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The biosimilar regulatory framework across Europe: An approach to facilitate tailored clinical programmes for biosimilars

Elena Guillén Benítez

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CERTIFY: That the present doctoral thesis, submitted by Elena Guillén Benítez, with the title: " The biosimilar regulatory framework across Europe: An approach to facilitate tailored clinical programmes for biosimilars" has been conducted under their supervision.

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Abbreviations

ADCC	Antibody-dependent cellular cytotoxicity
ADCP	Antibody-dependent cellular phagocytosis
ACR 20	American College of Rheumatology Response
ADAs	Antidrug antibodies
AEs	Adverse events
AESi	Adverse events of special interest
AlphaLISA	Amplified Luminescent Proximity Homogeneous Assay
ALK	Anaplastic lymphoma kinase
AR	Assessment Reports
AUCinf	Area under the curve to infinity
AUClast	AUC from time of administration up to the time of the last quantifiable concentration
BAC	Boronate affinity chromatography
BLI	Bioluminescence imaging
BS	Biosimilar
BSA	Body surface area
CDC	Complement-dependent cytotoxicity
CDAI	Clinical disease activity index
CE-SDS	Capillary electrophoresis-sodium dodecyl sulfate
CEX	Cation exchange chromatography
cIEF	Capillary isoelectric focusing
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence interval
CGE	Capillary gel electrophoresis
CL/F	Oral clearance
Cmax	Maximum concentration
Ctrough	Trough plasma concentration
DAS	Disease activity score
D120	Day 120
DOR	Duration of response
DSC	Differential scanning calorimetry
DLS	Dynamic light scattering
EGFR	Epidermal growth factor receptor
ELAM-1	Endothelial-leukocyte adhesion molecule 1

ELISA	Enzyme-linked immunoassay
EMA	European Medicines Agency
EP	Endpoint
EPARs	European public assessment reports
EU	European Union
EULAR	European League Against Rheumatism
Fab	Fragment antigen binding
FAS	Full analysis set
Fc	Fragment crystallizable region
FcγR	Fragment crystallizable gamma receptor
FcRn	Neonatal Fc receptor
FDA	U.S Food and Drug Administration
FFF	Filed flow fractination
FRET	Förster (Fluorescence) resonance energy transfer
FT-IR	Fourier-transform infrared
HEK293	Human embryonic kidney 293 cells
HDX	Hydrogen–deuterium exchange
HDX-MS	Hydrogen/deuterium exchange mass spectrometry
HIC	Hydrophobic interaction chromatography
HILIC-	Hydrophilic Interaction ultra-performance liquid chromatography
UPLC	Ultra-high-performance liquid chromatography
HPLC	High-performance liquid chromatography
HMWS	High molecular weight species
HUVECs	Human umbilical vein endothelial cells
HR	Hazard ratio
HVs	Healthy volunteers
IBDs	Inflammatory bowel diseases
iCIEF	Imaged capillary electrophoresis focusing
ICAM-1	Intercellular adhesion molecule 1
IEC	Ion exchange chromatography
IL-6	Interleukin 6
IL-8	Interleukin 8
IP	Investigational product
ITT	Intention-to-treat
KDR	Kinase insert domain receptor

LC-ESI-MS/MS	Liquid chromatography electrospray ionization tandem mass spectrometry
LMWS	Low molecular weight species
mAb	Monoclonal antibodies
MAA	Marketing authorisation application
MCP-1	Monocyte chemoattractant protein-1
MFI	Micro-flow imaging
MIP-1- β	Macrophage inflammatory protein 1-β
MoA	Mechanism-of-action
MLR	Mixed lymphocyte reaction
mTNFα	Membrane-associated TNFα
MTX	Methotrexate
mTSS	Modified total sharp score
NA	Not applicable
NF-κB	Nuclear factor kappa B
NGHC	Non-glycosylated heavy chain
NK	Natural killer
NMR	Nuclear magnetic resonance
NOESY	NOE correlated spectroscopy
NP	Not presented
NR	Non-reduced
NRI	Non-responder imputation
NSCLC	Non-squamous non-small cell lung cancer
OS	Overall survival
ORR	Overall response rate
PASI	Psoriasis area and severity index
PIGF	Placental growth factor
PK	Pharmacokinetic
PD	Pharmacodynamic
PBMCs	Peripheral blood mononuclear cells
PFS	Progression-free survival
PopPK	Population PK
PP	Plaque-type psoriasis
PPS	Per-protocol set
QAs	Quality attributes
RA	Rheumatoid arthritis

RCI	Root cause imputation
RD	Risk difference
Red	Reduced
RGA	Reporter gene assay
RP	Reference product
RPLC-UV/MS	Reversed phase liquid chromatography-ultraviolet/mass spectrometry
RR	Risk ratio
SAEs	Serious adverse events
SD	Standard deviation
SDAI	Severity disease activity index
SEC	Size-exclusion chromatography
SEC-MALS	Multi-angle light scattering coupled with size-exclusion chromatography
SF-36	Short form health survey
sPGA	Static physician global assessment
SPR	Surface plasmon resonance
SV-AUC	Sedimentation velocity-analytical ultracentrifugation
sVCAM-1	Soluble vascular cell adhesion molecule-1
T ½	Half-life
TEAEs	Treatment emergent adverse events
TNFα	Tumor necrosis factor α
Tmax	Time to maximum effect
TP1	Treatment period 1
TP2	Treatment period 2
UV-280	Ultraviolet absorbance at 280 nm wavelength
VEGF	Vascular endothelial growth factor
VEGFR2	Vascular endothelial growth factor receptor 2
Vz/F	Apparent volume of distribution during terminal phase

Abstract

After more than 15 years of regulatory experience with biosimilars in the European Union, there is sufficient evidence to support a more flexible approach in clinical data requirements for biosimilars. However, stakeholders and healthcare professionals are still hesitant regarding clinical tailoring for more complex biosimilar substances such as monoclonal antibodies (mAbs). This thesis aims to provide a deeper understanding on the role of analytical/functional and clinical data for the conclusion of biosimilarity and the decision on marketing authorisation (MA) for complex biosimilars (mAb and fusion proteins). Therefore, we analysed the marketing authorisation applications (MAAs) of all 33 mAbs and 3 fusion proteins evaluated by the European Medicines Agency (EMA) between July 2012 and November 2022. Moreover, we studied more in-depth the analytical and functional similarity data, and the clinical packages available for a representative set of 25 biosimilar mAbs (all submitted adalimumab, bevacizumab, rituximab and trastuzumab biosimilar candidates until the timepoint of analysis), including withdrawn applications. Our analysis showed, that in all cases, the quality package was predictive of the MA outcome, and there were no instances where seemingly negative clinical data, including failed efficacy trials, led to a negative overall decision. Moreover, in no case was clinical efficacy data necessary to resolve residual uncertainties. In brief, this work calls into question the usefulness of comparative clinical efficacy studies for the purposes of regulatory decision making when approving biosimilar mAbs and fusion proteins. This further supports the argument that sufficient evidence for biosimilarity can be obtained from a combination of analytical and functional testing and pharmacokinetic studies.

Resumen

Después de más de 15 años de experiencia regulatoria con biosimilares en el mercado europeo, existe suficiente evidencia que apoye un enfoque más flexible en los requisitos sobre estudios clínicos confirmatorios para biosimilares. Sin embargo, aún existe cierta resistencia en la disminución de requerimientos clínicos para biosimilares más complejos, como los anticuerpos monoclonales (mAbs). Esta tesis tiene como objetivo aportar más datos sobre el papel que realmente juegan los datos analíticos/funcionales y clínicos en la conclusión sobre la biosimilitud y la decisión de autorización de comercialización para biosimilares complejos. Hemos analizado las solicitudes de autorización de comercialización de 33 mAbs y 3 proteínas de fusión evaluados por la Agencia Europea de Medicamentos (EMA) entre julio de 2012 y noviembre de 2022. Se ha analizado en mayor profundidad los paquetes analíticos/funcionales y clínicos disponibles para un conjunto representativo de 25 biosimilares mAbs (todos los candidatos biosimilares de adalimumab, bevacizumab, rituximab y trastuzumab), incluyendo también solicitudes que fueron retiradas. En todos los casos, se observó que la evaluación del paquete de calidad era predictiva del resultado de la autorización de comercialización, y no hubo ningún caso en el que la presencia de datos clínicos negativos (incluidos los estudios clínicos de eficacia fallidos), dieran lugar a una decisión de autorización global negativa. Además, en ningún caso datos de estudios confirmatorios clínicos de eficacia ayudaron a resolver incertezas residuales en la evaluación de estos productos. En resumen, este trabajo cuestiona la utilidad de los estudios comparativos de eficacia en la toma de decisiones regulatorias y la aprobación de biosimilares de mAbs y proteínas de fusión. Esto respalda el argumento de que existe suficiente evidencia de biosimilitud con la combinación de un paquete analítico/funcional robusto, y estudios farmacocinéticos.

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1.1. THE INTRODUCTION OF BIOSIMILARS IN THE HEALTHCARE LANDSCAPE

Biologics are defined as medicinal products that are manufactured using biological systems, such as living cells or organisms. They are distinct from traditional chemical drugs because they are large and complex molecules derived from living sources (e.g., monoclonal antibodies (mAb)) (1). Biologic medicines have emerged as a crucial cornerstone in modern healthcare, proving highly effective in addressing a wide array of intricate medical conditions, such as cancer and immunological diseases. Their unique ability to target specific biological pathways and mechanisms has revolutionised the therapeutic landscape, offering new approaches and improved quality-of-life for patients with complex medical conditions, that otherwise would have limited therapeutic options (in many cases with low efficacy and high toxicity rates) (2).

Despite these achievements, the accessibility of pioneering biologic therapeutics remains notably disparate across global healthcare landscapes, with price being one of the main hurdles. Only in Europe, biologics constitute a substantial 35% share of pharmaceutical expenditure, exhibiting a noteworthy compound annual growth rate (CAGR) of 11.3% over the past quinquennial period. This compares to a 6.3% CAGR for the total market, demonstrating the exponential importance and growth in comparison to non-biological medicines(3).
Ultimately, such a growth in pharmaceutical expenditure and the lack of price competition has an enormous impact on patient´s access to these treatments.

Biosimilars have arisen as a promising therapeutic alternative to address the ongoing challenges surrounding accessibility to biological medications.
They are defined as biological copy products that contain a highly similar version of the active substance of an already authorised original biological medicinal product (RP, reference product), providing patients with products of the same quality, safety, and efficacy(4).

Biosimilars differ from generic drugs due to their biological source, the size of the active substance, their complexity, and the nature of the manufacturing process.

Same as generics, biosimilars can be introduced in the market once the market exclusivity for the original biologic ends, i.e., loss of basic patent and any associated supplementary protection certificate.

The entrance of biosimilars into the European market has led to competitive price reductions for both biosimilars and their respective biological originator, facilitating increased patient access. However, fostering biosimilar competition, i.e., having multiple suppliers of the same active substance, is necessary to curtail overall healthcare costs and to avoid supply shortages(3).

1.2. COMPARISON OF THE DEVELOPMENT PROCESS OF BIOSIMILARS AND ORIGINATOR BIOLOGICS

Because the current concept of developing complex molecules as biosimilars differs greatly from generic drugs, so does their regulatory approval process.

Generics are considered identical copies of an already approved product, and therefore mainly bioequivalence studies (together with routine quality documentation) are key for their approval, eliminating unnecessary and unethical safety/efficacy studies(5). Biosimilars however, due to their biological source, complexity, manufacturing process and the direct impact that the manufacturing process itself can have on the quality profile of the active substance, will not be identical but highly similar versions of an already approved RP. Therefore, for their approval high similarity must be shown beyond bioequivalence, considering also efficacy and safety, which includes the demonstration of absence of clinically meaningful differences(4).

Although demonstration of biosimilarity is more complex than the demonstration of bioequivalence for generics, the main goal in the development process of a biosimilar is high similarity to an already approved biologic, not patient benefit. Therefore, the development process of originator biologics and biosimilars differs on the weight and importance of each step.

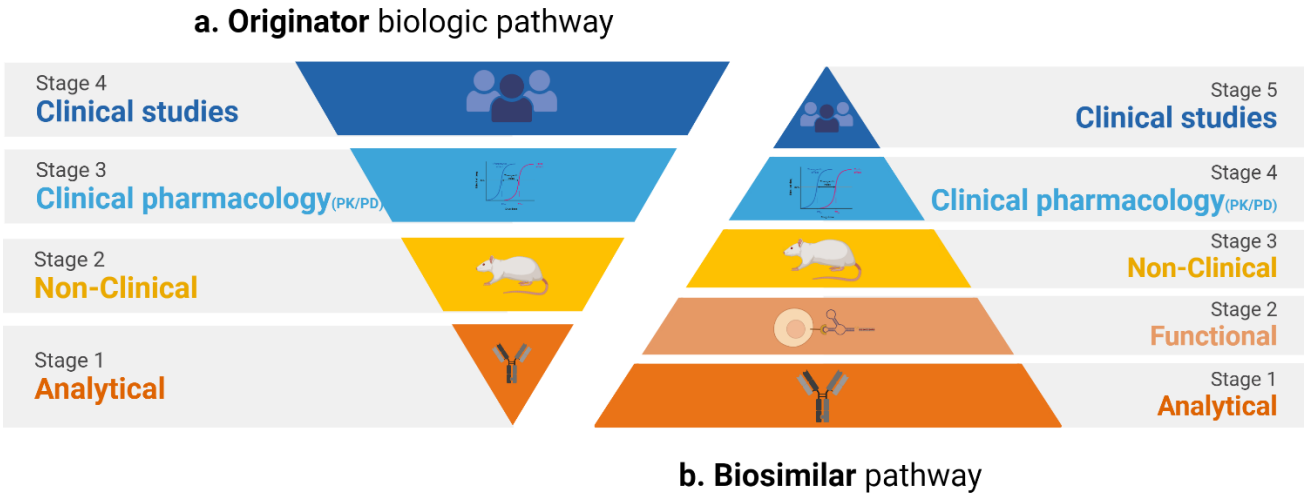
Figure 1 represents a comparison of the development process between originator biologics and biosimilars. The size of each individual stage is directly proportional to the weight that step has in the development process of the product, i.e., clinical efficacy/safety studies are paramount for originator biologics.

In the development process of any new molecule (i.e., originator biologic), the main goal is to establish safety and efficacy in a specific indication (**Figure 1a**). This includes i) physicochemical and functional characterization, ii) thorough preclinical tests, including understanding of mechanism of action (MoA) and animal toxicology, iii) establishing a therapeutic dose, and finally, iv) clinical studies to test safety and efficacy per indication. Each indication will require an individual phase 3 trial with carefully chosen patient population, endpoints, and length of observation time. The last stage, testing that the product is safe and efficacious in the patient population (and in the context of available standard therapies), is the main goal.

However, as mentioned before, in biosimilar development the main goal is to demonstrate biosimilarity (i.e., high similarity) to the RP, not patient benefit (**Figure 1b**). Consequently, the cornerstone in their development process is a comprehensive demonstration of high physicochemical and functional (analytical) similarity to their RP (stages 1 and 2). If comparability cannot be ensured at a physicochemical and functional level, and absence of clinically meaningful differences cannot be guaranteed, other data (nonclinical and clinical) will be needed (stages 3-5). Overall, the different steps of the comparability exercise will ensure that the proposed biosimilar does not exhibit any clinically significant differences in terms of quality, safety, and efficacy when compared to the RP.

Of note, throughout this thesis, the terms comparability and (bio-)similarity exercise are used synonymously.

