Biosimilars in the Next Era:
Hope or Hype for Biosimilar Monoclonal Antibodies in the Near Future?

Master thesis

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Abstract

Biosimilars, defined as biological medicinal products comparable in quality, safety and efficacy to reference products, follow the independent regulatory pathway in the EU for marketing authorizations after the patent expirations of the reference products. With the recent advent of biosimilar monoclonal antibodies (mAbs), the evolving EU guidelines on biosimilars are about to have a new regulatory landscape. The development of guideline on biosimilar mAbs is considered as a regulatory challenge due the tremendous complexity of mAbs. MAbs are highly complex molecules with secondary and tertiary structures subject to post-translational modifications, which are often heterogeneous and vulnerable to slight change in manufacturing process. Thus, to what extent of the similarity a biosimilar mAb should demonstrate, compared with its reference product, is currently the most controversial regulatory issue. This review discusses this issue by raising the questions in tiers of quality, non-clinical, clinical issues. In principle, the similarity issue on mAbs will be extensively discussed and justified on case-by-case basis. Most importantly, evaluation of biosimilar mAbs should be conducted with a holistic approach, i.e. rigorous interpretation between structure-function relationships to reduce unnecessary clinical trials while providing comprehensive post-marketing risk management plans. In the long run, biobetters might be gradually taking over biosimilars on the established regulatory track and leading to better access to biological medications.

Biosimilars

Since the advent of biotechnology era heralded by the launch of recombinant human Insulin, biologicals, as revolutionary medications, have continuingly made several achievements. These compounds, e.g. erythropoietin (EPO), insulin, growth hormones, granulocyte colony-stimulating factor (G-CSF), monoclonal antibodies (mAbs), have successfully transformed the ways in treating many severe diseases, such as anaemia, diabetes, cancer, hepatitis and multiple sclerosis.[1] Like small molecule drugs, biologicals also inevitably confront patent expirations and potential threats from copycat competitors. Patents for many biologicals have either expired or are about to expire. Thus, the playing fields of biopharmaceutical market have opened to “generic-like” versions of these products, which are called biosimilar in the European Union (EU) and follow-on biologicals in the United States (US). As a pioneer, the European Medicines Agency (EMA) has established regulatory pathways specific for biosimilars since 2004, while the counterpart legislation in the US is still under preparation and contentious discussion.

Biosimilars, defined as biological medicinal products comparable (but not identical) in quality, safety and efficacy to reference products, follow the independent regulatory pathway in the EU for marketing authorizations after the patent expirations of the reference products.[2] Biosimilar are considered differently and exhibit distinct features from conventional generic versions of small chemical products. Generic products, compared with reference products, are evaluated under the criteria whether they equip pharmaceutical equivalence (i.e. identical active substances) and bioequivalence (i.e. comparable pharmacokinetics), in which formal clinical efficacy and safety studies are not usually essential. By contrast, biosimilars require much more extensive assessment for comparability, in which the boundaries of criteria (usually on ad-hoc basis) are not usually well-defined, due to the complex nature of biologicals itself and its manufacturing process. Firstly, biologicals are composed of polypeptides and have molecular weights ranging from 3.5 to 150 kilodaltons, which could be hundreds times larger than that of small chemical drugs. These biologicals with high molecular weight are complex molecules with secondary and tertiary structures subject to post-translational modifications such as glycosylation. Secondly, biologicals are usually recombinant proteins produced in living cells with controlled and tailored gene expression systems by recombinant DNA technology.[3] The active substances of drugs its self and its final product tend to be heterogeneous and often incorporate variants, which might vary by as much as 1000 daltons.[4] Thus the dynamic systems— which are vulnerable to subtle changes, e.g. pH in cell cultures, temperature and culture media ingredients— could make manufacturing processes of biologicals highly complex and involve several specific isolation and purification steps;[5] as a result, the clinical properties of biological products are highly influenced by the methods of production.[6]

Thirdly, unlike small molecules, biologicals are often protected by multiple patents, e.g. DNA, vectors, hosts organisms of expression systems, humanization techniques, medical indication etc. The intellectual properties of developing a biological might held by different companies and pooled

by virtue of the licensing deals.[7] These “defense lines” make it more difficult for copycat companies to break through to duplicate biologicals. All of above account for a fact that biosimilars are considerably challenging than conventional generics to produce so that the demonstrating the comparability of a biosimilar to its reference product will always stay in the spotlight. Moreover, the safety concerns on biologicals are always noted, especially the issues related to potential immunogenicity.[8] Between 1998 and 2002, several cases of antibody-mediated pure red cell aplasia (PRCA) were identified to be associated with Eprex (epoetin alfa). In this case, the neutralizing antibodies were raised by the immunogenicity caused by a minor change in the formulation of the epoetin alfa products. A number of patients developed anti-epoetin antibodies which neutralised not only injected epoetin but endogenous erythropoietin.[9] This example can illustrate the difficulty in developing biosimilars, in which subtle change during manufacturing process might lead to severe clinical consequences

Table 1. The current approved biosimilar products in the EU[10]

