophilization; excipient

INTRODUCTION

The first US FDA-approved monoclonal antibody (mAb) drug (approved in 1986 for marketing) is muromonab-CD3 (Orthoclone OKT3) with low protein concentration formulation at only 1 mg/mL (1). Ever since, as of October 2021, FDA has approved 103 therapeutic antibody drugs including antibody-based therapeutics such as antibody drug conjugates in the past 35 years for marketing (2). Most of these 103 antibody drugs are formulated with low protein concentrations ($\leq$50 mg/mL) partially because of technical challenges in developing high-concentration formulation for antibody drugs. These technical challenges have been discussed and reviewed in several excellent review articles and book chapters (3–7). More than one decade later after FDA approved the first mAb, FDA approved in 1998 the first mAb formulated with 100 mg/mL protein concentration (Synagis, Palivizumab) (8). While drafting this article, we notice that an excellent review of formulations of commercially available antibodies approved by global regulatory agencies was already published in March 2021 (9). Our article, as indicated in the title, is focused only upon the US FDA-approved therapeutic antibodies with high-concentration formulation.

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Developing therapeutic antibodies with high-concentration formulation is now highly demanded in biopharmaceutical industry because of the following several reasons. This demand is partially driven by increasing subcutaneous (SC) administration for antibodies approved for commercial and/or late clinical stage. Other reasons are to extend shelf life and patent protection period of approved biologics with increased protein concentration (10). One of the examples is Merck & Co. (MSD)'s Keytruda (pembrolizumab), which was initially approved by the US FDA in 2014 for marketing. This is a lyophilized product, and can be reconstituted at 25 mg/mL protein concentration. Merck & Co. started several years ago to develop high concentration formulation for SC administration for its already marketed Keytruda. Merck reported most recently the phase I clinical trial results of Keytruda with SC delivery at the 2021 Annual Meeting of the American Association for Cancer Research (AACR abstract #: CT143) (11).

Therapeutic antibodies with high-concentration formulation can also offer other benefits, such as suitable for advanced administration (for instance self-administration with auto-injection devices). Therefore, reduction in administration time and medical resource utilization benefits both patients (especially for those with chronic diseases) and the entire healthcare system (12).

It is rarely defined how high is the high-concentration formulation (13). General speaking, equal to and more than 100 mg/mL protein concentration is widely regarded as high-concentration formulation and lower than 50 mg/mL is typically regarded a low concentration formulation. However, the range in between 50 and 100 mg/mL is usually a controversial grey range. Thus, in this review article we set $\geq$100 mg/mL as a criteria of high-concentration formulation for our data analysis. Based upon the publicly available information (dominantly from FDA website), we comprehensively analyzed the 34 FDA-approved therapeutic antibodies with at least 100 mg/mL protein concentration (as of October 2021). The raw data of these 34 antibody drugs are compiled in Table 1. The analysis of these data shown in Table 1 includes (a) FDA approval number versus approved year, (b) formulation composition analysis, (c) dosage form, (d) concentration analysis, (e) administration route analysis, (f) top sponsors of FDA-approved therapeutic antibodies with high-concentration formulation and (g) IgG type. In this article, we discussed some interesting and/or unexpected conclusions. We also proposed our own perspectives and insights based upon these results, and trends from the data analysis.

SUMMARIES

FDA approval number vs. approval year

As shown in Fig. 1, the FDA approval numbers are significantly increased since 2015. The approval number since 2015 (twenty six) accounts for 76% of the total approval number (thirty four), suggesting the increasing demand of high-concentration formulation in recent years from biopharmaceutical industry.

Formulation excipient analysis

The formulation excipients of the 34 therapeutic antibodies approved by the US FDA with high-concentration formulation were analyzed. The frequency of the formulation excipients used in each formulation composition in these 34 antibodies were calculated, and only formulation excipients with greater than 5% frequency are presented in Fig. 2. The major formulation excipients with more than 10% frequency are polysorbate (94%, including both polysorbate 20 and polysorbate 80), histidine (82%), sucrose (48%), arginine (27%), sodium chloride (NaCl, 18%), methionine (18%), acetate (12%) and trehalose (12%). Other formulation excipients with less than 10% frequency are proline, sorbitol, sodium phosphate, poloxamer 188, ethylenediaminetetraacetic acid (EDTA), citric acid, mannitol, glutamate, glycine, sodium citrate, sodium succinate and lactic acid.

Dosage form presentation

As demonstrated in Fig. 3, the dosage forms of the 34 antibodies with high-concentration formulation may be categorized as the following: liquid solution (Liq) only, lyophilized powder (Lyo), pre-filled syringe (PFS) only, and both PFS and Lyo. Please note that Liq dosage form is liquid in a vial, and the PFS represents also liquid but in pre-filled syringe. The Liq and the PFS presentations take up predominantly the market shares at 43% and 39%, respectively, followed by both PFS and Lyo at 12%. Unexpectedly, only 6% (2 products) of these 34 antibodies are formulated as lyophilized powders (Lyo) only.

Recent trends in the pharma injectable market have been observed such as a shift to self-medication to enhance patient convenience; tightened regulatory scrutiny and quality requirements for patient safety; and continuous exploration in patient experience and compliance. These trends are linked to an increased demand on the easy-to-use delivery devices such as prefilled syringes (PFS) (14–16). Additionally, after 2015, the FDA-approved high-concentration antibody products have been all either in liquid in vial or liquid in PFS format.

Concentration analysis

We analyzed the protein concentration in two concentration ranges, i.e., 100–150 mg/mL, which includes the lower limit of 100 mg/mL, but not the upper limit of 150 mg/mL; and 150–200 mg/mL, which includes both the lower limit of 150 mg/mL and the upper limit of 200 mg/mL. The formulations in the range of 100–150 mg/mL (56%) are slightly more than those of 150–200 mg/mL (44%). The highest protein concentration is at 200 mg/mL from products Benlysta (Belimumab) and Cimzia (Certolizumab pegol).