Figure 1. Comparison of the development process between a) originator biologic, where the main goal is to establish safety and efficacy and **b) biosimilar**, where the main goal is to establish biosimilarity to the reference product.



1.3. THE EMERGANCE OF BIOSIMILAR REGULATION

Biosimilar comparability studies (and their evaluation process) were initially developed based on the ICH Q5E guidance on biotechnological/biological products subject to changes in their manufacturing process(6). This guidance lays out the principles and requirements for bridging of data between manufacturing changes from highly complex molecules to ensure that changes in manufacturing process will not have an adverse impact on the quality, safety and efficacy of the drug product. This consistency is guaranteed throughout the comparability exercise between pre-change and post-change product batches.

As stated in the ICH Q5E guidelines(6), “The demonstration of comparability does not necessarily mean that the quality attributes (QAs) of the pre-change and post-change product are identical, but that they are highly similar, and that the existing knowledge is sufficiently predictive to ensure that any differences in QAs have no adverse impact upon safety or efficacy of the drug product”. The ICH Q5E guideline already establishes that, although analytical testing and biological assays are the cornerstone of the comparability exercises, in some cases, bridging nonclinical and/or clinical studies might be needed. The extent and nature of nonclinical and

clinical studies is determined on a case-by-case basis, taking into account several factors, i.e., quality findings, the nature of and level of knowledge of the product and existing nonclinical and clinical data relevant to the product. These may include pharmacokinetic (PK), pharmacodynamic (PD), clinical efficacy, specific safety, immunogenicity and pharmacovigilance studies.

Same as inter-batch variability, changes between a biosimilar and its originator occur and are expected and accepted. Same as inter-batch variability, the assurance that these changes do not affect the efficacy and safety of a biosimilar candidate is also done via comparability studies, following the ICH Q5E guidance. Therefore, comparability is fundamentally based on physicochemical and analytical testing, but if necessary, may also require relevant nonclinical and clinical data.

In this context, the regulatory pathway of biosimilars (1,7,8) emerged following a unique framework which adheres to the principle of considering the 'totality of evidence'(9). This principle follows a stepwise approach that relies on the accumulation of knowledge and understanding of the proposed biosimilar compared to its RP. This allows to interpret any differences identified between them, and to ensure that residual uncertainties arising at any step can be addressed during the development pathway(1,9,10).

Figure 1b represents the different stages of a biosimilar development programme, as it was established in the first biosimilar guidelines published by different regulatory bodies, in early 2000 (11–17). Again, a stepwise approach was recommended throughout the development process, with the extent and nature of nonclinical and clinical data depending on the level of evidence obtained in the previous step(s).

The following paragraphs lay out the principles covered in these first guidelines:

The first stages of biosimilar characterisation rely on analytical and functional comparability (steps 1 and 2 of **Figure 1b**), which is based mainly on extensive comparison of QAs with the RP. Critical QAs (CQAs) are defined as specific measurable characteristics (physicochemical, functional and/or biological) of the

biological product that are essential to ensure its safety and efficacy. All other QAs are considered to further define the safety, efficacy and overall, the quality profile of the product (18).

As for nonclinical development (step 3 of **Figure 1b**), the choice and extent *of in vitro* and *in vivo* studies is decided on a case-by-case basis. Comparative *in vitro* studies to assess differences in binding or functions should be conducted first. If an *in vivo* study is deemed necessary, the focus of the study depends on the need for additional information, and the availability of a relevant animal model.

Once analytical and functional comparability and bioequivalence is established, similarity with regard to efficacy and safety usually has to be confirmed in one “model” indication in a comparative efficacy and safety study. Again, the extent and nature of the clinical programme depends on the level of evidence obtained from preceding steps which support comparability.

In principle, the most sensitive model and study conditions should be used in a homogeneous patient population. A comparative PK study in a sufficiently sensitive and homogeneous study population (healthy volunteers or patients) normally forms an initial step of biosimilar development. PD parameters (if there is a relevant PD measure) may contribute to the comparability exercise in certain indications (step 4 of **Figure 1b**).

Therefore, for the assessment of comparability a comparative human PK and PD studies, if there is a relevant PD measure(s), and a clinical immunogenicity assessment are expected. In certain cases, the results of these studies may provide adequate clinical data to support a conclusion that there are no clinically meaningful differences between the proposed biosimilar product and the RP. However, if residual uncertainty about biosimilarity remains after conducting these studies, an additional comparative clinical study or studies would be needed to further evaluate whether there are clinically meaningful differences between the two products (step 5 of **Figure 1b**).

As for the expectations on clinical efficacy data, in the development of a biosimilar medicinal product, the choice of clinical endpoints and time points of analysis of

endpoints may deviate from the guidance for new active substances. As stated in regulatory guidelines(8,12), similar clinical efficacy between the similar and the RP should be demonstrated in adequately powered, randomised, parallel group comparative clinical trial(s), preferably double-blind. In general, an equivalence design is advised, and the trial is usually not time related and conducted in a one-year time frame. Regarding endpoints, they may differ, as it is not necessary to use the same primary efficacy endpoints as those that were used in the MAA of the RP. However, it is advisable to include some common endpoints (e.g., as secondary endpoints) to facilitate comparisons to the clinical trials conducted with the RP. Regarding immunogenicity, the type and amount of immunogenicity data will depend on the experience gained with the RP and the product class.

Other indications of the RP can usually be extrapolated, thus avoiding repetition of clinical trials already carried out with the RP(19).
For example, adalimumab originator has currently 9 indications which required 9 separate clinical phase 3 trials for each condition(20). All approved adalimumab biosimilars were granted marketing authorisation (MA) for all the 9 approved indications of its originator, based on the comparability demonstrated through quality comparison and clinical PK and efficacy trial, which confirmed biosimilarity.

1.4. THE EVOLUTION OF THE BIOSIMILAR REGULATORY PATHWAY

Figure 2 represents the evolution of the biosimilar regulatory pathway since the approval of the first biosimilars until today.

The biosimilar development process in Europe began in 2004, with the first approval of biosimilars two years later(21). At the initial stages of biosimilar development, many uncertainties remained regarding the translation of analytical and functional similarity to the clinical level. Therefore, the early regulatory framework followed a conservatory 5-step development process very similar to that of other complex molecules (**Figure 2a**). Consequently, a comparative clinical efficacy study was routinely required as a safeguard and precautionary measure to ensure that

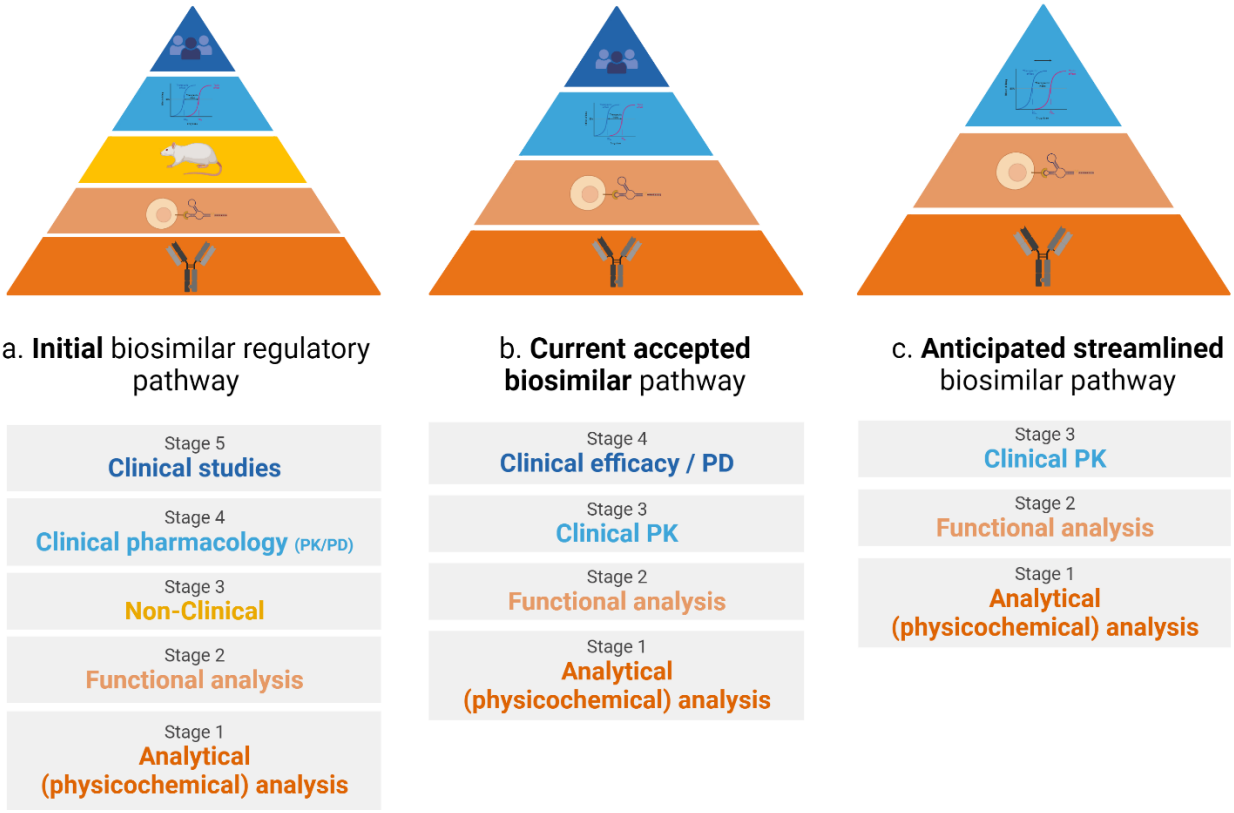
biosimilarity demonstrated at the analytical and functional (quality) level indeed translated into biosimilarity at the clinical level.

After cumulative experience, in the following years it has been made clear that nonclinical animal (*in vivo*) studies are no longer necessary for biosimilar development (**Figure 2b**). The guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: nonclinical and clinical issues(10) addresses the extent of the nonclinical studies required to confirm biosimilarity. It is stated that differences observed in the physicochemical and biological analyses will require additional *in vitro* studies, taking into consideration the MoA of the active substance in all the authorised indications of the RP and pathogenesis of the diseases included in the therapeutic indications. However, nonclinical animal studies are not considered to add to the totality of the evidence. Recent guidelines(22) make an even clearer statement “no *in vivo* studies from animals are requested as these are not relevant for showing comparability between a biosimilar candidate and its RP”. This eliminates default nonclinical animal (*in vivo*) work, which, if necessary, in any case will be determined on a case-by-case basis and depending on the level of evidence obtained in the previous steps.

This current 4-step regulatory pathway is already accepted worldwide. However, after more than a decade of experience with biosimilars, it is becoming more evident that when an adequate comparability exercise is established at an analytical/functional level (and the MoA of the originator is well known and established), confirmatory clinical efficacy studies also add little or no information that is useful for regulatory purposes(21). Therefore, a further streamlining of the biosimilar pathway is anticipated (**Figure 2c**).

Figure 2. The evolution of the biosimilar regulatory pathway: a) 5-step initial biosimilar regulatory pathway, similar to that of original molecules, b) current 4-step accepted biosimilar

regulatory pathway, without the inclusion of nonclinical animal studies, c) 3-step anticipated streamlined biosimilar pathway, without the inclusion of clinical confirmatory efficacy studies.



The reasons for further streamlining biosimilar development have become clear in the past years: the advancement of analytical sciences and the regulatory experience gained since the first approval of a biosimilar product, which have shown that confirmatory efficacy trials add very little to the totality of the evidence approach.

Regulatory guidelines developed in the past years, already consider a more flexible approach in the need of confirmatory clinical efficacy studies(11,22–24). These establish that when there is a convincing characterisation of a biosimilar in the initial stages of the comparability exercise (physicochemical and functional) performed with orthogonal and sensitive methods, together with PK and/or PD comparison, a confirmatory efficacy study may be waived. In other words, the requirements for a clinical efficacy trial, as stated in regulatory guidelines, is mainly to address residual uncertainty if there are clinically meaningful differences between the proposed product and the RP.

US biosimilar guidelines establish those factors that may influence the type and extent of the comparative clinical study data needed(8):

i. The nature and complexity of the RP, the extensiveness of structural and functional characterization, and the findings and limitations of comparative structural, functional, and nonclinical testing, including the extent of observed differences.
ii. The extent to which differences in structure, function, and nonclinical pharmacology and toxicology predict differences in clinical outcomes, in conjunction with the degree of understanding of the MoA of the RP and disease pathology.
iii. The extent to which human PK or PD is known to predict clinical outcomes (e.g., PD measures known to be relevant to effectiveness or safety)
iv. The extent of clinical experience with the RP and its therapeutic class, including the safety and risk-benefit profile (e.g., whether there is a low potential for off-target adverse events), and appropriate endpoints and biomarkers for safety and effectiveness (e.g., availability of established, sensitive clinical endpoints).
v. The extent of any other clinical experience with the proposed product (e.g., if the proposed product has been marketed outside the United States).

It further explains that sponsors should provide a scientific justification for how it intends to use these factors to determine what type of clinical study(ies) are needed and their design, and how certain aspects may be modified. For instance, since PD measures are more sensitive than clinical endpoints, there may be situations when the assessment of multiple PD measures in a comparative clinical study will enhance the sensitivity of the study and facilitate the conduct of a smaller study of limited duration.

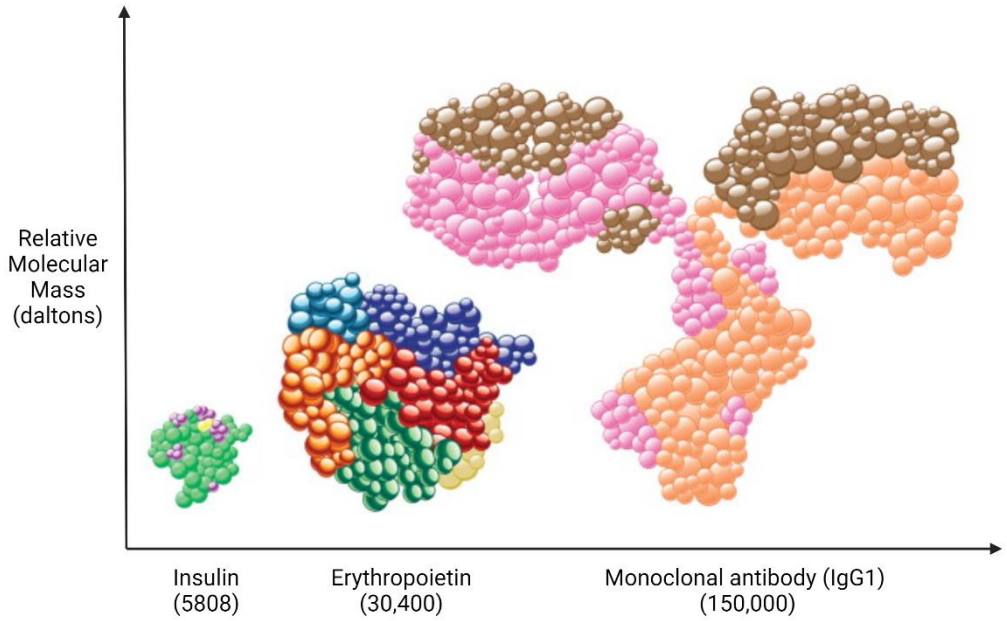
Therefore, there are several aspects of clinical efficacy trials that can be "cut down" for streamlining biosimilar development if the previous steps have shown adequate comparability. This includes shorter trials, less immunogenicity data for low immunogenicity products, conducting a clinical trial mainly focused on safety and immunogenicity endpoints and efficacy as a secondary endpoint, or not conducting

a clinical trial at all.