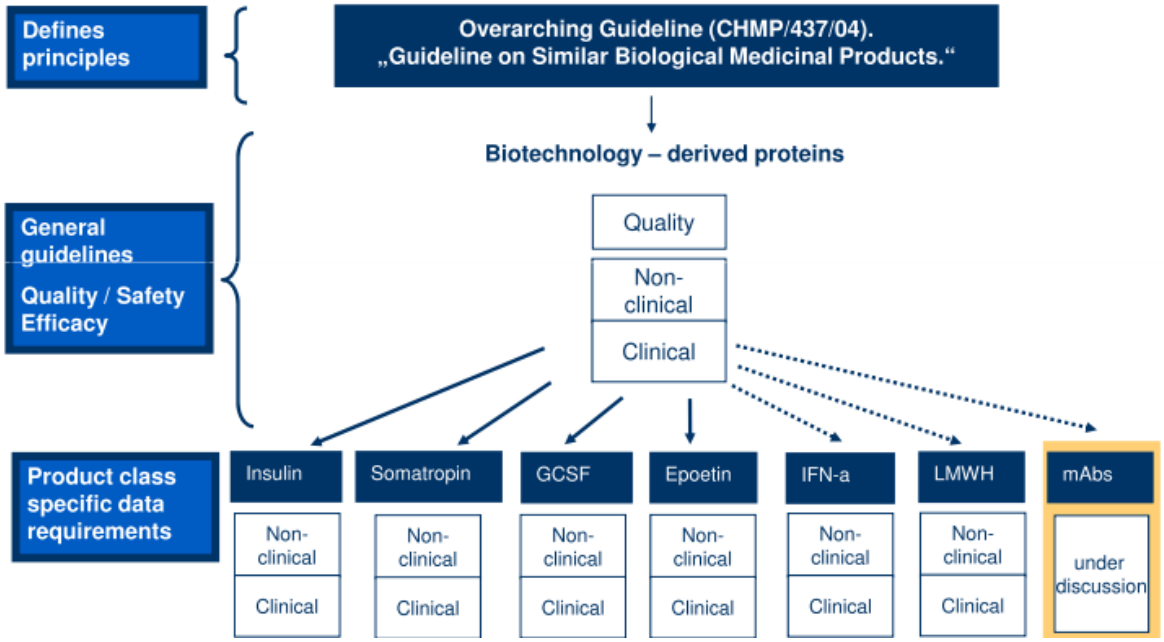
International Non-proprietary Name	Trade name	Company	Approval date	Indication
Somatropin	Omnitrope	Sandoz GmbH	12-Apr-06	Growth hormone deficiency in children and adults
	Valtropin	BioPartners GmbH	24-Apr-06	
Epoetin alfa	Binocrit	Sandoz GmbH	28-Aug-07	Anemia in kidney failure and cancer patients
	Epoetin alfa Hexal	Hexal AG	28-Aug-07	
	Abseamed	Medice Arzneimittel Putter GmbH	28-Aug-07	
Epoetin zeta	Retacrit	Hospira UK Ltd.	18-Dec-07	
	Silapo	STADA Arzneimittel AG	18-Dec-07	
Filgrastim	TevaGrastim	Teva Generics GmbH	16-Sep-08	Reducing duration of neutropenia in cancer patients
	Biograstim	CT Arzeimittel	16-Sep-08	
	Ratiograstim	Ratiopharm GmbH	16-Sep-08	
	Filgrastim ratiopharm	Ratiopharm GmbH	16-Sep-08	
	Filgrastim Hexal	Hexal AG	13-Feb-09	
	Filgrastim Zarzio	Sandoz GmbH	13-Feb-09	
	Nivestim	Hospira UK Ltd.	8-Jun-10	

To date, the EMA have granted 14 marketing authorizations for biosimilar products in the EU, such as recombinant human growth hormone, granulocyte-colony stimulating factor (G-CSF), erythropoietin (Table 1). Since 2004, the EMA and the Committee for Human Medicinal Products (CHMP) have developed three categories of guidelines on biosimilars, including (1) A overarching guideline to define the principle for biosimilar product, (2) General guidelines addressing quality (e.g. manufacturing processes and quality control), non-clinical and clinical issues, (3) Annex guidelines specific to different classes of biosimilars (Figure 1).[2, 11, 12] The approval process differs greatly depending on the products due to the significant differences between them. Therefore, the key requirement for a marketing authorization application of a biosimilar is to demonstrate the comparability to its reference product in terms of quality, safety and efficacy. However, the margins for acceptable differences between biosimilar and reference products in these three major attributes

are not clearly delineated in the guidelines; therefore, only the evaluation of what the EMEA CHMP approves and rejects will define what a biosimilar is.[13] The same mode of regulatory pathway is being applied to more complex biologicals, such as mAbs. A concept paper has been published for open discussions on the upcoming EU guidance on biosimilar mAbs. The new formal EU guideline on biosimilar mAbs is expected to be issued in the second half of 2011 and put into effect soon afterwards.[14] Indeed, the current methods for characterization of mAbs are proceeding to be more sophisticated; yet the ability to compare a biosimilar to a reference mAb on an analytical level remains limited. Thus the design of a clinical development program for a biosimilar mAb will be significantly challenging and of vast room to discuss for both manufacturers and regulators. So far, the EMA has received requests for scientific advice on six biosimilar mAbs; this relatively small number accounts for the difficulties of making such biosimilar medicines.[14]

In this review, we attempt to outline the current developing framework of EU regulation on biosimilar mAbs, and further discuss several regulatory considerations by raising the questions in tiers of quality, non-clinical, clinical issues with regard to biosimilar mAbs. At last, we cast a though about the feasibility and the trend of the development of mAbs in the near future.

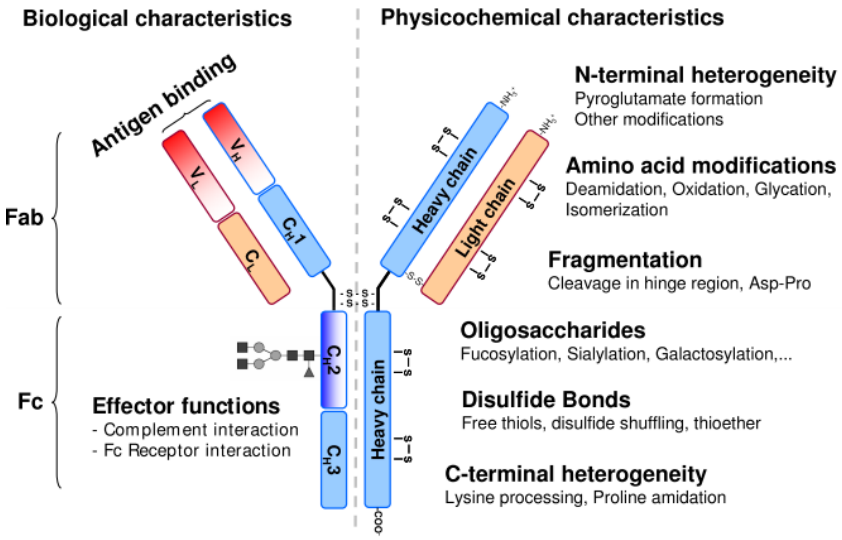
Figure1. The current guidelines on biosimilar products in the EU[15]