Administration route analysis

The Administration routes of the 34 antibodies with high-concentration formulation consist of four types: intravitreal injection (IVI), intravenous (IV), intramuscular (IM) and subcutaneous (SC). As shown in Fig. 4,

Table 1. Summary of US FDA-approved antibodies with high-concentration formulation

Product name	Dosage form	Anti-body concen-tration (mg/mL)	IgG Type	Formulation (per mL for liquid formulation if not specified)	Indication	Approved year	Admin-istra-tion route	Sponsor
Susvimo (Ranibizumab)	Liquid	100	IgG1	1 mg/mL histidine HCl, 0.1 mg/mL polysorbate 20, 82 mg/mL sucrose, pH 5.5	Neovascular (wet) Age-related macular degeneration (AMD)	2021	IVI	Roche/Genentech
Aduhelm (Aducanumab-avwa)	Liquid	100	IgG1	31.60 mg/mL L-arginine hydrochloride, 0.60 mg/mL L-histidine, 3.39 mg/mL L-histidine hydrochloride monohydrate, 1.49 mg/mL L-methionine, 0.50 mg/mL polysorbate 80, pH 5.5	Alzheimer's disease	2021	IV	Biogen and Eisai
Evkeeza (Evinacumab-dgnb)	Liquid	150	IgG4	14.8 mg/mL L-arginine hydrochloride, 0.74 mg/mL L-histidine, 1.1 mg/mL L-histidine monohydrochloride monohydrate, 30 mg/mL L-proline, 1 mg/mL polysorbate 80, pH 6	Homozygous familial hypercholesterolemia	2021	IV	Regeneron
Enspryng (Satralizumab-mwge)	PFS	120	IgG2	26.1 mg/mL L-arginine, 3.1 mg/mL L-histidine, 0.5 mg/mL poloxamer 188, 0.02 M L-aspartic acid, pH 6	Neuromyelitis optica spectrum disorder	2020	SC	Genentech
PHESGO (Pertuzumab, Trastuzumab, and Hyaluronidase-zzxf)	Liquid	120 (600 mg each)	IgG1	2000 Units hyaluronidase, 39.7 mg/mL α, α-trehalose, 0.44 mg/mL L-histidine, 3.61 mg/mL L-histidine hydrochloric monohydrate, 1.49 mg/mL L-methionine, 0.4 mg/mL polysorbate 20, 34.2 mg/mL sucrose, pH 5.5	1. Early breast cancer (EBC) 2. Metastatic breast cancer (MBC)	2020	SC	Genentech
Vyepti (Eptinezumab-jjmr)	Liquid	100	IgG1	1 mg/mL L-histidine, 2.8 mg/mL L-histidine hydrochloride monohydrate, 0.15 mg/mL polysorbate 80, 40.5 mg/mL sorbitol, pH 5.8	Migraine	2020	IV	Lundbeck
Beovu (Brolucizumab-dbll)	Liquid	120	scFv	0.02% Polysorbate 80, 10 mM sodium citrate, 5.8% sucrose, pH 7.2	Neovascular (wet) AMD	2019	IVI	Novartis
Herceptin Hylecta (Trastuzumab and hyaluronidase-oysk)	Liquid	120	IgG1	2000 Units hyaluronidase, 0.39 mg/mL L-histidine, 3.67 mg/mL L-histidine hydrochloride monohydrate, 1.49 mg/mL L-methionine, 0.4 mg/mL polysorbate 20, 79.45 mg/mL α, α-trehalose dihydrate, pH 5.5	HER2-Overexpressing breast cancer	2019	SC	Genentech
Aimovig (Erenumab-aooe)	PFS	140 (70 also available)	IgG2	2.0 mg/mL (0.025 M) acetate, 0.10 mg/mL polysorbate 80, 65 mg/mL sucrose, pH 5.2	Migraine	2018	SC	Amgen/Novartis
Ajovy (Fremanezumab-vfrm)	Liquid	150	IgG2	0.136 mg/mL disodium ethylenediaminetetraacetic acid dihydrate (EDTA), 0.543 mg/mL L-histidine, 2.62 mg/mL L-histidine hydrochloride monohydrate, 0.2 mg/mL polysorbate 80, 66 mg/mL sucrose, pH 5.5	Migraine	2018	SC	TEVA PHARMS USA
Emgality (Galcanezumab-gnlm)	PFS/pre-filled pen	120	IgG4	0.5 mg/mL L-histidine, 1.5 mg/mL L-histidine hydrochloride monohydrate, 0.5 mg/mL polysorbate 80, 8.8 mg/mL sodium chloride, pH 5.8	1. Migraine 2. Episodic cluster headache	2018	SC	Eli Lily
Ilumya (Tildrakizumab-asmn)	PFS	100	IgG1	0.495 mg/mL L-histidine, 1.42 mg/mL L-histidine hydrochloride monohydrate, 0.5 mg/mL polysorbate 80, 70.0 mg/mL sucrose, pH 5.7–6.3	Plaque psoriasis	2018	SC	Sun Pharma Global
Takhzyro (Lanadelumab-flyo)	Liquid	150	IgG1	4.1 mg/mL citric acid monohydrate, 7.8 mg/mL L-histidine, 0.1 mg/mL polysorbate 80, 5.3 mg/mL sodium chloride, 5.3 mg/mL sodium phosphate dibasic dihydrate, pH 6	Hereditary angioedema	2018	SC	Dyax Corp.
Trogarzo (Ibalizumab-uiyk)	Liquid	150	IgG4	10 mM L-histidine, 0.045% polysorbate 80, 52 mM sodium chloride, 5.2% sucrose, pH 6	HIV	2018	IV	Thera
Dupixent (Dupilumab)	PFS	150	IgG4	5.25 mg/mL L-arginine hydrochloride, 3.1 mg/mL L-histidine, 2 mg/mL polysorbate 80, 1 mg/mL sodium acetate, 50 mg/mL sucrose, pH 5.9	1. Atopic dermatitis 2. Asthma 3. Chronic rhinosinusitis with nasal polyposis	2017	SC	Regen-eron/Sanofi

(Continued)