In the EU, this streamlined approach has already been established for less complex biologics, such as recombinant granulocyte colony stimulating factors, low molecular weight heparins or insulins. For these biologics, several product specific guidelines have been revised in the past years, in light of analytical advancements and regulatory experience (14–17,25). For instance, insulin specific guidelines, revised in 2012(17), state that “there is no anticipated need for specific efficacy studies since endpoints used in such studies, usually HbA1c, are not considered sensitive enough to detect potentially clinically relevant differences between two insulins”. Similarly, low molecular weight heparin guidelines, revised in 2016(26), state that “whereas the parent guideline required a comparative clinical trial by default, the revised guideline focusses on demonstration of biosimilarity based on a strong and convincing physicochemical and functional data package and comparable PD profiles. Moreover, pre-marketing clinical immunogenicity data may not be necessary if the immunogenic potential can be adequately characterized in suitable and sensitive *in vitro* tests”. Product specific guidelines for recombinant granulocyte colony stimulating factors, also revised in 2017(27), show a similar message: “whereas the previous version of this guideline requested a comparative clinical trial in most cases, the revised guideline focusses on demonstration of biosimilarity based on a strong and convincing physicochemical and functional data package and comparable PK and PD profiles”.

However, in the particular case of other complex biosimilars such as mAbs and fusion proteins, this reduction of clinical data still has not been routinely established worldwide, due to the fact that, these are molecules with a much higher structural complexity compared to other biologicals (**Figure 3**). Highly complex molecules have been linked to more uncertainties in the translatability of analytical/functional comparability to a clinical level, and the safety and immunogenicity data extracted from the previous steps of the comparability process.

Figure 3. Structural comparison of different biologics by relative molecular mass. Monoclonal antibodies (IgG1) are structurally more complex than small-molecule agents and lower molecular weight biologics. *Figure modified from Mellstedt H. Clinical considerations for biosimilar antibodies. EJC Suppl. 2013 Dec;11(3):1-11.*



The EU guideline on similar biological medicinal products containing monoclonal antibodies, nonclinical and clinical issues(11), published by the European Medicines Agency (EMA) in 2012, consider some flexibility on clinical data: “to establish comparability, deviations from disease-specific guidelines issued by the Committee for Medicinal Products for Human Use (CHMP) may be warranted (choice of endpoint, timepoint of analysis of endpoint, nature and dose of concomitant therapy, etc.)”. The guiding principle is to demonstrate similar clinical efficacy and safety compared to the reference medicinal product, not patient benefit *per se*, which has already been shown for the reference medicinal product”. It states clearly that a step-wise approach is recommended, with clinical efficacy studies considered a second step after PK/PD trials. However, no further guidance is provided and this flexibility in terms of clinical efficacy trial requirements is not clear and seems to be still considered a default requirement: “normally, similar clinical efficacy should be demonstrated in adequately powered, randomised, parallel group comparative clinical trial(s), preferably double-blind, normally equivalence trials”.

There is cumulative evidence that streamlining of biosimilar development does not

affect the comparability of the biosimilar with its RP. Many authors have studied the extent of information that quality versus clinical efficacy data provides in biosimilar development (25,28–31). This streamlining of biosimilar development follows the totality of evidence approach, meaning that it is not less evidence that is wanted, but just a shift away from clinical burden to more extensive quality analysis.

However, biosimilar streamlining has not been implemented yet in all healthcare landscapes, and revision of current regulatory guidelines is necessary as the reduction of demands on clinical trials is not covered in most available guidelines, especially regarding more complex biologics. This shows that there are still residual uncertainties regarding biosimilar streamlining, and that more data is needed to demonstrate exactly what role quality vs. clinical data plays in the evaluation process of biosimilars for complex biosimilars such as mAbs and fusion proteins.

1.5. THE BENEFITS OF STREAMLINING BIOSIMILAR DEVELOPMENT PROGRAMMES

The benefits of further streamlining biosimilar development are of interest worldwide and have become a more pressing matter in recent years.

Firstly, it is unethical to subject patients to redundant clinical trials that offer little real contribution to the scientific community. Secondly, it would allow for the reduction of the cost of biosimilars and enable the allocation of these resources towards the use of truly innovative therapies. Thirdly, it would allow for new biosimilar development and competition among the field of biologics. Currently, estimates for the development cost of a biosimilar range from 100 to 300 million US Dollars(32), contrasting sharply with the 1–5 million US Dollars associated with a small molecule generic. This significant disparity primarily stems from the substantial clinical development expenditures (33–35). These large costs can make biosimilar development unattractive to pharmaceutical companies. In fact, it has been estimated that in the future more than 50% of biologicals with loss of market exclusivity will be without competitors (3). Some of the reasons for this include high

development costs for biologic medicines, difficult trial designs or small patient populations to be treated. The lack of new biosimilar development and biosimilar competition has fatal consequences for both patients and healthcare systems as it promotes inequality of access to important and innovative treatments.

In summary, if biosimilar development is not further streamlined soon, patient accessibility to new available therapeutic treatments will not be possible.

Objectives & Hypothesis

02

The underlying **hypothesis** of this thesis is that analytical/functional data (together with properly powered PK studies) has sufficient robustness and strength *per se* on the final conclusion on biosimilarity and the decision on MA for even complex biosimilars, calling into question the usefulness of routine confirmatory clinical efficacy trials. Moreover, it is hypothesised that analytical methods are more sensitive tools to detect differences between biosimilars and its RP than the clinical efficacy studies routinely performed, as in many cases these are not powered to detect such differences.

The general **aim** of this thesis is to provide deeper understanding on the role of analytical/functional and clinical data for the conclusion of biosimilarity and the decision on MA for mAb biosimilars. To achieve this aim, two specific objectives were proposed:

1. Review, categorise and analyse the analytical and functional similarity data, and the clinical data packages available for complex biosimilar mAb classes with different indications and MoA: a total of 25 biosimilar mAbs, including also withdrawn applications.
2. Analyse the outcome of Marketing Authorisation Applications (MAA) and the list of questions raised by the CHMP in the first regulatory assessments of all 36 mAbs and fusion protein biosimilar candidates evaluated by EMA up to November 2022.

Methods & Results

03

The provision of deeper understanding on the role of analytical/functional and clinical data for the conclusion of biosimilarity and the decision on MA for mAb and fusion protein biosimilars has been carried out through two independent studies. The methodology of each of these studies is detailed in the corresponding publications in the following sections of the thesis. However, in this section, a summary of the design and method of each study is provided.

Publication 1: A data driven approach to support tailored clinical programmes for biosimilar monoclonal antibodies.

An observational retrospective study of a cohort of all approved adalimumab and bevacizumab biosimilars by EMA until September 2021 was carried out.

The analysis included analytical/functional and clinical data packages of seven adalimumab biosimilars (Amgevita/Solymbic, Imraldi, Hyrimoz/Halimatoz/Hefiya, Hulio, Idacio/Kromeya, Amsparity, and Yuflyma) and five bevacizumab biosimilars (Mvasi, Zirabev, Aybintio/Onbevzi, Alymsys/Oyavas, and Abevmy/Lextemy). Data was extracted from product dossiers, which are confidential as they are submitted to EMA for MA (analytical/functional data) and European public assessment reports (EPARs), which are publicly available at the EMA website (clinical data).

Analytical/functional biosimilarity analysis involved the review, categorisation, and anonymisation of QAs extracted from product dossiers. Categorisation was systemized using mainly four colour-coded groups based on the degree of biosimilar batches with values within the similarity range of the RP: dark green (100% similarity), light green horizontal stripes (90–99%), light blue diagonal stripes (50–89%), and dark blue dots (less than 50% or when data was not available).

Clinical biosimilarity analysis involved the review and categorisation of clinical data extracted from EPARs. The information extracted was presented as raw data in tables. PK, efficacy, safety and immunogenicity parameters were presented descriptively.

For both analytical/functional and clinical data, the uncertainties that were raised during their regulatory evaluation were reviewed and analysed separately. A summary of the uncertainties and how these were resolved, were presented descriptively in separate tables.

Publication 2: Do the outcomes of clinical efficacy trails matter in regulatory decision making for biosimilars?

For the second study, an observational retrospective analysis of all evaluated mAbs and fusion proteins by EMA up to November 2022 was carried out. The analysis included biosimilar candidates that either received a MA, a negative opinion by the CHMP, or were withdrawn by applicants before a CHMP opinion, and for which an EPAR or Withdrawal AR is available on the EMA website.

Firstly, an analysis of the MAAs and first regulatory assessment reports (ARs) for all 36 mAbs and fusion proteins was carried out. Concerns related to quality (biosimilarity, general quality) or clinical aspects (PK/PD, efficacy/safety/immunogenicity) were reviewed, analysed and categorised in into five possible scenarios, indicating whether these aspects were acceptable or not to the EMA. Additionally, an analysis of the first regulatory ARs was conducted. All questions raised by the CHMP during the first phase of the centralized MA procedure were reviewed and classified as critical (those that could preclude authorisation if unresolved i.e., major objections (MO)) or other concerns (OC). The number of MO and OC related to quality or clinical aspects was counted and analysed descriptively.

Secondly, an in-depth analysis of the analytical/functional and clinical data packages of all evaluated rituximab and trastuzumab biosimilars by EMA up to November 2022 was carried out, following the same methodology of the first publication. This included three rituximab biosimilars (Ruxience, Rixathon/Riximyo, Blitzima/Truxima/Ritemvia and Rexeful/MabionCD20) and seven trastuzumab biosimilars (Ogivri, Zercepac, Trazimera, Ontruzant, Herzuma, Kanjinti and

Tuznue), which in contrast from the first publication, covered also withdrawn applications.

Same as publication 1, analytical/functional data was extracted from product dossiers, and clinical data from EPARs. For approved products, analytical/functional QAs were reviewed, anonymised and categorised into four colour-coded groups based on the degree of biosimilar batches with values within the similarity range of the RP. For withdrawn applications, we also looked at quality issues of the biosimilar itself affecting e.g., performance and consistency of the manufacturing process, and we categorised them into a table, indicating whether quality requirements considered key were met or not. Clinical biosimilarity analysis included the review and categorisation of clinical data descriptively as raw data in tables. For both analytical/functional and clinical data, the uncertainties raised during their regulatory evaluation were reviewed, analysed and presented descriptively in separate tables.

3.1. Publication 1: A data driven approach to support tailored clinical programmes for biosimilar monoclonal antibodies.

Guillen E, Ekman N, Barry S, Weise M, Wolff-Holz E. A Data Driven Approach to Support Tailored Clinical Programs for Biosimilar Monoclonal Antibodies. *Clin Pharmacol Ther*. 2023 Apr;113(4):933. doi: 10.1002/cpt.2785.

3.1.1. ABSTRACT

Biosimilar mAbs have been approved in the European Union (EU) since 2013 and have been demonstrated to reduce healthcare costs and to expand patient access. Biosimilarity is mainly established on the basis of demonstrated similarity of relevant QAs, determined by comprehensive physiochemical and functional analyses, and demonstration of bioequivalence. In addition, comparative efficacy/safety studies have been requested for all approved biosimilar mAbs so far, although the EMA Guidelines state that such confirmatory clinical trials may not be necessary in specific circumstances. In order to evaluate the degree of analytical similarity, how residual uncertainty regarding biosimilarity was resolved, and the value of clinical data, we analyzed the quality and clinical data packages for authorised adalimumab (7 products) and bevacizumab (5 products) biosimilars. The percentage of biosimilar batches meeting the similarity range for QAs, as defined by the biosimilar manufacturer based on a comprehensive characterization of the EU-RP, was determined and clinical data were reviewed. Our analyses show that QAs of approved adalimumab and bevacizumab biosimilars have varying concordance with the EU-RP similarity range. In this study, we found that clinical efficacy data played a limited role in addressing quality concerns. Therefore, we encourage a regulatory review of the standards for clinical data requirements for mAb and fusion protein biosimilars. This study outlines a quality data driven approach for facilitating tailored clinical programmes for biosimilars.

3.1.2. INTRODUCTION

Biosimilars are biological medicinal products that contain a highly similar version of the active substance of an already authorised original biological product (RP). They differ from generic drugs due to their biological source, in the size of the active substance, their complexity, and the nature of the manufacturing process. The mainstay of any biosimilar development is the comprehensive demonstration of close physicochemical and functional similarity as well as bioequivalence with their RP. In addition, comparative efficacy/safety studies have so far been requested for all approved biosimilar mAbs to confirm the absence of clinically meaningful differences compared with the RP(9,12). Typically, confirmation of comparable clinical efficacy in one “model” indication is required and other indications of the RP can be extrapolated(19), which leads to reduced development costs(2) and allows for competitive price reductions for biosimilars thus facilitating patient access(36).

In this paper, the terms comparability and (bio-)similarity exercise are used synonymously.

The biosimilar regulatory framework was initially developed with the conservative stance that one comparative efficacy study will always be required as a safeguard and precautionary measure to ensure that biosimilarity demonstrated at the analytical and functional (quality) level indeed translates into biosimilarity at the clinical level. However, in recent years, due to the advancement in the analytical sciences and the vast experience gained, the extent and usefulness of this clinical confirmation has been questioned, and regulators have started to adopt a more flexible approach where the extent of clinical data required can vary depending on the product class(14,17,25,26). Recent guidelines(13,22,23) state that a pre-licensing efficacy study may be waived in case biosimilarity can be convincingly concluded based on physicochemical and functional characterization studies using sensitive, orthogonal, and state-of-the-art analytical methods, together with comparison of the PK and/or PD profiles of the biosimilar and the RP.

In the particular case of mAbs, this has been considered challenging, given their relative complexity(29). However, since assessment and EU marketing

authorisation (MA) of the first biosimilar mAb in 2013(37), the physiochemical and functional assays have continued to evolve, with greater understanding of the relevant QAs and increased sensitivity of analytical methods to detect relevant differences(28,38). Therefore, the extent of analytical data routinely provided in biosimilar dossiers currently may give sufficient assurance that a biosimilar is indeed highly similar to the RP such that no difference in clinical performance is expected. As such, the default requirement for confirmatory clinical studies could be questioned also for mAbs, and alternative regulatory pathways and/or guidance may be warranted. Streamlining developments to become more cost and time efficient and sparing patients from entering unnecessary and redundant clinical trials is of foremost importance from an ethical point of view and at a time when public and patient resources are becoming increasingly strained(39,40).

In an effort to provide a deeper understanding of the magnitude and strength of the analytical and functional similarity data available for mAbs, data of two biosimilar mAb classes were analyzed: seven approved adalimumab and five approved bevacizumab biosimilars. In addition, clinical efficacy and safety comparability data were reviewed on a product basis by studying the EPARs.

The aim of this study was to analyze whether, and to which degree, QAs were within the similarity ranges established by the biosimilar developer based on a comprehensive characterization of the RP and what role clinical data played in the final conclusion of biosimilarity.

3.1.3. METHODS

We reviewed, categorised, and anonymised the analytical and functional similarity data and analyzed the clinical data packages for approved adalimumab and bevacizumab biosimilars. The analysis included seven adalimumab biosimilars (Amgevita/Solymbic, Imraldi, Hyrimoz/Halimatoz/Hefiya, Hulio, Idacio/Kromeya, Amsparity, and Yuflyma)(41–47) and five bevacizumab biosimilars (Mvasi, Zirabev, Aybintio/Onbevzi, Alymsys/Oyavas, and Abevmy/Lextemy)(48–52). The data lock point for the analysis was September 2021. We only included biosimilars that were authorised at the time of study analysis. Adalimumab and bevacizumab were selected as representative examples of widely used biologicals which cover different therapeutic areas (autoimmune and oncologic indications).