Monoclonal antibodies (mAbs)

Being described as “magic bullets”, monoclonal antibodies (mAbs) have been making numerous clinical progress and achievements in the past decade. At present, mAbs are approved to treat several serious and life-threatening diseases, such as cancers, cardiovascular diseases, inflammatory diseases, macular degeneration, transplant rejection, and viral infection, all of which fail to be managed effectively by conventional small drugs. Therapeutic mAbs act by specifically binding to its disease-related antigens on the surfaces of target cells, leading to the physiological blockade of the cells or stimulation of the patient's immune system to attack those cells. A mAb can recognize antigen with the Fab regions, which represent the variable domains of a mAb (Figure 2), and mediate cytotoxicity by Fc regions, which bind and activate complement (complement-dependent cytotoxicity, CDC) or interact with Fc receptors (FcR) on antigen presenting cells (antibody-dependent cellular cytotoxicity, ADCC).[16, 17]

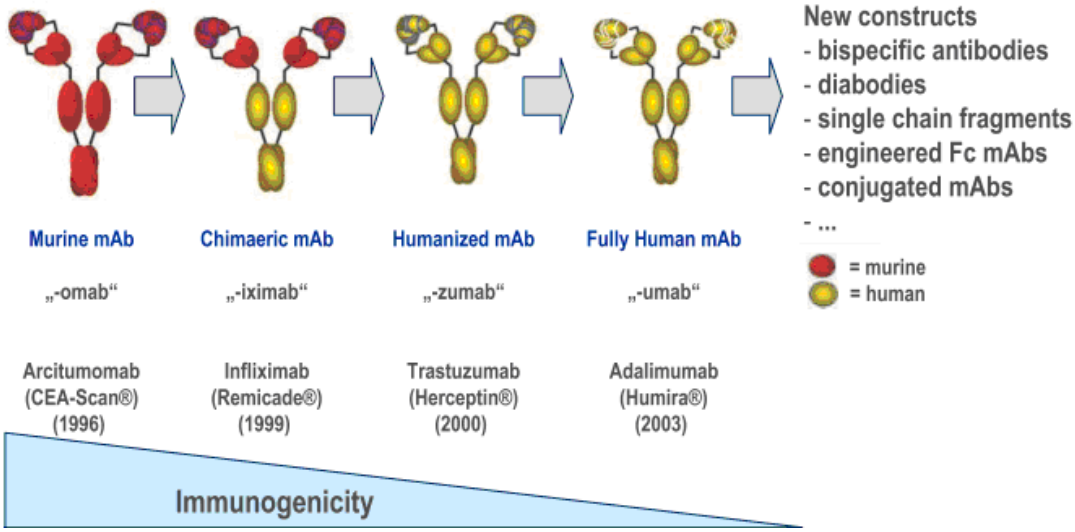
Figure2. Biological characteristics and physicochemical characteristics of mAbs[15]



Throughout the progression of development, mAbs have been evolving toward less immunogenicity for better efficacy and safety. According to their composition, mAbs can be classified into four major types: murine, chimeric, humanised and human mAbs (Figure 3). To reduce adverse immune response raised by mAbs, murine mAbs were engineered into chimeric mAbs (suffix: -ximab, e.g. infliximab) by replacing the non-human Fc regions with the human counterparts. Then, chimeric mAbs stepped forward into humanized mAbs (suffix: -zumab, e.g. trastuzumab) by maneuvering large parts of the Fab regions into human counterparts. More recently, the advance in transgenic mouse technology and development phage display technique has made fully human mAbs possible (suffix: -umab, e.g. adalimumab); however, while being reduced, the immunogenicity of human mAbs still exists to some extent. Thus, several strategies have been adopted to further tackle the

immunogenicity of mAbs, such as humanization of glycosylation pattern [18], chemical modification like PEGylation,[19] or engineering of the molecule to remove immunogenic T cell epitopes.[20] It should be noted that the role of glycosylation on mAbs has recently gained increasing attention.[21]

Figure 3. Evolution of mAbs[22]



While being as a prominently growing sector in the global pharmaceutical market share, therapeutic mAbs and mAb-like fusion proteins are confronting their upcoming patent expiry. In the United States (US), the biological blockbusters, such as Enbrel (etanercept), Remicade (infliximab), Herceptin (trastuzumab), Humira (adalimumab), Avastin (bevacizumab), are about to lose their patents by 2012, 2014, 2015, 2016, 2017, respectively.[23] However, unlike the smaller and simples biologicals, e.g. somatropin and epoetin, so far in the US and the European Union (EU), mAbs and infusion proteins have no biosimilar competitors due to their complexity in composition and structures. The relevant legislation is still under debate and many challenges remain to be conquered.

Current challenges and development of regulatory pathway for biosimilar mAbs

Being extraordinarily complex is the most unique feature of mAbs, both as regards their structure and their mechanism of action. Full-length mAbs have an average molecular weight of 150 kDa; by contrast, somatropin, 22kDa. The structure of higher molecular weight proteins usually come with more complex secondary and tertiary structures, subject to higher post-translational modifications, such as glycosylation and fucosylation, resulting in higher product heterogeneity and impurities.[16] Active substance of mAbs and its formulated final products often contain mAb variants with different glycans, namely so-called microheterogeneity. This imperfection can be characterized, to some extent, by the evolving modern technology;[24] however, it is believed that the microheterogeneity is inevitable and has significant influence on mAbs functional and pharmacological characteristics, leading to potential inconsistency in safety and efficacy in clinical testing.[16] Moreover, product-related impurities (e.g. fragments and aggregates) or process-related impurities (e.g. residual host cell DNA and proteins that sometimes might not be removed sufficiently) can cause safety problems in clinical trials, such as infusion reactions or enhanced immunogenicity.