Table 1. Continued

Product name	Dosage form	Antibody concentration (mg/mL)	IgG Type	Formulation (per mL for liquid formulation if not specified)	Indication	Approved year	Administration route	Sponsor
Hemlibra (Emicizumab-kxwh)	Liquid	150	IgG4	26.1 mg/mL L-arginine, 3.1 mg/mL L-histidine, 0.5 mg/mL poloxamer 188, pH 6	1. Hemophilia A	2017	SC	Genentech
Kevzara (Sarilumab)	PFS/pre-filled pen	131/175	IgG1	7.84 mg/mL Arginine, 3.25 mg/mL histidine, 2 mg/mL polysorbate 20, 50 mg/mL sucrose, pH 6	Rheumatoid arthritis	2017	SC	Sanofi
Rituxan Hycela (Rituximab and Hyaluronidase human)	Liquid	120	IgG1	2000 Units hyaluronidase, 0.53 mg/mL L-histidine, 3.47 mg/mL L-histidine hydrochloride monohydrate, 1.49 mg/mL L-methionine, 0.6 mg/mL polysorbate 80, 79.45 mg/mL α, α-trehalose dihydrate, pH 5.5	1. Follicular lymphoma 2. Diffuse large B-cell lymphoma 3. Chronic lymphocytic leukemia	2017	SC	Genentech
Siliq (Brodalumab)	Liquid	140	IgG2	4.3 mg/mL glutamate, 0.1 mg/mL polysorbate 20, 24 mg/mL proline, pH 4.8	Plaque psoriasis	2017	SC	Valeant Pharmaceuticals
Tremfya (Guselkumab)	PFS	100	IgG1	0.6 mg/mL L-histidine, 1.5 mg/mL L-histidine monohydrochloride monohydrate, 0.5 mg/mL polysorbate 80, 79 mg/mL sucrose, pH 5.8	Plaque psoriasis	2017	SC	Janssen
Anthim (Obiltoxaximab)	Liquid	100	IgG1	6.2 mg/mL L-histidine, 0.1 mg/mL polysorbate 80, 36 mg/mL sorbitol, pH 5.5	Anthrax infection	2016	IV	Elusys Therapeutics
Zinbryta (Daclizumab)	PFS	150	IgG1	0.3 mg/mL Polysorbate 80, 5.84 mg/mL sodium chloride, 5.94 mg/mL sodium succinate, anhydrous, 0.35 mg/mL succinic acid, pH 6	Multiple Sclerosis	2016	SC	Biogen
Cosentyx (Secukinumab)	1. PFS 2. Lyophilized	150	IgG1	1. 3.103 mg/mL L-histidine/histidine hydrochloride monohydrate, 0.746 mg/mL L-methionine, 0.2 mg/mL polysorbate 80, 75.67 mg/mL trehalose dihydrate, pH 5.8. 2. After reconstitution with 1 mL WFI: 4.656 mg/mL L-histidine/histidine hydrochloride monohydrate, 0.6 mg/mL polysorbate 80, 92.43 mg/mL sucrose, pH 5.8	1. Plaque Psoriasis 2. Psoriatic arthritis 3. Ankylosing spondylitis 4. Non-radiographic Axial spondyloarthritis	2015	SC	Novartis
Nucala (Mepolizumab)	1. PFS 2. Lyophilized	100	IgG1	1. 0.95 mg/mL Citric acid monohydrate, 0.019 mg/mL EDTA disodium dihydrate, 0.2 mg/mL polysorbate 80, 4.16 mg/mL sodium phosphate dibasic heptahydrate, 120 mg/mL sucrose, pH 6.3 2. After reconstitution with 1.2 mL WFI: 0.67 mg/mL polysorbate 80, 7.14 mg/mL sodium phosphate dibasic heptahydrate, 160 mg/mL sucrose, pH 7	1. Severe asthma 2. Eosinophilic granulomatosis with polyangiitis 3. Hypereosinophilic syndrome	2015	SC	Glaxosmithkline
Praluent (Alirocumab)	PFS	150 (75 also available)	IgG1	6 mM Histidine, 0.1 mg/mL polysorbate 20, 100 mg/mL sucrose, pH 6	1. Primary hyperlipidemia 2. CV Disease 3. Homozygous familial hypercholesterolemia	2015	SC	Sanofi
Repatha (Evolocumab)	PFS	140	IgG2	1.2 mg/mL Acetate, 0.1 mg/mL polysorbate 80, 25 mg/mL proline, pH 5	1. Dyslipidemias 2. Hypercholesterolemia	2015	SC	Amgen
Benlysta (Belimumab)	PFS	200	IgG1	5.3 mg/mL L-arginine hydrochloride, 0.65 mg/mL L-histidine, 1.2 mg/mL L-histidine monohydrochloride, 0.1 mg/mL polysorbate 80, 6.7 mg/mL sodium chloride, pH 6	Systemic lupus erythematosus	2011	SC	GlaxoSmithKline
Actemra (Tocilizumab)	PFS	180	IgG1	0.2 mg/mL polysorbate 80, 1.7 mg/mL L-histidine, 1.9 mg/m L-histidine monohydrochloride, 21 mg/mL L-arginine and L-arginine hydrochloride, 4.4 mg/mL L-methionine, pH 6	1. RA 2. GCA 3. Polyarticular juvenile idiopathic arthritis (PJIA) 4. Systemic juvenile idiopathic arthritis (SJIA) 5. Cytokine release syndrome (CRS)	2010	SC	Chugai (Roche)
Ilaris (Canakinumab)	1. Lyophilized 2. Liquid	150	IgG1	1. Lyophilized powder after reconstitution with 1 mL WFI: 2.8 mg/mL L-histidine, 1.7 mg/mL L-histidine monohydrochloride, 0.6 mg/mL polysorbate 80, 92.4 mg/mL sucrose. 2. Liquid solution 2.1 mg/mL L-histidine, 1.3 mg/mL L-histidine monohydrochloride, 49.2 mg/mL mannitol, 0.4 mg/mL polysorbate 80.	1. Periodic fever syndromes 2. Still's disease	2009	SC	Novartis

(Continued)