3.1.3.1 Comparison of analytical biosimilarity across products

QA characterization data were extracted from raw data of the biosimilar product dossiers submitted to the EMA for MA approval. The data were anonymised due to confidentiality.

The comparative QA data extracted were categorised into four pattern and colour-coded categories: depending on the degree of similarity with the RP (see **Table *1***) This categorisation was performed considering the percentage of analyzed biosimilar batches with values within the similarity range: solid dark green for QAs with 100% biosimilar batches within the similarity range; light green horizontal stripes for QAs with 90–99%, light blue diagonal stripes for QAs with 50–89%, and dark blue dots for QAs with < 50% of the batches within the similarity range or when the data was lacking. This crude categorisation was chosen by the authors to allow for meaningful differentiation of similarity ranges, without losing the anonymity of products.

Some assays are product specific (e.g., human umbilical vein endothelial cells (HUVECs) antiproliferation and human vascular endothelial growth factor (VEGF) binding for bevacizumab) and are therefore represented in the gray grid in **Table *1*.**

The reference (similarity) range for establishing analytical similarity is determined by the biosimilar manufacturer based on characterization data of the RP. Similarity ranges are usually calculated based on statistical analysis of the RP dataset and may be based on ranges such as mean $\pm 3 \times$ SD (standard deviation), tolerance intervals, or a minimum-maximum range(53). The approach for setting similarity ranges may vary between products, however, all the statistical approaches used were individually justified and assessed during the respective MA procedures.

Batch results outside the similarity range were conservatively counted as being “non-similar” regardless of how far outside of the similarity range the results were. The number of biosimilar batches analyzed per product varied between 8 and 20, for most QAs. **Table 1** includes mainly the analysis of quantitative QAs. In some cases, QAs were presented graphically in the MA, if the profiles were considered to be similar (i.e., the profiles of the biosimilar and RP overlap and are visually comparable), in addition, these were categorised in solid dark green.

For purity/impurity-related QAs, one-sided similarity ranges were considered, that is, if the biosimilar exhibited higher level of purity/lower level of impurities compared to the RP, this was considered *de facto* to be comparable. In such cases, 100% biosimilar batches were considered to be within the similarity range (=solid dark green).

Additional QAs, for example, amino acid sequence, secondary and higher order structure, etc., are not included in this analysis because, in many cases, the data submitted were not entirely quantitative. For other QAs related to protein modifications, such as oxidation or deamidation, a quantitative comparison across products was also not possible due to different methodologies used by applicants. **Table 1** and **Table 2** summarize all the QAs tested, including also qualitative tests (information extracted from the EPARs).

Table 4 provides a summary of the instances where < 100% of batches were within the reference range (data on file at the EMA), and how the resulting uncertainty was resolved. In each case, the reason why these differences were accepted by the EMA is explained.

3.1.3.2 Comparison of clinical biosimilarity across products

Clinical data are presented as raw data in **Table *5*** and **Table *6*** (the product columns are not in the same order as **Table 1** to maintain anonymity). Clinical data were extracted from EPARs, which contain public information that can be found on the EMA website and therefore anonymisation is not necessary (EMA. Find medicines).

For PK and efficacy parameters, acceptance ranges for comparability were defined before study start in the statistical analysis plan. Population PK (PopPK) analysis in patients was not model-based but descriptive. Safety and immunogenicity parameters are presented as raw data and were compared descriptively.

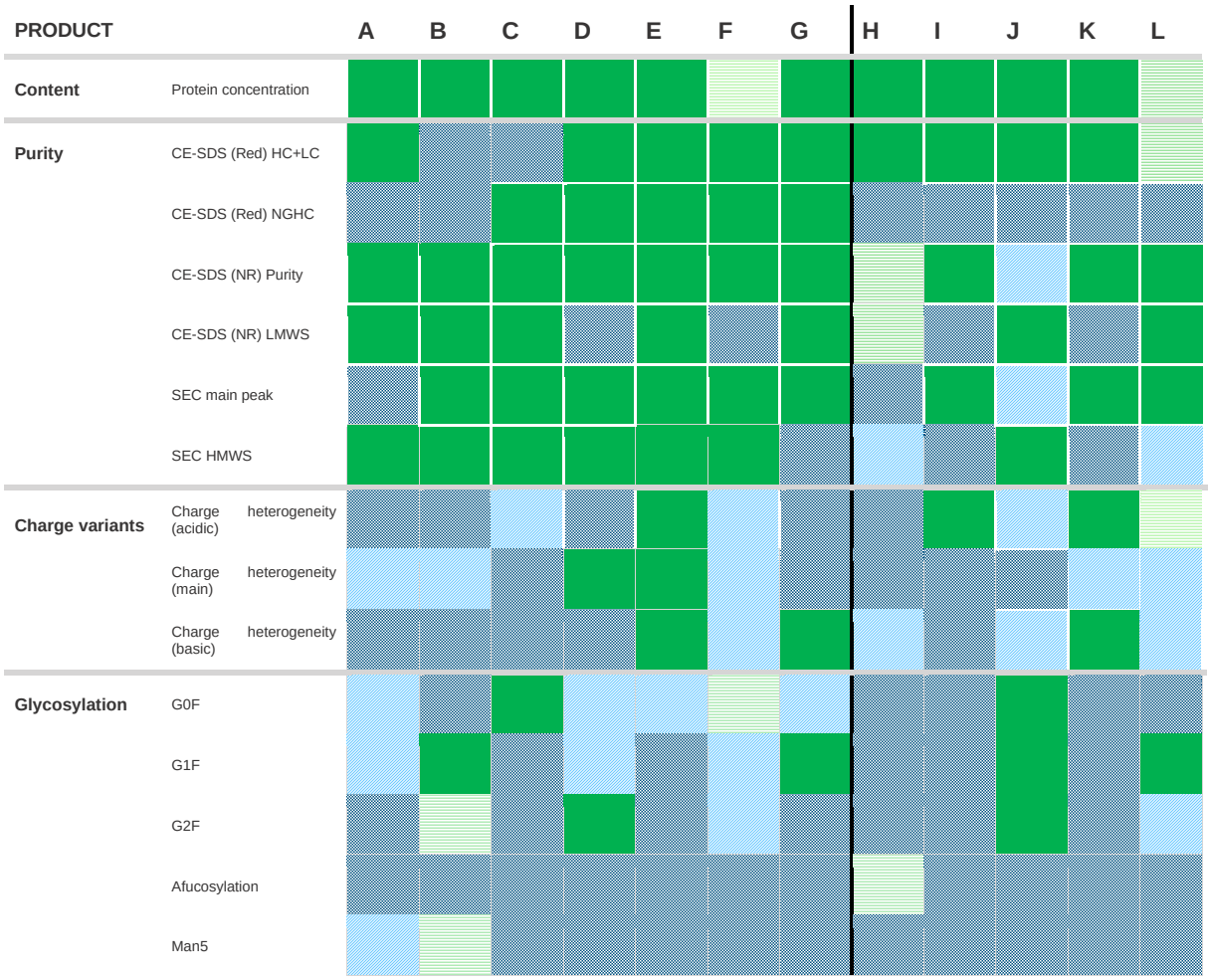
The few instances where uncertainties arose in the similarity of a specific clinical parameter are highlighted and discussed in context of other findings. **Table *7*** provides a summary of all uncertainties stemming from clinical data and how they were resolved.

3.1.4. RESULTS

3.1.4.1 Results of analytical comparability

Table 1 provides a summary of the QAs considered for adalimumab (products A–G) and bevacizumab (H–L) biosimilars. For each adalimumab and bevacizumab biosimilar, the percentage of batches within the established similarity range for each individual QA is categorised in a colour and pattern. The analytical similarity packages of the adalimumab and bevacizumab biosimilars comprised between 35 and 85 individual assays per product (for complete list see **Table 2** and **Table 3**). For most of the QAs, orthogonal analytical methods were used.

Table 1. Similarity of QAs for all adalimumab (A-G) and bevacizumab (H-L) biosimilars. Colour and patterns indicate the percentage of biosimilar batches within the similarity range derived from the EU reference product: solid dark green for 100%, horizontal light green stripes for 99-90%, diagonal light blue stripes for 89-50%, dark blue dots for <50% and also when the QA was not assessed. Gray grid represents product specific QAs which reflect the mabs main MoA. Dark green vertical stripes represent QAs that were tested but not found (in line with the mabs MoA).




Afucose + HM
Sialic acid
Potency
Potency (cell-based assay)
HUVEC anti-proliferation assay
Fab mediated
VEGF121 binding
VEGF165 binding
VEGF189 binding
VEGF206 binding
Soluble TNFα binding
Transmembrane TNFα binding
Fc Funcionality
ADCC
FcγRI binding
FcγRIIa binding
FcγRIIb binding
FcγRIIIa (158 f/f) binding
FcγRIIIa (158 v/v) binding
FcγRIIIb binding
FcRn binding
Complement Related
CDC
Additional functional assays
Apoptosis induction
Apoptosis inhibition
MLR
HEK293 VEGF reporter assay
VEGFR2 phosphorylation
HUVEC migration
HUVEC apoptosis

Protein content

Protein content is a highly critical QA which must be fully comparable between the biosimilar and the RP. For all biosimilars examined, 100% of biosimilar batches were within the reference range, except for one adalimumab (product F) and one bevacizumab (product L) biosimilar (≥ 90%).

Fragment antigen binding mediated functions

a. Binding to soluble tumor necrosis factor

Adalimumab is an IgG1 mAb that binds, via its fragment antigen binding (Fab) domain, to tumor necrosis factor α (TNFα) and prevents it from binding to its receptors TNFR1 and TNFR2, thereby blocking TNF-induced inflammation(54,55). This is the primary MoA for adalimumab across all approved indications. The biological activity of adalimumab is determined by a combination of binding assays and a cell-based TNFα cytotoxicity inhibition assay. In addition, some applicants used a nuclear factor kappa B (NF-κB) reporter gene assay, which is viewed as supportive data (not included in **Table 1** but for those biosimilars where this assay was used, ≥ 90% of biosimilar batches were within the reference range). As shown in **Table 1**, for all 7 adalimumab biosimilars studied, 100% of the batches were within the similarity range for binding to soluble TNFα with the chosen assays.

b. Binding to transmembrane TNF and reverse signaling

In addition to binding to soluble TNFα, adalimumab can bind to membrane-associated TNFα (mTNFα) and mediate reverse (or outside-to-inside) signaling. Binding of adalimumab to mTNFα does not appear to be important for therapeutic efficacy in all indications, however, it may contribute to the clinical efficacy of adalimumab in inflammatory bowel diseases (IBDs). Several possible mechanisms explain the contribution of reverse signaling to the efficacy of adalimumab in IBD. For example, adalimumab-mediated apoptosis of lamina propria T cells may represent an additional key MoA of adalimumab in IBD indications and is thought to be mediated by reverse signaling, although it may also be mediated by binding to soluble TNF, which is in turn bound to its receptor(56). Anti-TNF agents, such as adalimumab and infliximab, are also known to induce CD14+ CD206+ M2-type wound-healing macrophages (regulatory macrophages) which may contribute to mucosal healing in

IBD(57,58). Induction of regulatory macrophages can be assayed by measuring antiproliferative effects in a mixed lymphocyte reaction (MLR).

In all cases, 100% of adalimumab biosimilar batches were within the reference range for binding to mTNF.

c. Binding to VEGF

Bevacizumab is an IgG1 mAb which binds to VEGF-A and prevents the signaling of VEGF receptors(59). VEGF comprises at least 16 different isoforms due to alternate mRNA splicing. Inhibition of VEGF-A blocks the proliferation of vascular endothelial cells and angiogenesis. Although soluble VEGF isoforms ($VEGF_{121}$ and $VEGF_{165}$) are the most predominant isoforms in tumors, cell-associated VEGF (such as $VEGF_{189}$ and $VEGF_{206}$) is also expressed in a significant number of lung and colon cancers(59).

For all bevacizumab biosimilars, 100% of batches were within the reference range for at least 2 of 3 VEGF isoforms. Binding to $VEGF_{165}$ could be demonstrated for all batches and binding to $VEGF_{121}$ and $VEGF_{189}$ was observed for all but one product (product L and product H, respectively). Binding to $VEGF_{206}$ was frequently not performed by applicants which was accepted because this isoform is seen as less important(60).

Cell-based assays

Cell-based potency assays are considered highly important for determination of biosimilarity. In the absence of comparable biological activity, a product cannot be approved as a biosimilar. For adalimumab biosimilars, the functional cell-based assays were based on measuring adalimumab inhibition of TNFα mediated cell death. In 6 out of 7 adalimumab biosimilars, all 100% of batches were within the similarity range and for one product (product F), ≥ 90% of batches were within the similarity range. For bevacizumab biosimilars, an HUVEC-based antiproliferation assay was used, and, in all cases, 100% of batches were within the similarity range.

Fc-related assays

Adalimumab is known to induce antibody-dependent cellular cytotoxicity (ADCC) through the binding of the Fab region to mTNFα and the fragment crystallizable region

(Fc) region to FcγRIIIa, which is expressed on effector cells, such as NK cells (mainly via high affinity receptor genotype 158 v/v)(61). It is well-known that the binding of IgGs to FcγRIIIa is influenced by the glycan profile of the antibody. For example, levels of afucosylated glycans are generally correlated with ADCC activity. All seven adalimumab biosimilar applicants performed one or more comparative ADCC assays, which usually included peripheral blood mononuclear cells (PBMCs) or natural killer (NK) effector cells. As shown in **Table 1,** 100% of biosimilar batches were within the reference range for ADCC for all products. Although most applicants used 1 or 2 ADCC assay formats, for 1 biosimilar product, ADCC activity was measured using more than 20 different ADCC assay setups (see **Table 2**). This is an example of the large variety of assays that can be used to study a single QA.

Activation of complement-dependent cytotoxicity (CDC) is also viewed as a relevant MoA for adalimumab. For all adalimumab biosimilars, 100% of batches were within the reference range for CDC activity.

Bevacizumab is theoretically capable of mediating Fc-related effector functions. However, none of the authorised bevacizumab biosimilars displayed ADCC or CDC activity (represented as green vertical stripes in **Table 1**, meaning it was tested but not found), which is in line with previously published results for originator bevacizumab.

Fc binding assays

Neonatal Fc receptor (FcRn) has been shown to play a role in regulating IgG levels in the serum through recycling of bound antibodies, with an impact on the serum levels of therapeutic mAbs(62). For this reason, binding to FcRn is considered as a critical QA(63). In all cases except one bevacizumab biosimilar (product I), 100% of batches were found to be within the similarity range.

The results of binding assays for five other FcγR (FcγRIa, FcγRIIa, FcγRIIb, FcγRIIIa (158 f/f), and FcγRIIIb) showed a variable percentage of batches lying within the reference range. Only 3 of 7 adalimumab products had ≥ 90% or 100% of batches within the similarity range for all 5 FcR binding assays (products D, E, and G). Binding to the FcγRIIIa by therapeutic mAbs is known to enhance ADCC activity (relevant MoA for adalimumab). For the high affinity FcγRIIIa 158 v/v genotype, 6 out of 7 adalimumab biosimilars had 100% of batches within the similarity range and for one

product ≥ 90% of batches were within the similarity range (product A). For bevacizumab biosimilars, the results were more variable, with between one (product L) and 4 (products J and K) FcγR binding assays having < 90% batches within similarity range. However, as discussed above, bevacizumab does not exhibit effector function, and therefore binding to FcγR is not considered a critical aspect of biosmiliarity(51,52).