Table 2. The current EMA regulatory guidelines on biosimilar mAbs

General guidelines
Guideline on similar biological medicinal products. *CHMP/437/04; effective October 2005*
Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues *EMEA/CHMP/BWP/49348/2005; effective June 2006.*
Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues *EMEA/CHMP/BMWP/42832/2005; effective June 2006*
Specific to mAbs
Guideline on development, production, characterization and specification for monoclonal antibodies and related products *EMEA/CHMP/BWP/157653/2007; effective July 2009*
Concept paper on immunogenicity assessment of monoclonal antibodies intended for in vivo clinical use *EMEA/CHMP/BMWP/114720/2009; deadline for comments June 2009*
Concept paper on the development of a guideline on similar biological medicinal products containing monoclonal antibodies *EMEA/CHMP/BMWP/632613/2009; deadline for comments January 2010*

Due to the aforementioned difficulty and limitation in the development of mAbs, drawing up a regulatory pathway for biosimilar mAbs is challenging, and yet, still desirable and imperative. In principle, the three categories of data in medicinal product dossier are assessed by regulators for approval of clinical trial applications and marketing authorizations: quality (also known as CMC: Chemistry, Manufacturing and Control), non-clinical and clinical data, all of which are inherently interlinked, and have to be evaluated together. Since the complexity of mAbs tend to sophisticate the quality attributes and its interpretation to the subsequent categories, a holistic approach is particularly critical for the development of biosimilar mAbs. Since the EU overarching guideline for biosimilars (CHMP/437/04) was launched in 2005, several product-specific guidelines on biosimilars have been developed and taken into effect. Not until October 2009 did the EMA announce the conceptual guideline on biosimilar mAbs (EMEA/CHMP/BMWP/632613/2009) for

interested parties' consultation. Another two recently established mAb-specific guidelines, focused on quality control and immunogenicity assessment (Table 2), offer additional insight into the possible regulatory pathways for biosimilar mAbs in the near future. However, the feasibility of the development and authorization of biosimilar mAbs in the EU regulatory pathways is still under discussion. To what extend can the comparability of biosimilar mAbs in relent attributes be acceptable needs to be further specified. Several critical issues around the upcoming regulatory pathway for biosimilar mAbs are enumerated in the following sections:

Current regulatory issues on biosimilar mAbs: Chemistry, Manufacturing and Controls (CMC)

Can mAbs be considered as "well-characterized" biologicals?

Although current tools for physicochemical and biological assays enable considerable evaluation of biosimilarity on the quality level, whether mAbs can be classifies as well-characterized biologicals is still questionable. The term “well-characterized” is hardly applicable and quietly subjective to define biologicals. At present, the primary structure of mAbs can be determined by DNA sequence and fully accessible to analytical verification.[25] The identity/amount of related variants and glycosylation profiles of mAbs can be comprehensively characterized by analytical methods with high sensitivity. The precise and relevant assays for biological functions of pivotal Fab and Fc of mAbs are also readily available.[5] Thus, with the increasing advance in analytic methods, the greater sensitivity and resolution can be surely achieved; however, the more differences between biosimilar and reference products will inevitably be detected.

For physicochemical characterization of mAbs, to what extent are current methods sensitive enough to detect differences? To what extent might the differences in structure be acceptable?

It has been understood that mAbs can be characterized considerably in the aspects of their primary structure, identity and related variants including aggregates, glycosylation profiles by current physicochemical analytic methods (Table 3). The physicochemical tools allow detecting batch differences of innovative mAb products so as of biosimilars. According to the biosimilar quality guideline, it is not expected that the quality attributes in reference and biosimilar products are identical.[11] For instance, slightly structural differences in the active substance, such as variability in the post-translational modifications (PTMs) may be acceptable but need to be justified. The differences might have potential influence on the subsequent non-clinical and clinical data, which might be required for further satisfactory justification of the safety and efficacy of the biosimilar products.[11] Thus, the key questions here will be the ability to detect differences but the determination of their clinical relevance, such as: Whether the difference will affect the final outcomes? Which differences will play the roles? How will they act? And at what levels do they matter? These could only be answered by combining physicochemical results with functional/biological assays and the qualification in preclinical and clinical studies.[26]

Table 3. The current methods for physicochemical characterization of mAbs[27]

Molecular Parameter	Attributes	Methods for control and characterization
Primary structure	Sum formula: Mass of light chain, heavy chain and of intact mAb	LC-ESI-MS/MS
	Amino acid sequence	Orthogonal peptide maps with high resolution MS and MS/MS sequencing
	Disulfide bridging	Non-reducing Peptide map
	Free cysteines	Ellman's, Peptide map
	Thioether bridging	Ellman's, Peptide map
High order structure	Secondary and tertiary structure	CD spectroscopy, DSC, H/D exchange, FT-IR
Glycosylation	Glycan isoforms	NP-HPLC-ESI-MS of 2AB-labeled glycans, exoglycosidase digestion, MALDI TOF/TOF-MS
	Sialic Acids including NANA and NGNA	NP-HPLC, WAX, HPAEC; RP-HPLC (DMB-label)
	Aglycosylated mAb	CGE, Peptide map
Heterogeneity	C- and N-terminal: ±Lys, pyroGlu	CEX; Papain-IEX; Peptide Map, RP-HPLC
	Glycation of Lys	Boronate affinity; LC/MS; Peptide map
	Oxidation	RP-HPLC; Papain-HIC; Peptide map
	Deamidation	CEX; Papain-IEX; Peptide map
	Aggregation	SEC, FFF, MALLS, DLS, AUC; imaging, particle charge
	Fragmentation at disulfides (HL, H2L, H, L) and in amino acid chain (p100, p50)	CGE, SDS-PAGE, SEC, RP-HPLC

Note: NANA, N-acetylneuraminic acid; NGNA, glycolylneuraminic acid; LC-ESI-MS/MS, liquid chromatography-electrospray ionization-tandem mass spectrometry; CD, circular dichroism; DSC, differential scanning calorimetry; H/D exchange, hydrogen/deuterium exchange; FT-IR, Fourier transform infrared spectroscopy; NP-HPLC-ESI-MS, normal phase-high performance liquid chromatography-electrospray ionization-mass spectrometry; MALDI TOF, matrix-assisted laser desorption/ionization/time-of-flight; WAX, weak anion exchange; HPAEC, high performance anion exchange chromatography; RP, reversed phase; DMB, 1,2-Diamino-4,5- methylenedioxybenzene; CGE, capillary gel electrophoresis; CEX, cation-exchange chromatography; IEX, ion-exchange chromatography; LC/MS, liquid chromatography/mass spectrometry; HIC, hydrophobic interaction chromatography; SEC, size-exclusion chromatography; FFF, field flow fractionation; MALLS, multi-angle laser light scattering; AUC, analytical ultracentrifugation; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis

To what extent should glycosylation on biosimilar mAbs be comparable?