Table 1. Continued

Product name	Dosage form	Anti-body concen-tration (mg/mL)	IgG Type	Formulation (per mL for liquid formulation if not specified)	Indication	Approved year	Admin-istra-tion route	Sponsor
Simponi (Golimumab)	PFS	100	IgG1	0.88 mg/mL L-histidine and L-histidine monohydrochloride, 0.16 mg/mL polysorbate 80, 41 mg/mL sorbitol, pH 5.5	1. RA 2. Psoriatic Arthritis 3. Ankylosing spondylitis 4. Ulcerative colitis	2009	SC	Janssen
Cimzia (Certolizumab pegol)	1. PFS 2. Lyophilized	200	Fab	1. PFS/solution 1.36 mg/mL sodium acetate, 7.3 mg/mL sodium chloride, pH 4.7. 2. Lyophilized powder after reconstitution with 1 mL WFI: 0.9 mg/mL lactic acid, 0.1 mg/mL polysorbate, 100 mg/mL sucrose, pH 5.2	1. Crohn's disease 2. RA 3. Psoriatic arthritis 4. Ankylosing spondylitis 5. Non-radiographic Axial spondyloarthritis 6. Plaque psoriasis	2008	SC	UCB
Raptiva (Efalizumab)	Lyophilized	100	IgG1	After reconstitution with 1 mL WFI: 82.1 mg/mL sucrose, 4.5 mg/mL L-histidine hydrochloride monohydrate, 2.9 mg/mL L-histidine, 2 mg/mL polysorbate 20, pH 6.2	Psoriasis	2003	SC	Merck Serono/-Genentech
Xolair (Omalizumab)	1. PFS 2. Lyophilized	1. 150 2. 125	IgG1	1. PFS/solution 42.1 mg/mL L-arginine hydrochloride, 1.37 mg/mL L-histidine, 2.34 mg/mL L-histidine hydrochloride monohydrate, 0.4 mg/mL polysorbate 20. 2. Lyophilized powder After reconstitution with 1.4 mL WFI: 1.08 mg/mL L-histidine, 1.75 mg/mL L-histidine hydrochloride monohydrate, 0.33 mg/mL polysorbate 20, 90 mg/mL sucrose, pH 6	1. Asthma 2. Nasal polyps 3. Chronic idiopathic urticaria (CIU)	2003	SC	Novartis/Genen-tech
Synagis (Palivizumab)	Liquid	100	IgG1	0.5 mg/mL chloride, 0.1 mg/mL glycine, 3.9 mg/mL histidine, pH 6.0.	Prophylasis of Respiratory diseases	1998	IM	MedImmune

The pH values of the lyophilized antibodies in the table are referred to the pHs in reconstituted antibody solutions. The pH of reconstituted Ilaris (Canakinumab) is not available from the public available information. The pH of reconstituted Ilaris is calculated as around approximately 6.3, based upon the disclosed formulation excipients of this product.
IVI: Intravitreal injection; IV: intravenous; IM: intramuscular; SC: subcutaneous. scFv: single chain variable fragments; Fab: fragment antigen binding. WFI: water for injection.

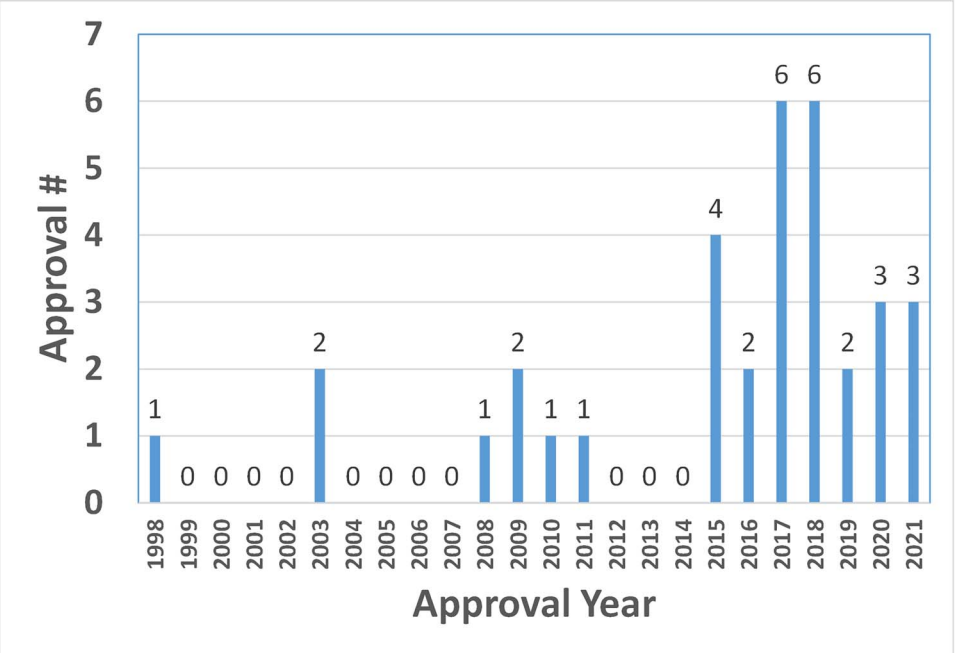

Figure 1. Yearly US FDA approval numbers of therapeutic antibodies with high-concentration formulation during 1998–2021. The 2021 year's data are as of October 2021. Only antibodies with ≥100 mg/mL protein concentration are counted in the data analysis. The first therapeutic antibody with high-concentration formulation (i.e., Synagis/Palivizumab, 100 mg/mL) was approved by US FDA in 1998. Thus, the data analysis in Fig. 1 was started from 1998.