Glycosylation profile

Tests for glycosylation profile included as a minimum: G0F, G1F, G2F, afucosylation, sialylation, and high mannose content. It can affect the immunogenicity and, in some cases, (adalimumab) also the functionality of the mAb. In most cases, < 90% of batches tested were within the similarity range or the assay was not performed. This is not unexpected, because it is known that the glycoprofile is highly dependent on the cell line that is used as expression system, media, and several growth conditions(64,65). Although differences in glycoprofile could impact the ADCC activity, in all cases for adalimumab, the ADCC activity was shown to be highly similar.

Purity testing

The purity/impurity profile is viewed as critical by the EMA as certain impurities may impact on safety and immunogenicity. Protein impurities can be measured by size exclusion chromatography (SEC) and capillary electrophoresis-sodium dodecyl sulfate (CE-SDS) under reduced and non-reduced conditions to detect several relevant impurities, such as fragmentation, truncation, and aggregation. For both the adalimumab and bevacizumab biosimilars, there were several instances where < 50% of the batches were inside of the similarity range. However, during the assessment process these differences were judged to be irrelevant in terms of safety and efficacy. In most cases, this was due to the fact that the absolute difference in impurity levels was so small as to not to be clinically meaningful. Furthermore, the purity is controlled by the release specification.

Charge variants

Due to the complex contribution of numerous QAs to the overall charge profile of mAbs, charge variations may quantitatively differ between biosimilars and their RP. As can be seen in **Table 1**, in most cases, the charge profile differed, with only one

adalimumab biosimilar (product E) with 100% batches within similarity range. Differences in charge profile can generally be accepted, provided applicants justify why any observed charge differences would not have an adverse clinical impact.

Additional assays

Additional assays include, for example, inhibition of TNFα-induced apoptosis and inhibition of release of IL-8 or sVCAM-1 in cell culture (not included in **Table *1***) for adalimumab, and induction of HUVEC migration or apoptosis, site-specific phosphorylation of VEGFR2, and HEK293 VEGF reporter assay for bevacizumab biosimilars. These assays are not considered mandatory but can be useful to strengthen the claim of biosimilarity.

For the majority of adalimumab biosimilars, 100% of batches were within the reference range when measuring induction of apoptosis. For one biosimilar (product A), this function was not addressed or outside the similarity range. Data from MLR studies were provided for all adalimumab biosimilars, with 3 products (C, D, and G) showing < 90% batches within range. Only one bevacizumab biosimilar (product J) had all additional functions assessed and with 100% batches within range.

However, due to the inherent variability of these assays and the low numbers of batches tested, the evidence provided by these assays was considered supportive only.

Table 2. Summary of analytical assays performed for adalimumab biosimilars.

Quality attribute (and analytical method/s) for comparative characterization	
Content	Protein Content (UV-280)
Primary structure	Molecular weight/intact mass (RPLC-UV/MS)
	Amino acid sequence (Peptide mapping)
	N-terminal sequencing (Peptide mapping, Edman sequencing)
	C-terminal sequencing (Peptide mapping)
	Peptide mapping (Peptide mapping)
	Disulfide bond analysies (Peptide mapping)
	Free thiols (Ellmans test, FLR)
Higher Order Structure	Secondary structure (FTIR)
	Secondary- and tertiary structure (Far and Near UV Circular Dichroism)

	Protein folding (Intrinsic and extrinsic fluorescence)
	Thermal stability (DSC)
	Tertiary structure (1D 1H NMR, 2D 1H-1H NOESY NMR, 2D-NMR, HDX, X-ray crystalography, Antibody conformational array)
Protein modifications	N-term Pyroglutamate (Peptide mapping)
	C-terminal lysine (Peptide mapping, CEX)
	Iso-aspartate (Peptide mapping)
	Deamidation (Peptide mapping)
	Oxidation (Peptide mapping)
	Glycation (BAC)
	Succinimidation (Peptide mapping)
	Isomerisation (Peptide mapping)
	Proline amide (Peptide mapping)
	Thioether (Peptide mapping)
	Cysteinylation (Peptide mapping)
Glycosylation	N-glycan profile (LC-ESI-MS/MS, 2-AB labelling HILIC-UPLC)
	Afucosylation (LC-ESI-MS/MS, 2-AB labelling HILIC-UPLC)
	High mannose (LC-ESI-MS/MS, 2-AB labelling HILIC-UPLC)
	Sialylation (LC-ESI-MS/MS, 2-AB labelling HILIC-UPLC)
	G0F (LC-ESI-MS/MS, 2-AB labelling HILIC-UPLC)
	G1F (LC-ESI-MS/MS, 2-AB labelling HILIC-UPLC)
	G2F (LC-ESI-MS/MS, 2-AB labelling HILIC-UPLC)
	Galactosylation (LC-ESI-MS/MS, 2-AB labelling HILIC-UPLC)
Purity/impurity profile and charged variants	Size heterogeneity (SEC, CE-SDS reducing and non-reducing, SV-AUC, SEC-MALS, DLS, FFF)
	Hydrophobic heterogeneity (HIC)
	N-linked glycosylation site (LC-ESI-MS/MS)
	Charge heterogeneity (CEX-HPLC, iCIEF, iCE, cIEF, IEC-HPLC)
Fab mediated	soluble TNF-binding (ELISA, SPR, FRET)
	membrane TNF-binding (cell-based assay)
	TNF-α neutralisation (NF-kB reporter, viability/cell death)
Fc and complement mediated	ADCC *e.g.for one product, up to 20 assays were performed, including: ● NK-PBMC ADCC using healthy and patient blood ● Whole blood ADCC using healthy and patient blood ● FcγRIIIa ADCC reporter ● Addition of serum to these assays ● Addition of IgG to these assays
	FcγRI binding (SPR)
	FcγRIIa (131H, 131R) binding (SPR)

	FcγRIIb binding (SPR)
	FcγRIIIa (158F, 158V) binding (SPR, AlphaLISA, RGA)
	FcγRIIIb binding (SPR)
	FcRn binding (SPR)
	CDC (cell-based assay)
	C1q binding (ELISA)
Additional functional assays	Apoptosis induction, reverse signalling (cell-based assay)
	Apoptosis inhibition in intestinal epithelial cells
	MLR (T cell proliferation, regulatory macrophages (CD14/CD206))
	IL-8 release from HUVECs
	IL-8 release from PBMCs
	IL-8 release from keratinocytes
	IL-8 release from intestinal epithelial cells
	IL-6 release from synoviocytes
	sVCAM-1 release from HUVECs
	ICAM-1 expression on HUVECs
	ELAM-1 expression on HUVECs
	MIP-1β release from whole blood
	MCP-1 release from whole blood
	Lack of impact on Lymphotoxin α

Table 3. Summary of analytical assays performed for bevacizumab biosimilars.

Quality attribute (and analytical method/s) for comparative characterization	
Content	Protein Content (UV-280)
Primary structure	Molecular weight (RPLC-UV/MS)
	Intact mass/reduced mass (LC-ESI-MS)
	Isoelectric point (cIEF)
	Amino acid sequence (peptide mapping)
	N-terminal sequencing (Peptide mapping, Edman sequencing)
	C-terminal sequencing (Peptide mapping)
	Amino acid sequence (Peptide mapping)
	Disulfide bond analysies (Peptide mapping)
	Free thiols (Ellmans test)
	Secondary structure (FTIR, Far and Near UV Circular Dichroism)
Higher Order Structure	Tertiary structure (Far and Near UV Circular Dichroism, FL)

	Protein folding (Intrinsic and extrinsic fluorescence)
	Thermal stability (DSC)
	Epitope mapping (HDX-MS)
	Di-sulfide bridging (RP-HPLC-ESI-MS, non-reduced peptide mapping)
Protein modifications	Deamidation (Peptide mapping)
	Oxidation (Peptide mapping)
	Glycation (BAC)
	Aspartate Isomerisation (Peptide mapping)
	Thioether (Peptide mapping)
	Cysteinylation (Peptide mapping)
Glycosylation	N-glycan profile (peptide mapping, LC-ESI-MS/MS, HILIC-UPLC)
	O-glycosylation (peptide mapping)
	Ng-HC and p75 (CE-SDS, reduced)
	Afucosylation (NP-HPLC)
	Fucosylation (NP-HPLC)
	High mannose (NP-HPLC)
	Sialylation (NP-HPLC, UHPLC-FLR)
	G0F (LC-ESI-MS/MS, 2-AB labelling HILIC-UPLC)
	G1F (LC-ESI-MS/MS, 2-AB labelling HILIC-UPLC)
	G2F (LC-ESI-MS/MS, 2-AB labelling HILIC-UPLC)
	Galactosylation (NP-HPLC)
Purity/ impurity profile and charge variants	Size heterogeneity (SEC, CE-SDS non-reduced and reduced, CGE non-reducing and reducing, SV-AUC, SEC-MALS, DLS, FFF)
	Particles (MFI)
	Charge heterogeneity (CEX-HPLC, iCIEF, cIEF)
	Hydrophobic heterogeneity (HIC)
Fab mediated	VEGF121 binding (HUVEC-cell based assay, SPR, ELISA)
	VEGF165 binding (HUVEC-cell based assay, SPR, ELISA)
	VEGF189 binding (HUVEC-cell based assay, SPR, ELISA)
	VEGF206 binding (HUVEC-cell based assay, SPR, ELISA)
	VEGF B, C, D binding (BLI)
	HUVEC neutralisation assay (cell-based assay)
	VEGFR phosphorylation inhibition (cell-based assay)
	Cell signaling assay (HEK293 RGA)
	KDR/KDR dimerization assay (cell-based assay)
Fc and complement	ADCC (cell based assay)

mediated	FcγRI binding (SPR)
	FcγRIIa FcγRIIa (131H, 131R) binding (SPR)
	FcγRIIIa (158F, 158V) binding (SPR, AlphaLISA)
	FcγRIIIb binding (SPR)
	FcRn binding (SPR, ELISA)
	CDC (cell based assay)
	C1q binding (ELISA)
Off-target binding	VEGF-B (SPR)
	VEGF-C (SPR)
	VEGF-D (SPR)
	PIGF-1 (SPR)
	PIGF-2 (SPR)

Table 4 provides a summary of the instances where < 100% of batches were within the reference range. In each case, the reason why these differences were accepted by the EMA is explained.

Table 4. QAs with < 100% of batches meeting similarity ranges and how the resulting uncertainty during MAA and how this was resolved.

Adalimumab	QA	Percentage of batches within the similarity range	How resolved
Product F	Protein content	≥90% of batches	The small difference in protein content was concluded to be of no clinical relevance. Batch-to-batch variability of the biosimilar within the expected range.
Product F	Cell based potency assay	≥90% of batches	Minor deviation not expected to affect the clinical performance of the product.
Products A,B,C,F	Binding to several FcγReceptors (FcγRIa, FcγRIIa, FcγRIIb, FcγRIIIa-158 f/f and FcγRIIIb)	Variable, see Table 1	Minor differences in binding results, similarity confirmed in cell-based functional assays.
Product A	Binding to FcγRIIIa 158 v/v	≥90% of batches	Viewed as sufficient based on ADCC assay results
Product A – G (all)	Glycosylation (7 attributes)	Variable, often < 90%, see Table 1	Similarity confirmed in cell-based functional assays. No clinically significant difference in PK profile.
Products	Purity testing	Variable, often <	Based on regulatory experience, the

A,B,C,D,F (all except E)		90%, see Table 1	small difference was seen as negligible. In most cases purity of biosimilar was marginally increased.
Products A,B,C,D,F (all except E)	Charge variants	Variable, often < 90%, see Table 1	Acceptable based on product understanding.
Product A	Apoptosis induction	<50% of batches or not done	The assay is not considered as highly critical, accepted based on high similarity for binding to transmembrane TNFα. Alternative assay used as a functional readout of transmembrane TNF binding e.g. MLR.
Products B	Apoptosis inhibition	Variable, < 90% in one case, see Table 1	Additional orthogonal assays supported biosimilarity. Accepted based on the totality of evidence.
Bevacizumab	**QA**	**Percentage of batches within the similarity range**	**How resolved**
Product L	Protein content	≥90% of batches	The small difference in protein content was concluded be of no clinical relevance. Batch-to-batch variability of the biosimilar within the expected range.
Product L	Binding to VEGF 121	<50% of batches or not done	High similarity for binding to other VEGF isoforms confirmed using orthogonal methods
Product H	Binding to VEGF 189	<50% of batches or not done	High similarity for binding to other VEGF isoforms confirmed using orthogonal methods
Product H, I, J, L (all except K)	Binding to VEGF 206	Variable, often < 90%, see Table 1	High similarity for binding to other VEGF isoforms confirmed using orthogonal methods VEGF 206 is a less frequent isoform in human tissues (39)
Product I	Binding to FcRn	≥90% of batches	Based on regulatory experience and the results from the comparative PK study, the minor difference was seen as negligible.
Product H – L (all)	Binding to several FcγReceptors	variable, see Table 1	Binding to FcγReceptors are not considered critical for the mode of action of bevacizumab.
Product H – L (all)	Glycosylation (7 attributes)	Variable, often < 90%, see Table 1	Due to the lack of Fc functions for bevacizumab, glycosylation pattern is not critical for bevacizumab. The PK profiles demonstrated similar.
Products H – L (all)	Purity testing	Variable, often < 90%, see Table 1	Based on regulatory experience, the small difference was seen as

			negligible.
Products H – L (all)	Charge variants	Variable, often < 90%, see Table 1	Acceptable based on product understanding.
Products H,I,K,L (all except J)	Additional functional assays	Variable, often < 90%, see Table 1	The assays are not considered as highly critical, differences accepted based on the totality of evidence presented for similarity.

3.1.4.2 Results of Clinical Comparability studies

The clinical results obtained for each adalimumab biosimilar are provided in **Table 5**.

The clinical results obtained for each bevacizumab biosimilar are provided in **Table 6**.

Table 5. Main PK, efficacy and safety/immunogenicity results of adalimumab biosimilars compared to reference product.