Glycosylation patterns are likely to be one of the most crucial issues in the development of biosimilar mAbs. Glycosylation can arise at several stages in the manufacturing process and is vulnerable to slight change and differences during manufacturing process, such as scale-up of pilot process, batch alterations, use of different expression systems. These diverse factors, which could differentiate the glycosylation profiles of mAbs, are challenging for biosimilar mAbs to demonstrate that subtle differences from reference products have no adverse impact on clinical safety and efficacy. The alteration of glycosylation in mAbs, even a single substitution of a residue, could significantly influence the mAbs' solubility, stability, clearance, binding, immunogenicity and immune effector functions.[28, 29] For example, the level of fucosylation in rituximab has been identified to have impact on its ADCC activity[26]. Besides, the galactose-α-1,3-galactose on the Fab of cetuximab heavy chain is proven to lead to anaphylaxis by inducing activation of IgE antibodies.

This type of glycoform in cetuximab occurs when the mAb is produced in the murine SP2/0 cell line, but not in the CHO cells.[30]

However, as the advance in glycoengineering and protein expression systems, the knowledge of structure- function relationships between glycosylation and bioactivity of mAbs is being improved. A recently developed analytic method, GTO-QbD (mathematical Graph Theory and Ontology based on Quality by Design), aims to determine the comparability of complex biopharmaceuticals.[31] The GTO-QbD is based on the philosophy of QbD in ICH guidelines Q8 to tackle the complexity of post-translational modifications of biological products, especially the glycosylation of mAbs. The GTO-QbD maps and integrates several relationships between physicochemical, biological, process parameters, safety, efficacy attributes related to glycosylation, providing a systemic insight into designing biosimilar mAbs.[31] On the other hand, several achievements in glycoengineered yeast expression systems have made it possible to produce fully humanized sialylated structures or human N-glycosylation, which is critical for antibody-mediated effector functions of mAbs. Following the precedent case of Valtropin, the use of different host cells from a reference for a biosimilar product might be considered possible, but extensive characterization of glycosylation will be necessary.

As a crucial attribute, the impact of glycosylation differences between reference and biosimilar mAbs on clinical properties, in which mAbs' modes of action might be vary, should be justified. The acceptability of differences in glycosylation (e.g. due to different expression systems) should depend on the criticality of glycosylation.[32] The identity of the individual glycan structure should be the same, and the quantitative glycan composition should be comparable. However, the degree of acceptable differences in qualitative and quantitative composition would depend on the relevance of the respective individual glycan in biosimilar mAbs.[33]

To what extent do mAb variants have to be comparable between reference and biosimilar products?

According to the general guideline on biosimilar, in principle, a biosimilar must have the same amino acid sequence as its reference product. However, since (innovative or biosimilar) mAb products inevitably have microheterogeneity and contain post-translationally modified variants of mAbs, deviations from this rule are acceptable depending on the level of understanding of the clinical relevance of the variants.[34] A biosimilar mAb should contain the same variants in comparable amounts as its reference product.[33] An understanding of the process-related and product-related critical quality attributes is needed. For example, differences in levels of terminal lysine variants might not affect the biological function; glycosylation might be less important for some mAbs exhibiting no effector functions.[26] Thus, the relevance of major variants on clinical efficacy and safety needs to be established. Since the actual composition of the mixture cannot be reproduced by a different manufacturing process, comparative non-clinical and clinical data of biosimilar mAbs will always be necessary for further justification. In this case, differences may be acceptable based on the outcome from the overall comparability exercise..

Current regulatory issues on biosimilar mAbs: Non-clinical

What are the roles of biological assays in biosimilar comparison?

Biological/functional assays, such as potency assay, are considered as essential parts in biosimilar comparison. Current methods for examining mAb functionality are well-established, e.g. bioassays on inhibition of binding of mAb-targeted ligands to its receptors and downstream biological functions.[22] The assays can helpful for depicting critical structure-function relationships and might be equally important in the holistic evaluation of biosimilarity together with physicochemical, preclinical and clinical data.[25, 35] For example, a multiple evaluation of functional assays may complement physicochemical methods for determination of higher order structure so that reinforces overall product understanding and reduces potential risk in the preclinical and clinical programs.[25, 35] Because mAbs' modes of action (MoA) are usually complex and may interweave within multiple mechanisms, network and in vivo net contribution of different MoA of a mAb is often incompletely understood and may also vary in different indications.[33] As a result, the biological characterization of biosimilar mAbs should include both Fab- and Fc-mediated functions unless there is any justification.

To what extent could biological assays and physicochemical quality assays substitute for each other's data gaps in biosimilar comparison?

Even with the current state-of-art analytic methods, the detection and verification of the influence, raised by slight differences between reference and biosimilar products, still can be improved. Thus, relying on quality attributes to predict the impact of the differences on clinical efficacy and safety is difficult. Comprehensive bioassays might offer better insight in clarifying the relevance of physicochemical properties of biosimilar mAbs, such as the role of specific glycan variants on function of mAbs. For example, the batches of efalizumab produced respectively by XOMA and Genentech were identified to contain minor physicochemical differences, but ended up with notable differences in a bioequivalence study (pharmacokinetics) and clinical results. An additional Phase III study was requested, given the impact of changes in pharmacokinetics on safety and efficacy can not be predicted reliably.[36] On the other hand, for gaps in functional assay knowledge of mAbs, comprehensive quality data are likely to mitigate the risk of the uncertainty. For example, combing functional binding assays, such as complement dependent cytotoxicity (CDC) and antibody-dependent cell cytotoxicity (ADCC) assays, with sensitive quantitative glycan data could serve as a surrogate for unknown additional Fc functionality, which might not be directly implied by the functional binding assays.[33]

To what extent are non-clinical studies in relevant species required for biosimilar mAbs?