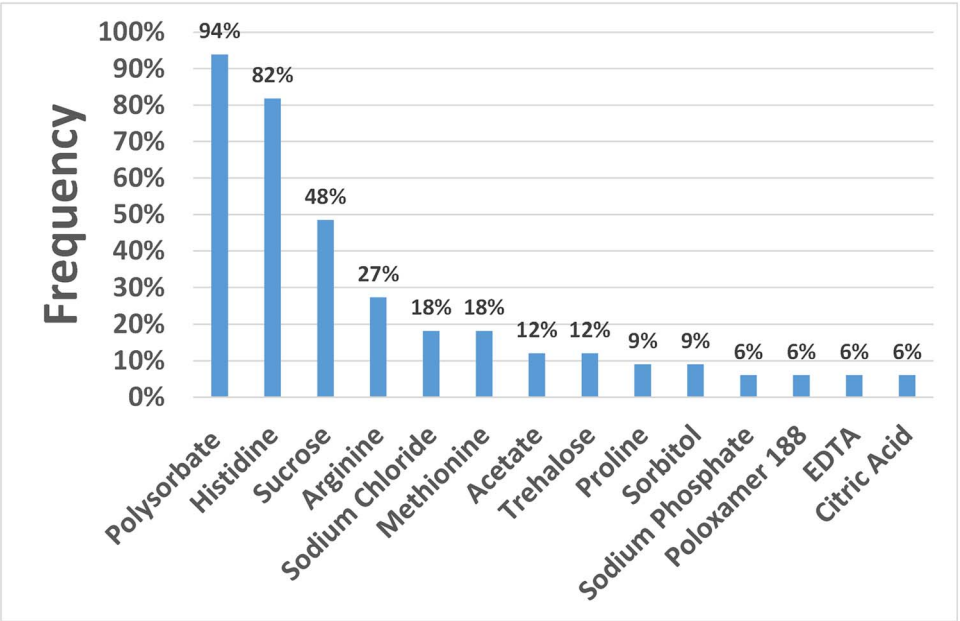

Figure 2. Formulation excipient analysis of US FDA-approved therapeutic antibodies with high-concentration formulation. The formulation excipients of 34 therapeutic antibodies approved by US FDA with high-concentration formulation were analyzed. The frequency of each formulation composition in these 34 antibodies were calculated, and only formulation excipients with more than 5% frequency were presented in Fig. 2.

the dominant administration route, as expected, is SC (26 products, 76%), followed by IV (5 products, 15%), IVI (2 products, 6%) and IM (1 product, 3%). The only antibody with IM administration route is Synagis (Palivizumab) which is, as mentioned above, the first FDA-approved high-concentration antibody product. The two IVI-administered antibody products are Beovu (Brolucizumab-dbll), and Susvimo (Ranibizumab), which the latter was approved in October, 2021 by US FDA for intravitreal use via ocular implant for the treatment of patients with neovascular or "wet" age-related macular degeneration (nAMD). Although both Beovu and

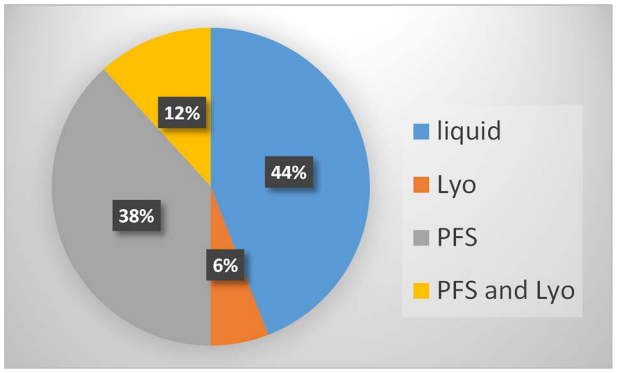

Figure 3. Dosage form analysis of US FDA-approved therapeutic antibodies with high-concentration formulation. Lyo: lyophilized powder; PFS: pre-filled syringe. Please note that liquid dosage form is liquid in a vial, and the formulation solution in PFS is also liquid.

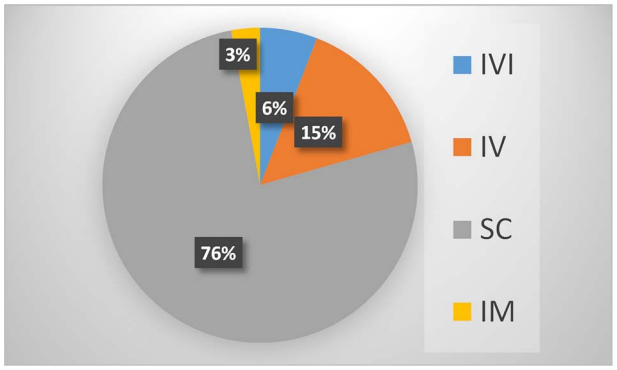

Figure 4. Administration route analysis of US FDA-approved therapeutic antibodies with high-concentration formulation. IVI: Intravitreal injection; IV: intravenous; IM: intramuscular; SC: subcutaneous.

Susvimo share the same indication (nAMD) and the same administration route (IVI), Susvimo (Ranibizumab) is delivered by a special system. This system is called Port Delivery System (PDS) which can continuously deliver the Ranibizumab medicine into the eyes through a refillable implant. Thus with just as few as two treatments per year, Susvimo could help patients with nAMD maintain their vision.

In addition, the above-mentioned antibody products with either IV, or IVI or IM administration routes are all in liquid dosage form, as shown in Table 1.

Top sponsors of FDA-approved therapeutic high-concentration antibodies

We also analyzed the sponsors of the 34 antibodies to identify the top players in high-concentration formulation segment of the industry. If two companies are the co-sponsors of an antibody, each of the two sponsors is counted with a 0.5 approval number. We propose to categorize these sponsors as three different tiers, i.e., tier 1 (8 approved products), tier 2 (1.5–4 approved products) and tier 3 (0.5–1 approved product). Only Roche and its subsidiaries (Genentech and Chugai) fall in the tier 1 category with 8 approved antibodies at high-concentration

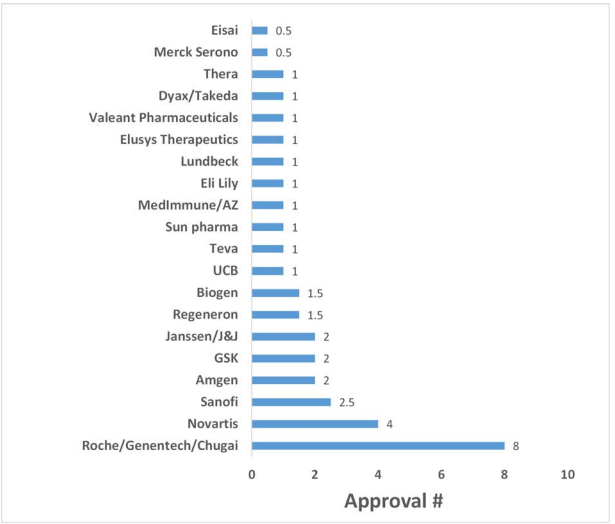

Figure 5. Sponsors vs. approval number analysis of US FDA-approved therapeutic antibodies with high-concentration formulation.