		Amgevita/Solymbic	Idacio/Kromeya	Amsparity	Yuflyma	Imraldi	Hyrimoz/Halimatoz/Hefiya	Hulio
PK STUDY	Sample size (total subjects)	+/- 200	+/- 200	B5381001: +/- 200 B5381007: +/- 350	+/- 300	+/- 200	GP17-101: +/- 200 GP17-104: +/- 300	+/- 150
	1°EP	Cmax, AUCinf	Cmax, AUCinf, AUClast	B5381001: Cmax, AUCinf, AUClast B5381007: Cmax, AUCinf, AUClast, AUC0-2wk	Cmax, AUCinf	Cmax, AUCinf, AUClast	GP17-101: Cmax, AUCinf, AUClast GP17-104: Cmax, AUCinf	Cmax, AUCinf, AUClast
	2°EP	Tmax, T ½	Tmax, Vz/F, T ½,CL/F	Tmax, Vz/F, T ½, CL/F	AUClast, AUC0-336, Tmax, Vz/F, λz, T ½, CL/F, %AUCextrap	AUC0-360, T ½	AUC0-360, Tmax, T ½, %AUCextra, CL0-last and Kel	%AUCextrap, Tmax, Vz/F, λz, T ½, CL/F
	Prespecified margins	0.80 to 1.25	0.80 to 1.25	0.80 to 1.25	0.80 to 1.25	0.80 to 1.25	0.80 to 1.25	0.80 to 1.25
	Cmax	0.96 (0.89, 1.03)	0.95 (0.87, 1.04)	B5381001: 1.07(0.97, 1.18) B5381007: 1.11 (1.04, 1.19)	0.96 (0.87, 1.05)	ANOVA: 1.17 (1.06, 1.29) ANCOVA: 1.07 (0.97, 1.17)	GP17-101: 1.151 (1.064 – 1,245) GP17-104: 0.95 (0.9, 1.01)	1.00 (0.94, 1.07)
	AUC last	0.99 (0.89, 1.10)	0.91 (0.81, 1.03)	B5381001: 1.11 (1.01, 1.23) B5381007: 1.07 (0.99, 1.15)	1.03 (0.91, 1.15)	ANOVA: 1.10 (0.99, 1.23) ANCOVA: 1.00 (0.9, 1.11)	GP17-101: 1.226 (CI: 1.085 – 1.385) GP17-104: 1.06 (0.97; 1.15)	1.01 (0.93, 1.09)
	AUC inf	1.04 (0.93, 1.17)	0.89 (0.80, 0.99)	B5381001: 1.13 (1.00, 1.28) B5381007: 1.05 (0.96, 1.15)	0.99 (0.89, 1.11)	ANOVA: 1.08 (0.96, 1.22) ANCOVA: 0.98 (0.87, 1.10)	GP17-101: 1.156 (CI: 1.017 – 1,314) GP17-104: 1.04 (0.96,1.13)	0.98 (0.90, 1.07)
	TEAES, any	35.8% vs. 41.8%	NA	B5381001: 46.4% vs. 62.95% B5381001: 57% vs. 40.3%	57.1% vs. 46%	58.3% vs. 65%	62.7% vs. 73.9%*	54.9% vs. 57.5%
	SAES, deaths, TEAES leading to discontinuation	0 vs. 0	NA	1 SAE overall (007)*	2 SAES*	1 SAE in each group*	3 SAES overall (only 1 drug-related)	3 SAES overall*
	patients with ADA positive samples at any time	53.7% vs. 67.2%	82.1% vs. 83.5%	85.5% vs. 90.0%	98.4% vs. 95.2%	69.5% vs. 73.3%	66.5% vs. 70.6% (pooled PK studies)	97.1 vs. 95.2%

		Amgevita/Solymbic		Idacio/Kromeya	Amsparity	Yuflyma	Imraldi	Hyrimoz/Halimatoz/Hefiya	Hulio
	Observation period (until last PK sample)	62 days		70 days	B5381001: 42 days B5381007: 49 days	70 days	64 days	72 days	71 days
PHASE 3 TRIAL	Sample size (total subjects)	+/- 500	+/- 300	+/- 500	+/- 600	+/- 600	+/- 600	+/- 450	+/- 600
	Indication	Moderate-severe RA despite MTX therapy >6m.	Moderate-severe PP	Moderate-severe PP	Moderate-severe RA despite MTX therapy >6m.	Moderate-severe RA despite MTX therapy >6m	Moderate-severe PP	Moderate-severe RA despite MTX therapy >6m	Moderate-severe RA despite MTX therapy >6m
	Efficacy 1°EP	ACR20 at w24.	PASI % improvement at week 16	PASI75 at week 16*	ACR20 at week 24.	co-primary EP: ACR20 at week 24 in FAS with RCI (EMA) or NRI (FDA)	PASI75 at week 16	ACR20 at week 12	ACR20 at week 24
	Efficacy 2° EP	ACR20 at earlier time pointsACR50 and ACR70 at each time point, change from baseline of DAS28-CRP	PASI % improvement at w32, 50, PASI75 response at w16, 32, 50, sPGA response and BSA involvement at different time points	%-change from baseline in PASI w16 and w24, PASI75 response at w24, PASI score absolute values, PGA response at w16 and w24	ACR20 at w52, ACR-N at w24, 52; AUC of ACR-N at w24, change from baseline of DAS28 at w24,52, AUC of change from baseline of DAS28 at w24,52, EULAR response at w24,52, ACR70 at w52, change from baseline of mTSS at w52	DAS28-CRP score at w24, ACR20, ACR50 and ACR70 week 0 – week 24, CRP, HAQ-DI	%improvement in PASI up to w16, PASI50, PASI75, PASI90, and PASI100, absolute and percentage change in PASI scores, proportion of IGA responders (every other week until w17 and every 6w thereafter until w51)	DAS28-CRP score, ACR20, ACR50 and ACR70 measured in eleven times up to 1y, EULAR good response, DAS remission, ACR/EULAR remission	DAS28-CRP score, ACR20, ACR50 and ACR70 measured in eleven times up to 1y, EULAR good response, CDAI, SDAI, SF-36
	Prespecified equivalence margin for 1°EP	0.738,1/0.738 (RR), 1° EP	±15%	±18%; ±15% for relative change from baseline	±15%	±13% (FDA -12%/+15%, ± 0.6 equivalence range for the DAS28-	±18%; ±15% for relative change from baseline	±14%	±15%

		Amgevita/Solymbic		Idacio/Kromeya	Amsparity	Yuflyma	Imraldi	Hyrimoz/Halimatoz/Hefiya	Hulio
		±10% (RD), 2º EP				CRP)			
	Primary analyses population for 1ºEP	ITT	ITT	PPS	PPS	ITT	PPS	ITT	ITT
	Results 1ºEP (95% CI); ITT population	1.04 (0.94, 1.15)	-2.18 (-7.39, 3.02)	2.79 (-4.00, 9.57%).	0.8% (-7.03%, –8.56%).	EMA (RCI): -1.3 (−7.6, 5.0); FDA (NRI): -1.8 (−7.3, 3.6)	2.2 (−6.79%, 11.10%)	-2.98 (-10.38%, 4.44%)	0.0 (-5.94, 5.94)
	Results 1ºEP (95% CI); PPS population	1.0 (0.89, 1.113)	-2.64 (-6.89, 1.60)	-1.86% (-7.82, –4.16%).	0.1% (-7.83% - 8.13%)	EMA (RCI): -0.4 (−6.7, 5.9)	1.8 (−7.46%, 11.15%)	–4.14% (-11.79%, 3.61%).	0.06 (-5.60, 5.78)
	Results 2º EP	ACR50 and ACR70 at w24: 0.948 (0.796, 1.128) and 1.13 (0.830, 1.538)	diference response PASI75 response w16: -7.729 (-16.62%, 1.163%), sPGA -7.36 (-17.2%, 2.47%), BSA 1.9 (-0.24%, 4.1%)	%improvement in PASI up to w16: 0.88 (-1.21, -2.98)	ACR50 and ACR70 at w24: -2% (-10.69% - 6.75%) and -1.3% (-8.41% - 5.8%)*	DAS28-CRP score: 0.01 (-0.17, 0.18)	%improvement in PASI75 up to w16: -0.8% (-3.15% - 4.84%)	DAS28-CRP score at w12: -2.2 (n=290; SD=1.20) vs. -2.3 (293; 1.26), difference <0.6	DAS28-CRP at w24: -0.01 (-0.19, 0.16)
	TEAES, any	18.9% vs. 21%	TP1: 24.7% vs. 24.9% TP2: 18.4% vs. 22.8% vs. 26%	78.3 vs. 76.5%	TP1: 35.8% vs. 40.7%. TP2: 52.2% vs. 56.4% vs. 54.3%	72.4% vs. 66.2%	61.3% vs. 64.9%	TP1: 48% vs. 47.8%. TP2: 43.5% vs. 44.4% vs. 38.3%	TP1: 52.2% vs. 56.8%. TP2: 39.9% vs. 45.4% vs. 48%
	Fatal TEAES	0% (both)	0% (both)	0% (both)	NA	NA	0% (both)	TP1: 0% vs. 0.3%. TP2: 0% (all)	0% (both)

		Amgevita/Solymbic		Idacio/Kromeya	Amsparity	Yuflyma	Imraldi	Hyrimoz/Halimatoz/Hefiya	Hulio
	TEAEs leading to discontinuation of IP	1.9% vs. 0.8%	TP1: 4% vs. 2.9%. TP2: 4.6% vs. 1.3% vs. 3.9%	OVERALL: 4.5% vs. 13.4%	TP1: 0.7% vs. 3.3%. TP2: 1.5% vs. 5.5% vs. 2.4%	8% vs. 4.5%	TP1: 1.7% vs. 3% TP2: 4.8% vs. 7%	TP1: 3.7% vs. 4.7%. TP2: 2.1% vs. 5.9% vs. 1.5%	TP1: 1.5% vs. 2.5%. TP2: 1% vs. 1.3% vs. 3.3%
	SAEs, any	3.8% vs. 5%	TP1: 3.4% vs. 2.9%. TP2: 2.6% vs. 5.1% vs. 5.2%	OVERALL: 9% vs. 6.7%	TP1: 1.1% vs. 2.9%. TP2: 3.4% vs. 5.9% vs. 4.7%	5.4% vs. 2.6%	TP1: 1.3% vs. 4.3%. TP2: 3% vs. 8.8%	TP1: 4% vs. 4.3% TP2: 1.4% vs. 4.4% vs. 2.3%	TP1: 3.1% vs. 4.9%. TP2: 2% vs. 2% vs. 3.3%
	Patients with ADA positive samples at any time	40.2% vs. 40.1%.	At week 16: 55.7% vs. 64.2%. Overall: 68.4% vs. 74.7% vs. 72.7%	88.1% vs. 88.4%	32.1% vs. 31.2%	At week 24: 62% vs. 59.4%	36.8% vs. 34.1%	44.4% vs. 50.5%	44.1% vs. 57.1%
	Population PK (done/not done)	yes	yes	yes	yes	yes	yes (study GP17-301)	yes	yes
	if yes, how	Assessment of Ctrough levels at week 2, 4, 12, 24, 26 for all patients	Assessment of Ctrough levels at week 4, 16, 20, 32, 52 for all patients	Assessment of Ctrough levels at at weeks 4, 8 12, 16, 24, 32, 40, 52, 60 for all patients	Ctrough levels obtained prior to dosing at weeks 0, 4, 8, 12, 16 and 24 for the first 65% enrolled subjects (356 subjects; 178 each group)	Statistical comparison for the Ctrough pre-dose concentrations in the target population (at weeks 4, 12, 20 and 24 and at weeks 12, 24 and 30 in the phase III studies FKB327-002 and 003 respectively)	Assessment of Ctrough levels at week 3, 7, 11, 17, 23, 29, 35, 41, 47 and 51	Planned with the drug concentration-time data prior to the week 26 injection using a nonlinear mixed effect modelling approach	Ctrough levels were obtained at week 0,2,6,10,14,18,22 of all patients who received at least one full dose of either of the study drugs and had at least 1 post-treatment concentration data(290 subjects)
	Switch (yes/no). If yes, timepoint of switch	no	Yes, at week 16 156 subjects	Yes, at week 17, 101 subjects were switched from EU-	Yes, at week 24, 125 subjects were switched from EU-	Yes, an extension trial (FKB327-003) was performed	Yes, at week 17, 126 subjects total were switched (63	Yes, at week 12, 134 subjects switched from EU-RMP to BS	Yes, at week 26, 152 subjects switched from EU-

		Amgevita/Solymbic		Idacio/Kromeya	Amsparity	Yuflyma	Imraldi	Hyrimoz/Halimatoz/Hefiya	Hulio
			were switched from EU-RMP to BS	RMP to BS	RMP to BS	with re-randomisation in each treatment arm for an additional 28 weeks.	in each group)		RMP to BS
	Observation period	24 weeks	52 weeks	54 weeks	60 weeks	24 weeks(up to 1 year in the extension trial)	51 weeks	52 weeks	52 weeks

Table 6. Main PK, efficacy and safety/immunogenicity studies of bevacizumab biosimilars compared to reference product.

		Zirabev	Alymsys/Oyavas	Abevmy/Lextemy	Mvasi	Aybintio/Onbevzi
PK STUDY	Sample size (total subjects)	+/-100	+/-100	+/-100	+/-200	+/-100
	1°EP	Cmax, AUCinf, AUClast	Cmax, AUCinf	AUCinf	Cmax, AUCinf	AUCinf
	2°EP	NP	AUClast, Tmax, T ½	Cmax, AUClast	AUClast	AUClast, Cmax, Tmax, Vz, λz, T ½, %AUCextrap
	Prespecified margin	0.80 to 1.25	0.80 to 1.25	0.80 to 1.25	0.80 to 1.25	0.80 to 1.25
	Cmax	1.04 (0.98, 1.10)	1.12 (1.03, 1.22)	0.90 (0.84, 0.94)	0.96 (0.92,1.00)	0.996 (0.93, 1.06)
	AUC last	1 (0.93, 1.05)	1.16 (1.09,1.22)	0.94 (0.89,0.99)	1.03 (0.98, 1.08)	0.89 (0.83, 0.95)
	AUC inf	0.99 (0.92, 0.105)	1.16 (1.1,1.22)	0.94 (0.89,0.98)	0.96 (0.92, 1.01)	0.89 (0.82, 0.95)
	TEAES	48.5% vs. 62.9%	63% vs. 66%	89% vs. 78%	47% vs. 61%	50% vs. 37.5%
	AESI, SAES, deaths	SAEs: 0 vs. 1*	0 vs. 0	0 vs. 0	0 vs. 0	SAE: 1* vs.0
	patients with ADA	NP	2 vs. 1 (treatment induced)	1 vs. 1 (predose)	0 vs. 0	1 vs. 4 (post dose)

	positive samples at any time					
	Observation period (until last PK sample)	100 days	100 days	99 days	85 days	85 days
PHASE 3 TRIAL	Sample size (total subjects)	+/-700	+/-600	+/-700	+/-600	+/-700
	Indication	Newly diagnosed Stage IIIB or IV NS-NSCLC	Newly diagnosed or recurrent Stage IIIB/IV NS-NSCLC	Newly diagnosed advanced (stage IV) /recurrent NS-NSCLC	Newly diagnosed advanced (stage IV) /recurrent NS-NSCLC	Newly diagnosed advanced (stage IV)/ recurrent NS-NSCLC
	EGFR/ALK status	excluded	excluded	excluded	included	excluded (30% known status)
	Asian Population	10% vs. 11%	22% vs. 17%	33% vs. 30%	9% vs. 8%	8.4% vs. 9%
	Efficacy 1°EP	RD of best ORR by week 19	RD of ORR by week 18	RD of best ORR by week 18	RR of best ORR by week 19	Rd of best ORR by week 24
	Efficacy 2° EP	PFS, DOR, OS	PFS, DOR, OS, OT, time to OR	PFS, OS, DOR, DCR week 18	PFS, DOR	PFS, OS, DOR
	Prespecified eq margin for 1°EP	± 13%	±12 %	± 12.5%	0.67, 1.5 (RR of ORR), ± 12.5% (RD of RR)	± 12.5%
	Primary analyses population for 1ºEP	ITT	ITT	ITT	ITT	PPS
	Results: RD of ORR (95% CI); ITT	0.65 (-6.60,7.9)	-4.02 (-11.76 to 3.71)	-1.6 (-9.0, 5.9)	-2.9 (-10.48, 4.67)	4.8%, (-2.3%, 11.9%)
	Results: RD of ORR (95% CI); PPS	0.79 (-6.53, 8.12)	-4.27 (12.92 to 4.38) not met	-2.4 (-10.2, 5.3)	-2.82 (-11.06, 5.42)	5.3%, (-2.2%, 12.9%)
	Results: RR of ORR (95% CI)	1.01 (0.86, 1.19)	0.91 (0.76, 1,09)	NP	0.93 (0.77, 1.12)	1.12 (90% CI 0.98, 1.28)
	Results PFS	HR 0.93 (0.77, 1.11); median (weeks) 39 (33.0%, 42.1%) vs. 33.6 (33.0%, 37.0%)	HR 1.2 (0.98, 1.46); median (weeks): 36 (33.6 - 36.9) vs. 43 (36.1- 45.1)	median (months): 7.8 (7, 9.5) vs. 7.3 (7, 8.9)	HR 1.03 (90% CI: 0.83, 1.29). median (months): 6.6 (6.3, 7.9) vs. 7.9 (6.6, 8.2)	HR 1.01 (0.84, 1.22; median (months): 8.5 (7.2, 9.7) vs. 7.9 (7.3, 9.4)