A non-clinical study with relevant species is a critical transition to first human clinical trial. A

non-clinical study could be a challenging task in evaluating comparability between reference and biosimilar products, in which relevant species are usually required for assessing the pharmacological and toxicological parameters of biological products, particularly mAbs. This is because mAbs tend to be highly species-specific regarding their sophisticated mechanism of action, which is significantly influenced by the post-translational modifications on mAbs.[16] A valid relevant species refer to subjects in which the test material is pharmacologically active due to the expression of certain receptors or, in case of mAbs, epitopes.[37] Thus the relevance of the species chosen to evaluate the toxicity of a mAb for human use should be demonstrated. Normally the primary amino acid sequence of the human mAb's epitope will be compared with the corresponding sequence of the candidate species. However, even with nearly identical sequence homology, it is not sufficient to conclude on the relevance of the species.[16] For example, alemtuzumab (Campath), an anti-CD52 mAb, has 16-fold higher affinity to its ligand in Cynomolgus monkey than in humans due to an amino acid deletion in sequence.[38]

Nevertheless, comparative pharmacokinetics/pharmacodynamics (PK/PD) studies obtained in a relevant species are still necessary, but should be combined to reduce the number of animals used. A head-to-head comparative PK/PD evaluation, if feasible, should be conducted to specify how in vitro PD results translate into in vivo effects. Toxicology studies should include one repeat dose study of minimal but sufficient duration to evaluate the toxicity profile in relation to that of the reference product.[39] The necessity of head-to-head comparative toxicology studies need to balance the extensive relevant species used in comparative studies and the capacity to verify unexpected toxicity of a biosimilar from a reference product. In addition, immunogenicity of biosimilar mAbs should be examined to explain potentially unexpected PK/PD profiles or toxicity. Safety pharmacology should be conducted on a case-by-case basis, e.g., cardiovascular endpoints should be included in a repeat dose toxicology study. Injection sites should be evaluated to determine local tolerance.[33]

Given the fact that relevant species for assessment of mAbs are often non-human primates (NHPs), the number of subjects per group is likely to be limited. In this case, the conduct of large comparative toxicity studies may not be feasible or ethically acceptable. Creation of animal models, such as transgenic mouse model, might be a solution to the limited size of NHP studies. Furthermore, experience gained with numerous existing mAbs will have to be considered, together with a differential discussion on the toxicity, in which product-related (e.g. mechanism of action) and process-related (e.g. impurities) toxicity should be justified. Thus, to what extent non-clinical pharmacodynamic studies are feasible and necessary in view of the clinical data to be gathered is important.[34] Since innovators have already established key factors, e.g. toxicity profile, for appropriate use of relevant species, the comparative assays such as dose-response tests are more suitably compared in non-clinical studies, rather than clinical trials. Most importantly, unnecessary duplication of toxicity studies with the reference product should be avoided as possible. The exploration of new methodologies, e.g., modeling, simulation, use of biomarkers, to optimize study

design (such as numbers of animals), should also be encouraged.[33] Further additional regulation, such as more rigorous binding with regulatory authorities for scientific advice, as well as more centralized committee for approval of NHP experiments, might be needed.

Current regulatory issues on biosimilar mAbs: Clinical

To what extent can safety and efficacy data be extrapolated from one indication to another and to what extent are comparative clinical trials necessary for biosimilar mAbs?

The clinical development of biosimilars is strictly comparative in nature. The designs of clinical trials need to be explored to provide sufficient reassurance of equivalent efficacy and comparable safety a biosimilar mAb to a reference mAb.[34] It may be possible to extrapolate a biosimilar's efficacy and safety data for one indication to other approved indications of its reference product, according to the current general guideline on clinical aspect of biosimilar products, only if the biosimilarity could be justified. The justification, including whether or not the same mechanism of action or the sample receptors are involved in all indications, will depend on clinical experience, available literature data and previous comparative assay (i.e. CMC and non-clinical studies).[12] However, the mechanism of action of biologic products could be disease-specific or not well-defined, particularly in the case of mAbs. Unlike biologicals of lower molecular weight, mAbs exhibit much higher complexity, in which the clinical activities could be determined by multiple properties of mAbs. Thus, the structure-function relationships of mAbs are often not quite intricate and need to be further specified.

With regard to mechanism of action, mAbs have diverse functional activities, in which the same molecule could interact differently with diverse receptors and lead to different scenarios, such as antigen neutralization, receptor blockade, ADCC, CDC, transport into specific tissues, etc.[40] Besides, mAbs could be used for quite dynamic and diverse indications, e.g. rituximab for non-Hodgkin's lymphoma, chronic lymphocytic leukemia and rheumatoid arthritis. Different indications might require specific ligand-receptor coordination in terms of different expression of target antigen and receptors, different reaction sites, over different time courses, under different concomitant medications.[41] Thus extrapolation between indications of biosimilar mAbs might not be as straightforward as that of smaller biosimilars. Justification for the extrapolation will be rather difficult because the differences will often exist and not likely to be excluded due to the size, complexity, mechanisms of action, and multifunctionality of mAbs. Thus comparative clinical trials will be necessary. The assay sensitivity has to be ensured and margins of trial design should be pre-specified primarily on clinical grounds Assay sensitivity has to be ensured.[41]

Which clinical endpoints should be used for assessing biosimilar mAbs as a general strategy?

As knowledge about the mechanism of action mAbs has been increased, the prediction of efficacy in new clinical indications should also be enhanced. Since the extrapolation of efficacy between indications of biosimilar and reference mAbs will still be evaluated on considerably on a case-by-case basis, utilization of sensitive and rapidly measurable clinical endpoints will be important for abridged development programs for comparative pivotal clinical studies. It might be possible to conduct a pivotal clinical trial as a comparative trial with the biosimilar against the reference mAbs in which differences in efficacy and safety can be detected and justified with sufficient accuracy and sensitivity. In this case, short-term efficacy studies with surrogate clinical endpoints are usually employed particularly when differences in hard endpoints are not detected with sufficient sensitivity. [34] However, this scenario might be without precedent and thus require discussion with regulators.