(including two approved antibodies co-sponsored with other companies). This Roche's approval number (eight) is far greater than any other sponsors' approval numbers. The tier 2 sponsors with approval numbers ranging from 1.5 to 4 include Novartis (4 approvals), Sanofi (2.5 approvals), Amgen (2 approvals), GSK (2 approvals), Johnson & Johnson including its subsidiary Janssen (2 approvals), Regeneron (1.5 approvals) and Biogen (1.5 approvals), as shown in Fig. 5. All other sponsors with a single or 0.5 approval, have gone to the tier 3 sponsor category.

IgG type

Not surprisingly, most of the 34 antibodies are IgG1 (22 out of 34), followed by IgG2 (5 out of 34), IgG4 (5 out of 34) and other IgG types (2 out of 34). Other IgG types include single-chain variable fragments (scFv, Beovu/Brolucizumab-dbll), and fragment antigen binding (Fab, Cimzia/Certolizumab pegol).

DISCUSSION AND PERSPECTIVES

Thanks to the technical advances in high-concentration formulation, our data in Fig. 1 clearly demonstrate an increasing trend of the FDA approval numbers of high-concentration antibody drugs in recent years. This trend is partially driven by clinicians and patients, as discussed in literature (12). Both clinicians and patients demand an SC delivery of antibody drugs because the SC injection is more convenient than the IV injection and yet less expensive. Such antibody drugs with high-concentration formulation may be administrated in clinic or even at patients' homes. However, developing high-concentration formulation of the antibody drugs for SC administration is not an easy task because of many challenges (e.g., limitation of 1–2 mL injection volume) (16, 19). We believe that the establishment of SC Drug Delivery and Development Consortium in 2018 accelerated progress on addressing these challenges associated with SC drug delivery and high-concentration

formulation of antibody drugs (18, 20). Eight problem statements were indeed proposed by this SC Consortium, which aim to identify and then address the critical gaps and issues in the SC delivery of high-dose/volume products (18). The SC Consortium also highlighted the needs to shift perceptions of the capabilities of technologies that enabled the SC delivery of large-volume (>3 mL) and/or high-dose biologics. Most of the key members of the SC Consortium are from big biopharmaceutical companies such as Pfizer, Sanofi, Amgen, Bristol-Myers Squibb, AstraZeneca, Eli Lilly, Johnson & Johnson Company, Novartis and Merck, nearly all of which are the sponsors of the FDA-approved high-concentration antibodies (except Pfizer), as shown in Fig. 5.

In addition to the technical advances in SC delivery as summarized in the review articles (16, 21–24), the recombinant human hyaluronidase (rHuPH20) developed by Halozyme may dramatically increase the SC injection volume (up to hundreds of milliliters) (25, 26). Halozyme can degrade hyaluronan (HA), a glycan that fills the interstitial space within the collagenous matrix and acts as a physical barrier to prevent bulk fluid flow, and therefore facilitates the dispersion and absorption of the drug and fluids that are injected under the skin. Three out of the 34 antibody drugs with high-concentration formulation listed in Table 1 are indeed co-formulated with recombinant human hyaluronidase, i.e., Phesgo (Pertuzumab, Trastuzumab and Hyaluronidase-zzxf), Herceptin Hylecta (Trastuzumab and hyaluronidase-oysk) and Rituxan Hycela (Rituximab and Hyaluronidase human). All of these three above mentioned antibody drugs were developed and sponsored by Genentech/Roche under the collaboration with Halozyme. It is noteworthy that Phesgo contains two different antibodies, i.e., Pertuzumab and Trastuzumab. This is the first two-in-one cocktail antibody drug approved by FDA in 2020 for the treatment of breast Cancer (27).

As shown in Table 1, a typical antibody formulation of the 34 antibody therapeutics consists of an antibody, a buffer and a surfactant, as well as an excipient to adjust tonicity or osmolality for solutions or a lyoprotectant for lyophilized powder. Other commonly used excipients in formulation compositions are sodium chloride, an ionic tonicity-adjusting excipient, and the non-ionic osmolality-adjusting excipients such as sucrose, trehalose, and sorbitol. Sucrose and trehalose are stabilizer and could be used as the lyoprotectants in amorphous formulations. The pH range of the 34 formulations is 4.7–7.2. The lowest pH 4.7 is found in the solution of Cimzia (Certolizumab pegol)'s PFS. Interestingly, as shown in Table 1, the formulations and the corresponding pHs of Cimzia's two dosage forms between PFS and lyophilized powder are different. The formulation of Cimzia's solution in PFS is 1.36 mg/mL sodium acetate, 7.3 mg/mL sodium chloride, pH 4.7, while the formulation of reconstituted lyophilized powder is 0.9 mg/mL lactic acid, 0.1 mg/mL polysorbate, 100 mg/mL sucrose, pH 5.2. The highest pH 7.2 is in the solution of Beovu (Brolucizumab-dbll).

Furthermore, the buffering systems of the 34 formulated antibodies are quite diverse, but histidine-HCl is the dominant buffer used (82% of all formulations). Other buffers or pH-modifying agents include citrate, succinate, acetate, phosphate, glutamate, aspartic acid and lactic acid. Based upon the above discussion, it would be rational to build a platform formulation that basically contains polysorbate, histidine and sucrose in order to accelerate the timeline and to enhance the efforts/efficiency on high-concentration formulation development for antibody drugs, especially at early stage.

Consistent with our observation, Robert Strickley and William Lambert suggested this year (2021) in their review article that "a typical subcutaneous solution formulation has 100 mg/mL antibody, sucrose at 50 mg/mL, buffered at pH 6 by 0.01M histidine, has the surfactant polysorbate 80 at 0.01 mg/mL, the viscosity lowered with 10 mg/mL arginine, and is packaged in a prefilled syringe" (9).