	Results DOR	HR 0.79 (0.600-1.039); median (weeks): 36.1 (30.4, 45.1) vs. 28.1 (26.3, 36.1)	HR 1.19 (0.91, 1.56) median (weeks): 30.3 (28.3, 38.4) vs. 37.1 (30.4, 39.6)	HR NA (p-value=0.09); median (months): 7.6 (7.0, 9.5) vs. 9.0 (7.2, 9.7)	HR 0.76 (90% CI: 0.51, 1.14); median (months): 5.8 (4.9-7.7) vs. 5.6 (5.1 -6.3)	mean (months): 6.33 (3.78) vs. 6.8 (4.2)
	Results OS	HR 0.92 (0.729, 1.15) median (weeks) 80.1 (71.1, -) vs. 77.4 (70.1, -)	HR 1.12 (0.826 to 1.483); median was not estimable for either group	HR 1.26 (0.94, 1.69) median not reached	HR 1.10 (90% CI: 0.75, 1.61)median was not estimable for either group	HR 1.08 (0.86, 1.35); median (months): 14.80 (13.00, 17.00) vs. 15.80 (13.80, 17.70).
	TEAES, any	97% in both arms	92.6% vs. 92.9%	91.3% vs. 91.1%	95.1% vs. 93.5%	92.1% vs. 91.1%
	Fatal TEAES	NP	7.4% vs. 7.7%	6.3% vs. 4%.	4% vs. 3.6%	5.8% vs. 7.1%
	TEAEs leading to discontinuation of IP	4.5% vs. 3.4%	13.5% vs. 10.6%	10.7% vs. 8.3%	18.8% vs. 17.2%	13.2% vs. 9.5%
	SAEs, any	23% vs. 22%.	18.6% vs. 17.4%.	17.6% vs. 16.7%	26% vs. 23%.	19.8% vs. 21.3%.
	Patients with ADA positive samples at any time	at week 55: 1.5% vs. 1.4%	overall (post-dose): 16.1% vs. 17%	at week 16: 1.4% vs. 3.7%	at week 19: 1.4% vs. 2.5%	at cycle 7: 13.5% vs. 10.1 %
	Population PK (done/not done)	yes	no	yes/insufficient	yes	yes
	if yes, how	Assesment of Ctrough at every cycle up to Cycle 17 and post dose cycle 1, 5	NA	Allrandomised patients who completed at least 1 dose and provide at least 1 evaluable post-dose drug concentration	Assesment of Ctrough at time baseline, and at weeks 4, 7, 13 and 19. ADA were determined at baseline, and at weeks 7, 13, 19 and at follow-up	Assesment of Ctrough/Cmax at cycles 1,3,5,7
	Switch (yes/no). If yes, timepoint of switch	no	no	no	no	no
	Observation period	55 weeks	52 weeks	42 weeks	64 weeks	48 weeks

In the following section, results of PK analyses (obtained in healthy volunteers (HVs) and patients), efficacy analyses of clinical trials in patients and safety, and immunogenicity evaluation (obtained from PK and efficacy studies) are presented.

PK studies

a. PK in healthy volunteers

In **Table 5** and **Table 6**, the primary end points with prespecified margins and all secondary end points, including safety and immunogenicity, are presented.

Observation period

For adalimumab biosimilars, the length of follow-up ranged from 62 to 71 days, and for bevacizumab biosimilars from 85 to 100 days, which represents ~ 5 half-lives.

Primary end point

In all instances the primary end points (area under the curve to infinity (AUCinf), maximum concentration (Cmax), and AUC from time of administration up to the time of the last quantifiable concentration (AUClast) were contained within the prespecified acceptance range of 0.8–1.25. For three adalimumab products (Hyrimoz/Halimatoz/Hefiya, Hulio, and Amsparity) and one bevacizumab (Alymsys/Oyavas) the end points were such that unity was not included in the 90% confidence intervals (CIs), which may be permissible(66). Root cause analysis for not being included in the 90% CI was performed by additional supplementary analyses on the primary end point and scrutinizing relevant QAs (for example, high mannose and sialic acid) with no negative signals.

In addition, for two adalimumab products (Hyrimoz/Halimatoz/Hefiya, and Hulio), initially failed and subsequently successful PK studies were submitted. Root cause analysis was performed, without finding analytical dissimilarities that could have explained the initial failure to show bioequivalence. Further, in both instances, a second, more strictly standardized PK study was conducted with reduced intersubject variability, and PK similarity was shown(25,29).

b. Population PK in patients

For some products, PopPK data were collected in a subset of patients as part of the clinical efficacy/safety study, with trough plasma concentration (Ctrough) as an end point, as recommended in the mAb guideline(11).

For adalimumab biosimilars, samples were typically collected at 5 timepoints (sparse sampling) for all patients in the initial 6 months study period and, in some instances, until week 50 or even 60. In all instances, PopPK results were considered comparable.

For bevacizumab biosimilars, Ctrough was typically collected at time zero (baseline), and at weeks 4, 7, 13, and 19. Acceptable PopPK data were provided in four of six cases; for Alymsys, PopPK analysis was not carried out, and for Abevmy, the data set was viewed as insufficient after assessment. In both cases, the insufficiency of comparative PopPK data in patients was justified by proven PK similarity in HVs and totality of evidence from other parts of the similarity exercise.

Clinical efficacy studies

Adalimumab is currently approved in 13 autoimmune indications(20). Four applicants chose to compare efficacy in subjects with rheumatoid arthritis (RA) as a model indication in the clinical trial. Three applicants chose chronic plaque-type psoriasis (PP) as the model indication. Both indications are viewed as sufficiently sensitive by the EMA to detect potential clinically relevant differences between the biosimilar and the RP due to the large treatment effect.

Bevacizumab is currently approved in six indications in the EU(67). All applicants chose newly diagnosed or recurrent stage (IIIB)/IV nonsquamous non-small cell lung cancer (NSCLC) as the most sensitive model indication due to the large treatment effect.

a. Observation period

The length of follow-up was typically 1 year for all adalimumab and bevacizumab biosimilars (for Hulio, follow-up was 24 weeks with an extension trial proceeding up to 1 year).

b. Primary end point

For adalimumab biosimilars, American College of Rheumatology Response (ACR 20) and Psoriasis Area and Severity Index (PASI) score were chosen as primary end points for RA and psoriasis, respectively. The equivalence margins for the risk difference (RD) varied between ± 10% and ± 15% for RA and ± 15% and ± 18% for psoriasis, depending on the number and nature of trials performed with the RP that were included in the meta-analysis to derive the equivalence margin. In all instances, the 95% CI for the primary end points were within the prespecified equivalence margins and all other secondary end points also supported similar clinical performance. Results obtained in both analysis sets (intention to treat (ITT); per protocol set (PPS)) were concordant in all instances. Secondary end points in the trials were ACR 50 and ACR 70 scores, and Disease Activity Score (DAS) 28 in RA, and PASI 50, 75, and 90 scores in psoriasis, as well as additional efficacy measurements at different timepoints.

For bevacizumab products, the predefined equivalence margin for the RD of overall response rate (ORR) varied between ± 12 and ± 13% depending on the chosen reference studies. The 95% CI for the ORR was fully contained within the prespecified acceptance range for all five substances. Results of secondary end points of progression-free survival (PFS) and duration of response (DOR) generally provided further support for biosimilarity. However, for Alymsys, PFS was seemingly worse for the biosimilar with a hazard ratio (HR) of 1.2 (0.98, 1.46); median (weeks): 36.0 (33.57–36.86) vs. 43.0 (36.14 to 45.14). Nevertheless, this finding was not viewed as critical as the primary end point was met and the study was not designed to demonstrate equivalence for PFS.

c. Secondary end points

Time-dependent end points were included as secondary end points, but are less sensitive and informative for conclusions on biosimilarity than end points reflecting the MoA, because they are likely influenced by patient-related factors, such as general health status. For bevacizumab biosimilars, median overall survival (OS) could not be estimated for either group in all instances due to limited observation time and due to the fact that > 50% of patients were still alive at the cutoff. In instances where the HR for OS was > 1.0 (e.g., Abevmy and Alymsys), suggesting higher mortality in the

biosimilar group, the OS results were viewed with caution by the EMA because the studies were neither adequately powered to demonstrate equivalence, nor to detect differences in OS, and no type 1 error control was included.

d. Clinical safety data

Safety parameters, such as treatment emergent adverse events (TEAEs), adverse events of special interest (AESi), serious adverse events (SAEs), deaths, and TEAEs leading to discontinuation were comparable between groups in most cases, as seen in **Table 5** and **Table 6**. Adverse events (AEs) were mild to moderate and the adverse episodes resolved in all instances with no deaths reported.

It should be noted that clinical trials are not powered for safety end points, because this is considered unnecessary and would usually require several thousand study participants.

e. Clinical immunogenicity data

Adalimumab is a highly immunogenic product and antidrug antibodies (ADAs) were detected in 30–88% of subjects across all trials. The variability may be explained by differences in study populations and sensitivity of antibody assays used. Importantly, patients with ADA-positive samples at any time were similar between treatment arms for all biosimilar products.

Bevacizumab is a low immunogenic product and bevacizumab ADAs and neutralizing antibodies were rarely detected, except for higher percentages observed for Alymsys/Oyavas and Aybintio/Onbevzi; however, they were similar between treatments arms and almost all ADAs were transient and appeared not to have effects on PKs or safety.

A summary of all observations with clinically deviating results is provided in **Table 7**.

Table 7. Discrepancies in clinical attributes and how they were resolved.

Adalimumab	Clinical attribute	Observation	How resolved
Hyrimoz/Halimatoz/ Hefiya; Hulio and Amsparity	PK	Unity was not included in the 90% CI	(1) Permissable (44) (2) Relevant QAs (high mannose, sialic acid) showed close similarity.

Hyrimoz/Halimatoz/ Hefiya; and Hulio)	PK	Initial study failed to meet predefined acceptance range	(1) Root cause analysis (2) Subsequently, successful PK studies were submitted.
Bevacizumab	**Clinical attribute**	**Observation**	**How resolved**
Alymsys/Oyavas	PK	Unity was not included in the 90% CI	(1) Permissable (44) (2) Relevant QAs (high mannose, sialic acid) showed close similarity.
Alymsys/Oyavas	Pop PK	Not carried out	(1) Pop PK only supportive (2) PK similarity proven in HV
Abevmy	Pop PK	Insufficient	(1) Pop PK only supportive (2) PK similarity proven in HV
Alymsys/Oyavas	PFS	HR of 1.2 (0.98, 1.46); median (weeks): 36.0 (33.57 - 36.86) vs 43.0 (36.14 to 45.14).	(1) Primary endpoint (ORR) met (2) Study not designed to demonstrate equivalence for PFS. (3) Totality of evidence in overall biosimilarity assessment
Abevmy, Alymsys/Oyavas	OS	HR for OS > 1.0; suggesting higher mortality in the biosimilar group	(1) Primary endpoint (ORR) met (2) Study not designed to demonstrate equivalence for OS. (3) Totality of evidence in overall biosimilarity assessment

3.1.5. DISCUSSION

In the early years of biosimilar development, it was considered that even with a convincing quality and PK package, there would always be some “residual uncertainty” which in most cases could only be addressed by a sufficiently powered efficacy study in patients. However, since then, the discriminatory power of the analytical methods used has vastly increased. EU-regulators have gained a large body of knowledge on the quality profile of several mAbs, adalimumab and bevacizumab being just two examples. As shown in **Table 2** and **Table 3**, the panel of analytical testing for biosimilars is very comprehensive, with numerous orthogonal methods used to analyze dozens of QAs. Therefore, every relevant aspect of the mAb structure and activity is interrogated to ensure that it is sufficiently aligned with the RP in order to guarantee comparable clinical efficacy and safety.

This is the first study that performs an in-depth analysis of all quality and clinical data for currently authorised biosimilars of two originator mAbs used in either oncologic (5 products) or in autoimmune indications (7 products).

Based on information provided to the EMA in the MA submissions, and following the scientific evaluation carried out by the Agency, we found that over 90% (and in most cases 100%) of the biosimilar batches met the EU-RP similarity range for critical QAs. A lower percentage of biosimilar batches were within the similarity range for QAs which may be considered less critical to safety and efficacy, such as glycosylation profile or charge variants (see **Table 1, Table 4** and **Table 5**).

The most critical QAs for the determination of biosimilarity are those that could have an impact on the PK profile, on safety (including immunogenicity) and efficacy. Therefore, for high criticality QAs, a high degree of similarity to the RP is expected. Of the numerous QAs studied, the ones which as part of the assessment process were considered by the EMA to be of high criticality for determination of adalimumab biosimilarity, and which have demonstrated high concordance (marked solid green) are: protein content, soluble TNFα binding, transmembrane TNFα binding, FcγRIIIa binding, FcRn, biological activity (as measured in cell-based TNFα neutralization assay), ADCC, CDC, and a functional read out of reverse signaling (e.g., apoptosis

induction). C1q binding is also considered critical, but these data were not presented in our analyses due to problems with anonymity. Similarly, high criticality for determination of bevacizumab biosimilarity (as considered by the EMA and marked solid green) are: protein content, biological activity (as measured in cell-based antiproliferation assay), binding to main VEGF isoforms, and FcRn binding.

If < 100% of batches were within the similarity range for these highly critical QAs, data from relevant additional analytical and functional assays were reviewed in order to establish that the variations will not lead to differences in clinical performance of the biosimilar (see **Table 4**).

For all adalimumab products (A–G in **Table 1**) > 90% of batches were within the similarity range for highly critical QAs: protein content, potency, ADCC, CDC, and binding to soluble TNFa, mTNFa, FcγRIIIa (158 v/v), and FcRn. Regarding less critical QAs, < 90% of biosimilar batches were within the similarity range (for instance, products A and E, indicated as dark blue dots or light blue diagonal stripes). This was considered acceptable and the PK trial demonstrated no impact on safety/immunogenicity. In addition, the clinical data were supportive of biosimilarity.

Regarding the differences found in glycosylation, although high similarity was observed for all adalimumabs compared with the RP Humira with regard to FcγRIIIa (158 v/v) binding, as well as for ADCC and CDC activity, none of the adalimumab biosimilars had a fully comparable glycoprofile to the RP Humira. The specific glycoprofile is highly dependent on the manufacturing process, including the cell line and growth conditions used, therefore manufacturing an mAb with a highly similar glycoprofile is challenging. In all cases, the minor differences in glycoprofile were justified not to have a functional impact through orthogonal methods, for example, leading to a difference in ADCC activity. Therefore, any observed differences in the glycoprofile between the biosimilar and the originator were justified not to affect the clinical performance of the biosimilar.