The selection of clinical endpoints is crucial and requires discussion to demonstrate biosimilarity, i.e. whether the endpoint should be the most sensitive endpoint (e.g. a surrogate endpoint) or a clinically relevant endpoint measuring patient benefit (e.g. overall survival) but might be less sensitive to detect differences. For endpoints used for demonstrating similarity, should these be rather conforming to guidelines, or could these be newly developed endpoints? Considerations for choosing endpoints have been proposed in the EMA conceptual guidelines for biosimilar mAbs (EMEA/CHMP/BMWP/632613/2009), including the severity of the diseases (e.g. cancer, or autoimmunity), the heterogeneity of the patient population that might hamper the biosimilarity exercise, and the feasibility of clinical trial design, such as the number of patients required. [34] Moreover, European Biopharmaceutical Enterprises (EBE) suggested that the EMA should to further define the “acceptance criteria” for biosimilarity in the major indications e.g. cancer, rheumatoid arthritis, psoriasis, etc. The future guideline should specify the EMA’s expectations for which endpoints should be employed and how clinically meaningful differences between endpoints should be defined in the comparative clinical studies biosimilar mAbs. Endpoints selected need to be both clinically meaningful and sensitive enough to detect any potential differences between the reference and biosimilar mAbs. Furthermore, the use of biomarkers or surrogate endpoints should be able to demonstrate biosimilarity and to support the extrapolation of indications of biosimilar mAbs.[32]

What is the role for Immunogenicity in the development of biosimilar mAbs

Unwanted immunogenicity is a major safety concern during the development of mAbs. Immunogenicity caused by mAbs might results from product- and process-related factors in which mAbs are recognized as foreign entities due to their impurities, large molecular size and complex structure, e.g. microheterogeneity and variable post-translational modifications which are different from that of endogenous proteins. Despite current advance in producing highly humanized and fully human mAbs, immunogenicity still can not be avoided.[42] The immunogenicity usually leads to development of anti-mAb antibodies, which might have clinical impact on the aspects of safety and efficacy of mAbs range from a lack of explicit consequences to life-threatening complications. These antibodies can act as neutralizing antibodies, which can affect pharmacokinetics and decrease biological activity of mAbs by binding directly to epitopes within or close to the active sites on mAbs. The existence of neutralizing antibodies may not result in an adverse clinical event but a reduction in efficacy, which requires administration of higher doses.[43] Unfortunately, the occurrence, the quantity, and the clinical relevance of developing specific anti-mAb antibodies (as identified in sera of patients) are extremely variable, and impossible to anticipate from the results of non-clinical safety studies.[43] Nonclinical evaluation of mAb immunogenicity usually demands for non human primates as relevant species due to the fact that targets for mAb are considerably species-specific. The tests on monkeys are cumbersome and relatively little is known of immunopathological responses in monkeys to ensure sufficient predictability of non clinical immunogenicity of mAbs in monkeys. Thus, the examination of specific anti-mAb antibodies in the sera of human subjects is crucial in the evaluation of immunogenicity of mAbs administrated.[44]

The recently established guideline on immunogenicity assessment of mAbs (CHMP/BMWP/114720/2009) provides additional consideration on mAbs than other classes of biologicals. For example, since anti-mAb antibodies can usually block mAb binding to its targets, competitive ligand binding assays of immunogenicity are often neutralizing assays for mAbs rather than classic bioassays. However, despite the fact that neutralizing antibodies against mAbs can be detected by testing patient sera in bioassays or cell-based assays, the clinical safety evaluation of immunogenicity for mAbs still need to be improved due to the limitations in the transition form animal to human studies.[45, 46] Not only immunological biomarkers of efficacy but also relevant biomarkers of safety in clinical trials need to be further defined. The biomarkers should ideally also be applicable to animal safety studies for facilitate the data extrapolation from animals to human. A recently developed method by combining in-silico and T-cell based assays, in which epitopes associated with induction or suppression of immune responses could be indentified, has shown potential for predicting immunogenicity of mAbs.[47]

Furthermore, risk-based approach for assessment of unwanted immunogenicity of mAbs has been suggested due to their considerably variability and uncertainty in clinical incidence, particular in the case of biosimilars. Some products are considered to be low risk; whilst others may be related to be

higher risk. However, since mAbs are varied greatly in immunogenicity and its clinical consequences, no defined criteria of risk value for mAbs can be applied on a product-class basis. Instead, risk assessment for mAb immunogenicity needs to be evaluated on a case-by-case basis.[48] Most importantly, with regard to biosimilar mAbs, the extra consideration is focused on determining product similarity of immunogenicity. Despite the current difficulty in defining comparative immunogenicity assessments, the relevant discussion might have to be specified in the upcoming guideline on biosimilar mAbs. The immunogenicity assessment should be designed to comparatively evaluate long-term immunogenicity of biosimilar and innovator mAbs using the same analytical standards.[32] In addition, immunogenicity of biosimilar mAbs will probably have to be evaluated separately according to their each indications in applicable.[48] Differences in immunogenicity across indications of a mAb are not well understood and can exist greatly between populations, disease states, concomitant medications, dose and route of administration.[32] The upcoming guideline on biosimilar mAbs should underline that human clinical data on immunogenicity in each indication are justified and extrapolation of immunogenicity could only be considered in ad-hoc circumstances.

Outlook and conclusion

Biosimilar, biobetters and 2nd generation mAbs in the near future

While everyone is awaiting the EU's upcoming legislation for biosimilar mAbs to take effect, biosimilar mAbs have come to the reality in other corners of the world. As the first wave of biosimilar mAbs, Reditux (a biosimilar rituximab developed by Dr. Reddy) and Clotinab (a biosimilar abciximab developed by ISU ABXIS) have been launched respectively in India and in South Korea.[49] Although no further information available to show how these biosimilar mAbs are comparable to the reference products, or whether they are under the pathways as rigorous as EU's regulatory framework, more companies have announced to deploy into the lucrative markets like the EU and US.[50] Further biosimilar mAb candidates might include copycat versions of blockbusters, such as infliximab, etanercep, cetuximab and trastuzumab.[51] With the soaring pharmaceutical expenditure, especially in the sector of therapeutic mAbs, the advent of forthcoming biosimilar mAbs in the EU and US is merely the matter of time. Healthcare payers worldwide are urgently looking for solutions to bridge the gaps brought by innovative biologicals with daunting price tags. On the other hand, pharmaceutical companies, while confronting predicaments of drying-out pipelines of new drugs and perceiving the upcoming patent expirations of many mAb blockbusters, are keen for the emerging market of biosimilars to replenish their portfolios. These are the driving forces to make the scenarios of biosimilar mAbs inevitably to happen in the near future, however, at the same time, leaving numerous debatable issues and challenges for regulators.