As discussed above, all the excipients in Table 1 are traditional and these have been used in the approved antibody drugs for many years. Thus far, no novel excipients have been used in any of the FDA-approved high-concentration antibody products for a few decades mainly because of the difficulty in getting approved by FDA (12). Interestingly, FDA's Center for Drug Evaluation and Research (CDER) launched in September 2021 the voluntary Novel Excipient Review Pilot Program (Pilot Program), which is intended to "allow excipient manufacturers to obtain FDA review of certain novel excipients prior to their use in drug formulations" (visit https://www.fda.gov/drugs/development-approval-process-drugs/novel-excipient-review-pilot-program for more detailed information). We expect that this Pilot Program and strategy would foster the development of novel excipients, followed by the application of these excipients in drug product development.

Two notorious technical challenges associated with high-concentration formulation are high viscosity and aggregation (29–31, 38–40). It is now well established that the viscosity of an antibody solution may increase exponentially with the increasing antibody concentration. Thus, the high viscosity of the high-concentration antibody may result in challenges during manufacturing the drug substance (especially ultrafiltration and diafiltration process) and the drug product (in particular mixing, sterile filtration and filling). If subcutaneous administration is used for administration of high-viscosity antibody drug, large bore size needles may have to be used because of the increase in back pressure, and thus may result in an increased pain for patients (9, 14).

The origin and mechanism of the viscosity in antibody solution have been studied extensively by many scientists in the world (28–30). Three authors in Japan reviewed recently the origin of solution viscosity and concluded that the solution viscosity increases by the attraction and repulsion between the antibody molecules (30). The authors further described the use of small molecules such as amino acids and salts to control the viscosity of protein solution. More importantly, the authors provided a practical problem solution that would control the experimental behavior of protein solution viscosity. As concluded in this literature (30), the most frequently used viscosity-lowering excipients are arginine, sodium chloride and proline. In fact, multiple literatures as well as our formulation excipient analysis in Fig. 2 consistently concluded that arginine is the most widely used viscosity-lowering excipient because it may reduce both electrostatic

interaction and hydrophobic interaction between protein molecules (9, 30, 35).

Although the mechanism of viscosity in solution has been extensively studied, a prediction of high viscosity remains a challenge. The authors at University of Delaware and Amgen proposed that larger activation energies for protein–protein interactions corresponded to larger activation energies for protein solution viscosity (η), and those became predictive with higher η value at higher protein concentrations (31, 32). This conclusion upon high viscosity prediction is largely consistent with the above-mentioned literature, which also shows protein–protein interactions (attraction and repulsion between protein molecules) as determinants of high viscosity in protein solutions (30).

A key risk for high-concentration antibody therapeutics is the antibody aggregation induced by high concentration as the antibody aggregation increases the immunogenicity of antibodies, leading to immune-mediated adverse effects. Thus, this risk has to be mitigated. The cause of antibody aggregation and the corresponding mitigation strategies have been widely studied and/or reviewed (38–39). The primary strategy for risk mitigation is to use surfactants such as polysorbates and Poloxamer 188. Indeed, our analysis data, as shown in Fig. 1, demonstrate that surfactant polysorbate 20 or polysorbate 80 is dominantly included in majority of 34 high concentration marketed products with a total of 31 (polysorbate 20 or polysorbate 80) formulations out of 34 formulations. The most commonly used surfactant is polysorbate 80 (22 out of 34) falling in a range of 0.1–2 mg/mL (i.e., 0.01–0.2%) followed by polysorbate 20 (8 out of 34) in a range of 0.1–4 mg/mL (i.e., 0.01–0.4%) and poloxamer 188 (2 out of 34, both at 0.5 mg/mL). The exact polysorbate type (i.e., either polysorbate 20 or polysorbate 80) is not specified in the disclosed formulation of Cimzia (Certolizumab pegol). Surprisingly, Synagis' (Palivizumab) formulation does not contain any surfactant, and its formulation only contains chloride, glycine, and histidine, as shown in Table 1. In addition to lowering the viscosity, as reported earlier, arginine may serve as an ionic strength adjuster and aggregate reducer, and is also a promising excipient used in lyophilization formulation (33, 34, 37).

Another commonly used strategy to minimize antibody aggregation in solution is to make lyophilized formulation for antibodies at high concentration (41–44). Unique challenges, such as long reconstitution time for a lyophilized high antibody/protein concentration drug product, as well as solutions have been reviewed/reported (36).

From patient point of view, prefilled syringe (PFS), as a ready-to-use drug delivery system, is recognized as a convenient method to enable at-home self-administration, which can in turn provide considerable cost savings compared to dosing in a clinical setting. From industry point of view, overfill of 20–25% drug product in vial, especially with high-concentration formulations, forms the major costs. The PFS allows the overfilling drop down to 2–3%, a significant saving on the active and raw material cost. Furthermore, PFS, combining with high-concentration formulations, can also bring advantages such as patient compliance, dosing accuracy and reduction of wasted drug. Both greater dose precision and minimizing the drug waste can lead to smaller overage requirement, then further result in cost savings (45–47). For such reasons, PFS systems have been gaining strong acceptance in the market. As shown in Fig. 3, presentations of PFS alone and both PFS & Lyo account for 38% and 12% of all high-concentration dosage forms, respectively. Summing up together, PFS products have taken half (50% in total) of the market share in the high-concentration injectables market.

With the development of high-concentration formulations in PFS, one of the significant challenges that brings in the process, manufacturing and administration difficulties is again the high viscosity of the solution, which requires silicone oil coating to avoid high gliding forces. To ease the delivery of the contents, the design of plunger-barrel of PFS comes with the syringe barrels coated with silicone oil to provide the sufficient and uniform glide force to the plunger. Since silicone can leach into protein during storage of product in silicone-coated PFS, some syringe manufacturers developed new silicone-coating techniques with specialized silicone grade. Another unique challenge when uses PFS includes the extraction of contaminants (e.g., tungsten particles) because of the direct contact of antibodies with the surface of a plunger and a barrel lubricant (12). One literature demonstrates that the source of tungsten in PFS is the tungsten pin used in barrel manufacture to insert the needle (6).