The major scenarios of the development of mAbs down the road, except innovative products with mechanism of action, are likely to include biosimilar, biobetter and 2nd generation mAbs. By definition, a biosimilar is a biological compound similar, in molecular and biological terms, to the active substance of the reference product.[52] Nearly all characteristics of a biosimilar mAb and its counterpart innovator product have to be as similar as possible, including mechanism of action, potency, immunogenicity, identical pharmacokinetics, etc. What a biosimilar is not, in fact, defines it more than what it is.[53] In another word, a biosimilar mAb can not be better than the reference product in any aspect; a biosimilar must be as good (or as bad) as the innovator. This would seem to be paradoxical, if a biosimilar mAb is declined due to its improvement in half-life or purity of the product. Therefore, for mAbs, the even more challenging and perplexing question is "how much similarity to the reference product does a biosimilar mAb has to demonstrate?" The margin for the answer is not straightforward, yet, because even with increasingly sophisticated analytical methodology to date, it is not possible to fully characterize mAbs with current technology owing to their structural complexity. Thus, biosimilar mAbs are considerably assessed on case by case and usually require further justification. Moreover, as comparative trials, which are essential for biosimilar mAbs to establish similarity of efficacy and safety, could require large number of patients; thus, certain biosimilar candidates might simply end up being infeasible. Also, most of the recent patent-expiring mAbs are murine or chimaeric antibodies, which are usually associated with higher

immunogenicity in the clinic; this makes the development of biosimilar mAbs not seem to be advisable. In such cases, the development of mAbs with comparably improved pharmacological properties, while sharing the same regulatory pathway with biosimilars, will be the possible scenario for the future.

However, as implied by the EMA guideline in the case of biosimilar interferon alfa-2b's setback, a baseline has been drawn: a biosimilar must have an identical amino acid sequence to its reference product, but a similar glycosylation profile is acceptable with justification.[2] Ever since, a biobetter mAb could be defined as an antibody which can target the same validated epitope, i.e. he same CDRs, as an existing antibody but with an optimized glycosylation profile, e.g. low fucose levels for enhanced ADCC or an engineered Fc domain to increase the serum half-life.[54] For example, a copies version of rituximab has been developed by using glycoengineered yeast strains, in which the tailored glycoforms of fucosylation on the mAbs can generate a 100-fold increase in ADCC.[55] Increasingly, more attention will be focused on the strategy for developing biobetters mAbs, in which improved glycoengineering and glycoprofiling methods are employed based on the principle of QbD (Quality by design) to tackle the complexity of mAbs.[56] More validation methods for specifying the structure-function relationships between glycosylation and the in vivo bioactivity of mAbs will also follow up. Therefore, it is reasonable that companies tend to develop biobetter, instead of biosimilar mAbs, with current state-of-the-art technology and knowledge, so that improved clinical performance and more competitive commercial profiles can be reached.

Another scenario may be the development of 2nd generation mAbs. A 2nd generation mAb, which might resemble its counterpart innovative product within a wider range than a biosimilar mAb does, can be designed to share the same molecular target, and exhibit slightly different properties such as lower immunogenicity, improved pharmacological properties (e.g., targets a different epitope, has higher affinity for the target with higher potency), or different structures (e.g., a humanized mAb versus a chimeric mAb).[33] One of the major distinctions between 2nd generation mAbs and the aforementioned biobetters mAbs is that a 2nd generation mAbs does not usually share identical primary amino acid sequence when compared with an existing innovative product. This feature, under current regulatory status, strongly implies that 2nd generation mAbs are illegible to be considered as comparable as biosimilar mAbs. However, there is a question about whether some concepts relevant to biosimilars are applicable to 2nd generation mAbs, such as those that are functionally equivalent but with subtly structural differences. The current consensus is that the slightly structural differences may come with uncertainty in modes of action or off-target effects of 2nd generation mAbs. For example, the TNF blocker, infliximab, shows potency in treating Crohn's disease patients whereas another TNF blocker Fc-fusion protein, etanercept, does not.[33] Therefore, 2nd generation mAbs are only applicable to new drug regulatory pathway, not biosimilar track. In the long run, as more humanized mAbs come to the market, the trend of development of mAbs will gradually shift from 2nd generation to biobetters mAbs, savoring the abbreviated regulatory pathway for biosimilars while taking less risk with existing knowledge of developing

mAbs..

In conclusion, the evolving EU guidelines on biosimilars are just to enter a new chapter: biosimilar mAbs. Despite being on the track to come to the reality, the development of guideline on biosimilar mAbs is considered as a regulatory challenge due the tremendous complexity of mAbs. They are vastly complex molecules with secondary and tertiary structures subject to post-translational modifications such as glycosylation. Vulnerable to even slight change during manufacturing process, mAb products are often heterogeneous with subtle mAb variants. Therefore, how similar a biosimilar mAb should reach, compared with its reference product, is currently the most concerned regulatory issue for all stakeholders. While comparative studies—in the aspects of CMC, non-clinical and clinical studies— are important for a biosimilar mAb, the margins for the acceptable differences between a biosimilar and reference mAb should be extensively discussed and justified. The discussions will be conducted on case-by-case basis between regulators and sponsors. Moreover, it is also crucial for evaluating biosimilar mAbs by a holistic approach, i.e. rigorous interpretation between structure-function relationships to reduce unnecessary clinical trials while providing comprehensive post-marketing risk management plans. In the long run, biobetters might be gradually taking over biosimilars on the established regulatory track and leading to better access to biological medications.

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