And metals such as tungsten can leak from rubber stoppers. Metal chelators such as EDTA can assist formulators to resolve these problems. This extractable concerns may be overcome by replacing the rubber stoppers with the inert substances such as Teflon. Additionally, polymeric syringe systems have emerged to provide a solution for products that require the absence of silicone and tungsten. Thanks to the technical advances in PFS technology, more antibody drugs are expected to be formulated and brought to market with PFS (45–47).

Autoinjectors together with PFS are now commercially available to quite a few of the 34 antibodies such as Actemra (Tocilizumab), Aimovig (Erenumab), Zinbryta (Daclizumab), Nucala (Mepolizumab) and Simponi (Golimumab). The injection volume for most of these autoinjectors is limited up to only 1.0 mL. However, delivering increased volume with innovative autoinjectors is available to patients. For example, Amgen utilizes an innovative single use Pushtronex ® system (on-body infusor with prefilled cartridge) for its Repatha, in addition to its regular PFS. Pushtronex comprises a complex engine that moves a plunger inside a cylindrical barrel containing the drug, with the reservoir being either prefilled or filled by the user (9). This system can deliver 3.5 mL of Repatha solution (420 mg single dose) within 5 minutes. Considering SC injection volume may be now increased up to hundreds of milliliters, thanks to the above mentioned Halozymes's hyaluronidase (rHuPH20), we expect that in the future more and more new autoinjectors with capability of delivering larger volumes of mAb solutions will be approved in the market to meet this unmet medical need.

Although an increasing number of mAbs are administered subcutaneously, many aspects of SC bioavailability of mAbs are still poorly understood. The major challenges of SC bioavailability of mAbs were already reviewed well in 2018, and the authors summarized the major

challenges including reliable models for predicting SC bioavailability of mAbs in humans. The scientists at Genentech and researchers at two universities in UK have recently made good progress in addressing these challenges. They established an *in vitro* model for predicting bioavailability of subcutaneously injected mAbs, and demonstrated the feasibility of this in vitro modeling strategy as a tool to identify drug and formulation properties that can define the performance of SC injected medicines (48). This model and approach may provide the potential for predicting clinical outcomes that could be useful for formulation selection and a first-in-human clinical dosing strategy. We expect that more progresses in SC bioavailability of mAbs will be further made in the years to come. In addition, the above-mentioned Merck's AACR abstract reported Pembrolizumab (Keytruda)'s bioavailability after subcutaneous administration (11). Merck/MSD demonstrated in this abstract that the PK of pembrolizumab's two SC formulations were similar with an estimated bioavailability of 64% (95% CI, 54–74); and this is consistent with the reported bioavailability of other SC monoclonal antibodies that dominantly range from 50 to 85% in humans (48).

It is understandable that SC is the dominant administration route for the high-concentration antibody products as high concentration is usually necessary to deliver desired dose in volume amenable to subcutaneous delivery. Although we do not fully understand why five high-concentration products are IV delivered, there are at least two potential reasons for IV administration. The first reason is high dose. For example, Trogarzo (Ibalizumab-uiyk) is administered intravenously as a single loading dose of 2000 mg followed by a maintenance dose of 800 mg every 2 weeks. Another example is Evkeeza (Evinacumab-dgnb) with a dose of 1200 mg. The second reason is that IV offers inline filters to address particle concerns. Although there are syringes equipped with inline filters, the use of them is not considered as convenient especially when viscosity is often an inherit characteristic of high-concentration products. Multiple antibody products (such as Aduhelm/Aducanumab-avwa) are indeed administered as IV via 0.2 or 0.22 micron inline filter.

As demonstrated in Table 1, 34 antibodies with high concentration (≥100 mg/mL), accounting for nearly one-third of the total FDA-approved antibody based drugs (not including Fc fusion protein), have been approved commercially by FDA as of October 2021. This indicates that achieving 100 mg/mL protein concentration in antibody formulation nowadays would not be much difficult within the biopharmaceutical industry. The highest protein concentration in solution among these 34 antibody drugs is 200 mg/mL. It is expected that therapeutic antibodies with higher than 200 mg/mL protein concentration will be approved in the future by the US FDA. The limitation to achieve such high protein concentration for a mAb may be partially due to the high viscosity. When the protein concentration is increased from 100 mg/mL or even from 50 mg/mL, the viscosity of mAb is typically increased exponentially. Regardless of the recent technical advances in high-concentration formulation, extremely high mAb concentration such as above 500 mg/mL, will still be very challenging to achieve (13). Some companies focusing upon new technology development of biologics formulation (e.g., Elektrofi) take advantage of such new technology—as using particle suspensions to make protein concentration reaching greater than 400 mg/mL without exceeding the viscosity limits while preserving the full activity of the biologic, as the company claimed at its website (elektrofi.com).

Interestingly, it is noted that all these 34 highly concentrated antibodies are either naked full length IgG or antibody fragments (scFv and Fab). No antibody drug conjugates (ADCs) are yet on the list as of to date. This is because of the special formulation challenges associated with ADCs such as stability of linker and solubility of the ADCs. In addition, all 11 ADC drug products approved by FDA so far are manufactured as lyophilized form at low protein concentration of the reconstituted ADC solutions, partially because of the above-mentioned stability and solubility issues (data resource: Drugs@FDA). Consequently, ADCs are unlikely to be administrated subcutaneously (49, 50).

Taken together, we believe that high-concentration formulations of antibody drugs will be the future trend of therapeutic antibody formulation development, regardless the challenges encountered for highly concentrated protein formulations. We also believe that all these challenges and problems are resolvable, thanks to the new formulation technology advancement and the aid of SC Consortium.

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CONFLICT OF INTEREST STATEMENT

Shawn Shouye Wang and Kin Ho are employees and shareholders of WuXi Biologics. Shawn Shouye Wang was the founding president, has been serving as a director in the Board of Directors for Chinese Antibody Society. Antibody Therapeutics is the official journal of Chinese Antibody Society. The authors report no other potential conflicts of interest for this work.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available.

ETHICS AND CONSENT

Consent was not required.

ANIMAL RESEARCH

This is not applicable.

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