For bevacizumab products (H–L), again, for highly critical QAs, ≥ 90% of batches were within the similarity range (i.e., protein content, HUVEC antiproliferation assay, and binding to FcRn and VEGF165). For products H and L, < 90% of biosimilar batches were within the similarity range for binding to VEGF189 and VEGF121, but this was

accepted given as binding to other VEGF isoforms was highly similar. For products I and K, differences were apparent in several purity and glycosylation attributes, but again this was accepted by the EMA during the MA evaluation as Fc functionality (glycosylation profile) is not critical for bevacizumab.

Assessing the PK trial demonstrated no impact on safety/immunogenicity of patients and further clinical data were also supportive of biosimilarity.

Regarding the differences found in purity, these need to be justified or appropriately clinically qualified as they may affect efficacy and safety, including immunogenicity. In some instances, there were minor differences in impurity levels between the biosimilars and RP, with some biosimilars showing slightly higher purity levels and some slightly lower. However, in all cases, differences were considered minimal in absolute numbers and were justified to have no impact on safety or efficacy. Further, no immunogenicity signals were observed in the clinical PK or efficacy trials.

As a general comment, the list of critical QAs known to be of high importance for determination of biosimilarity should not be interpreted in such a way that these are the only QAs of interest and other QAs do not need to be studied. Rather, if differences between the biosimilar and the RP are detected, the biosimilar applicant needs to justify the impact of the difference. Moreover, although we did not include them in our analysis because the data could not be categorised in a quantitative way, the amino acid sequence secondary and higher order structure of a biosimilar is expected to be the same or highly similar to the RP and therefore these are also considered critical QAs. Information presented on the number and type of conducted assays for all products should not be leveraged by future developers as the presented results are reviewed by regulators on a case-by-case basis.

Several scientists (25,28–31,68) pointed out limitations of indiscriminative efficacy and safety studies in light of technical advances in analytical methods which provide more discriminative research tools. Even large molecules can currently be thoroughly characterized using state-of-the-art analytical and *in vitro* functional testing. This thorough characterization is also routinely applied as part of comparability studies conducted for biological medicinal products following introduction of manufacturing

process changes(69–71). The recently established EMA tailored scientific advice pathway for biosimilars acknowledges these scientific advances(6).

For all adalimumab and bevacizumab biosimilars studied, a comprehensive clinical programme was submitted consisting of a PK trial and a clinical efficacy study, which confirmed biosimilarity.

Secondary efficacy end points, safety, PopPK, and immunogenicity end points were always descriptive in nature and results generally concordant with those of the primary end points. In those cases where a trend toward a possible difference was observed, it was judged to be negligible and/or likely due to immaturity of the data. In some cases, certain data were not obtained or incomplete. These deviating or missing results did not preclude approval, as similarity was shown for the relevant QAs and in dedicated PK studies and confirmed by clinical data in the efficacy study. As this paper analysed already approved biosimilar products, and subsequent experience with these products to date has not resulted in any safety or efficacy issues following their approval(28,72,73), it can be concluded that the regulatory decisions taken were correct.

Our study supports previous observations(68) that adequately powered PK trials, provide sufficient clinical safety and immunogenicity data, especially when close similarity in analytical and functional parameters together with comparable PK and impurity profiles can already largely predict similar safety and immunogenicity of the biosimilar and the RP.

As stated by Kurki *et al*.(68) the intrinsic immunogenicity observed for each RP was also observed for the respective biosimilars. In no instance did RPs with high immunogenicity have a biosimilar with low immunogenicity, or vice versa. Observations regarding comparability of immunogenicity made in the PK trial were, in all instances, confirmed in the efficacy/safety study. Similar observations were made with regard to safety parameters.

Furthermore, the EU pharmacovigilance systems and risk management planning are sufficiently robust(28,72,73) to detect safety signals in postmarketing use. However, safety signals (including reports on reduced efficacy) are not anticipated, because

more than a decade of clinical experience indicates that a new safety signal solely identified with a biosimilar is extremely unlikely(72,74).

For the biosimilars included in this study, differences in several QAs were found. As part of the EMA approval process, applicants were challenged to justify that the observed differences would have no impact on the clinical performance of the biosimilar. Importantly, in all cases, these questions were answered by applicants based either on quality data alone or on a combination of quality, PK, and immunogenicity data (**Table 4**). In no instance were data from the clinical efficacy, safety, and immunogenicity study required to justify the differences at the quality level. On this basis, we argue that for the adalimumab and bevacizumab biosimilars, clinical efficacy, safety, and immunogenicity data were not needed to address residual uncertainty remaining from the quality and PK studies.

This analysis adds to the ongoing debate about the role of clinical studies for biosimilars.

In the authors' opinion, the usefulness of clinical efficacy, safety, and immunogenicity data for the purposes of regulatory decision could be questioned. Where the quality, PK, and immunogenicity data are sufficiently robust and convincing for regulatory decision making, as in the case of the adalimumab and bevacizumab examples cited in this paper, then it is our contention that the current expectations for clinical efficacy, safety, and immunogenicity could be re-examined. Therefore, we encourage a regulatory review of the standards for clinical data requirements for biosimilars, and propose that clinical data requirements should be further tailored.

Given the 10 years of regulatory experience in assessing and approving biosimilars, and the positive performance of approved biosimilar mAbs on the market(25,29,68,75), the authors suggest to move to a concept of "tailored evidence," depending on the nature of the product and the available orthogonal assays for quality similarity. For example, this could include removing the standard requirement for equivalence trials, accepting wider equivalence margins, omitting PopPK studies, and/or reducing secondary clinical end points.

Such tailored approaches may prove particularly useful in the case of biosimilars for orphan medicines or other treatments where there is a small patient population or products with a narrow treatment effect where a comparative efficacy study may not be feasible due to the inability to recruit a sufficient number of subjects for any meaningful statistical analysis.

In the authors' opinion, if the efficacy study is omitted, sponsors may consider expanding their PK studies with regard to study size or observation period to gather additional safety/immunogenicity data. In other instances, a clinical study generating some limited safety and immunogenicity data in patients may be beneficial.

Where the quality package or the PK data are not sufficiently convincing, a root cause analysis would be necessary, potentially requiring changes to the manufacturing process of the biosimilar candidate or an improved design/power of the PK study, as was, for example, observed during biosimilar adalimumab development(29). Alternatively, a stand-alone application could be pursued. Whereas the analysis in our study is based on adalimumab and bevacizumab as representative examples, the principle could be generalized to mAbs as a class.

In conclusion, in the author's opinion, a tailored evidence approach for all biosimilars including mAbs and fusion proteins, where a robust and convincing analytical biosimilarity package is available in conjunction with an appropriately powered PK study that also provides safety and immunogenicity data, the extent of the clinical trial requirements can be further reduced, or such trials even omitted. This would allow for more rational use of clinical resources, reduce the type of clinical data analyzed or number of clinical trials, and streamline the development of biosimilar mAbs and fusion proteins to the benefit of patients and healthcare stakeholders which is also in line with the strategic priorities of the EMA(76).

Conflict of interest

The authors declared no competing interests for this work.

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3.2. Publication 2: Do the outcomes of clinical efficacy trials matter in regulatory decision making for biosimilars?

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3.2.1. ABSTRACT

Background: There is an increasing body of evidence supporting a more flexible approach in clinical data requirements for the approval of more complex biosimilar substances such as mAbs.

Objective: The aim of this paper is to further analyse the role of quality/chemistry, manufacturing and controls (CMC) and clinical data for the conclusion on biosimilarity and the decision on marketing authorisation (MA).

Methods: In the present study, we analysed the MAAs of all 33 mAbs and 3 fusion proteins evaluated by the EMA between July 2012 and November 2022 with special emphasis on all submitted rituximab (four products) and trastuzumab (seven products) biosimilar candidates, including withdrawn applications. For the two withdrawn applications, the comparative efficacy trials suggested biosimilarity, but the quality/CMC package was not accepted by EMA. We therefore investigated whether a negative MAA outcome could have been predicted based on the evidence generated in the quality/CMC packages, regardless of clinical trial data. For this purpose, we reviewed the respective EPARs or withdrawal ARs, and the first regulatory assessments for all these 36 MAAs (i.e. Day 120 of the centralized procedure), which are not publicly available. During EMA review, where significant issues are identified which would preclude a marketing authorisation, these issues are raised as questions to the applicant and are classified as major objections (MO).

Results: In 67% of cases, the outcome of the quality and clinical assessment was the same i.e. both the quality and clinical assessments either supported approval or did not support approval. In 11% of cases, MO were identified in the quality part of the submission but not in the clinical data. In 22% of cases, MO were raised on the clinical data package but not on the quality data. However, we found no instance where seemingly negative clinical data, including failed efficacy trials, led to a negative overall decision. In each instance, the failure to confirm similar clinical performance in all investigated aspects was eventually viewed as not being related to the biosimilar per se but as being due to imbalances in the trial arms, immaturity of secondary endpoint results, change in the RP, or even chance findings. Furthermore, when performing an in-depth analysis of the quality and clinical packages of trastuzumab and rituximab

biosimilars, we found that in no case were clinical trial data necessary to resolve residual uncertainties regarding the quality part.

Conclusion: The results further support the argument that sufficient evidence for biosimilarity can be obtained from a combination of analytical and functional testing and PK studies which may also generate immunogenicity data. This calls into question the usefulness of comparative efficacy studies for the purposes of regulatory decision-making when approving biosimilar mAbs and fusion proteins.

3.2.2. INTRODUCTION

Biologic medicines are effective treatment options for complex conditions such as cancer and an increasingly important component of health care solutions.

However, patient access to highly effective biological medicines is still unequally distributed across countries, often due to the high cost of these medicines.

Biologics represent 35% of medicine spending in Europe at list prices and have been growing at an 11.3% compound annual growth rate over the past five years (3). The expenditure on cancer medicines is growing at rates higher than the growth rates of the patient population and overall health expenditure (77).

Biosimilar competition, i.e. having multiple suppliers of the same active substance, is necessary to curtail overall healthcare costs and to avoid supply shortages. Although the savings from current biosimilar competition in the EU market and patient access are improving, a growing disparity is occurring across countries (3).

The past five years have shown a maturation of the biosimilars market. However, it is estimated that in the period 2023 – 2027, 55% of biologics with loss of market exclusivity will be without competitors (78). Products with low sales value are unattractive to biosimilar manufacturers due to clinical development costs, largely driven by large and lengthy clinical trials and procurement costs of reference product (RP) comparator batches. Cost estimates for developing a biosimilar range from 100 to 300 million US-Dollar (32), compared to 1–5 million US-Dollar for a small molecule generic, largely due to clinical development costs (33–35). This cannot be in the interest of stakeholders including regulators and contradicts the strategic priorities of the EMA (76), which has set out to allow for more rational use of clinical resources.

Recent efforts have resulted in alternatives to costly comparative clinical studies for certain biosimilars, where the extent of clinical data required can vary depending on the complexity and characterisation of the molecule (14,17,26). Also, several initiatives have been launched that could impact regulatory decision making and lead to revised guidelines such as omitting or reducing the size of studies involving human subjects for a larger set of biosimilar products (79,80).

In a recent publication (81), the degree of analytical similarity and the role of clinical data was analysed for authorised adalimumab and bevacizumab biosimilars. It was argued that clinical trial requirements for monoclonal antibody (mAb)-biosimilars can be further reduced, or such trials even omitted, where a robust and convincing analytical biosimilarity package is available in conjunction with an appropriately powered PK study that also provides safety and immunogenicity data.

The remaining question is whether product candidates with good or promising quality data could nevertheless translate into poor (i.e. those with high uncertainty) or failed clinical trials, which may prevent approval of the product where otherwise it would have been approved.

The aim of this paper is to further analyse the role of quality/chemistry, manufacturing and controls (CMC) and clinical data for the conclusion on biosimilarity in a broader setting and in more depth, including all classes of approved biosimilar mAbs and also refused or withdrawn biosimilar candidates, which likely would have failed marketing authorisation (MA).

We therefore analysed the final outcome of the submitted MAAs of all 36 mAbs and fusion protein biosimilar candidates evaluated by EMA up to November 2022, including those for which a withdrawal/refusal assessment report (AR) is available on the EMA website, i.e. one trastuzumab and one rituximab biosimilar candidate, and contextualised these findings by analyses of all approved rituximab and trastuzumab biosimilar products.

We also reviewed the regulatory assessment during the first phase of these MAAs (i.e. the day 120 assessment reports (D120 AR) of the centralised procedure), which are not publicly available, to analyse whether quality data is predictive for clinical outcome and how clinical assessment impacts the decision on MA.

3.2.3. METHODS

3.2.3.1. Analysis of MAA outcome

We analysed the outcome of the MAAs, as well as the available regulatory ARs of all biosimilar mAbs and fusion proteins, i.e. adalimumab, bevacizumab, etanercept, infliximab, ranibizumab, rituximab, and trastuzumab, which included 33 mAbs and 3 fusion proteins evaluated by the EMA between July 2012 and November 2022. This included biosimilar candidates, which received a MA or a negative opinion by the CHMP or which were withdrawn by applicants prior to a CHMP opinion, and for which a EPAR or a Withdrawal AR is available on the EMA website(37,41–52,82–104). Concerns regarding quality/CMC (biosimilarity, general quality) or clinical aspects (PK/PD, efficacy (E)/safety (S)/immunogenicity (I)), as indicated in the EPAR or withdrawal AR, were analysed. We categorised the 36 mAbs and fusion protein biosimilar candidates according to five possible scenarios (**Figure 4**) indicating whether the quality/CMC, PK and clinical aspects of the biosimilar MAA dossier were acceptable to the EMA (shown in green/no pattern) or not acceptable (shown as horizontal red stripes). Vertical green stripes indicates some remaining uncertainties that were discussed within the EMA scientific committees and working parties, but not severe enough to prevent MA. Furthermore, the outcome of the MAA is indicated as well as the active substance and the IgG class.

3.2.3.2. Analysis of first regulatory assessment reports

We analysed the list of questions (LoQ) raised by the CHMP in the D120 ARs of all above-mentioned 36 mAbs and fusion protein biosimilar candidates. D120 ARs are discussed and agreed by the CHMP during the first phase of the centralised MA procedure. They are shared with the applicant but are usually not publicly available(105).

They include formal regulatory aspects (see **Table 8**) and the scientific evaluation of all quality/CMC, non-clinical and clinical data and the risk management plan, that led to a LoQ regarding concerns and uncertainties that must be addressed by applicants before MA(106). These questions are classified as major objections (MO) and other concerns (OC). MO are defined as critical issues that will preclude authorisation if not

resolved(107), while OC should be resolved but are not severe enough to preclude authorisation per se.

We counted the number of MO and OC and classified those as either related to quality or clinical aspects, in the same manner as for the MAA outcome analyses (**Figure 5**). Formal regulatory aspects were not included in the analysis because these are not of direct scientific concern in the context of this study.

3.2.3.3. Evaluation of analytical and clinical biosimilarity for rituximab and trastuzumab biosimilars

We analysed the quality/CMC and clinical data packages for all submitted rituximab and trastuzumab biosimilars to contextualise the results observed for the two withdrawn products (99,100). The analysis included four rituximab biosimilars (87–89,99,108,109) and seven trastuzumab biosimilars (92–95,100,103,104). The data lock point for the analysis was February 2023.

Comparison of analytical biosimilarity (QA) for approved biosimilars was performed using the methodology of our previous paper. Briefly, we extracted raw data from the biosimilar product dossiers, anonymised and colour-categorised them depending on the percentage of analysed biosimilar batches with values within the similarity range of the RP (**Table 9**). For cases where less than 100% of batches were within the reference range it was analysed how the resulting uncertainty was resolved (**Table 10**).

For full details, see Guillen et al., 2023 (81).

For withdrawn applications, we also looked at quality issues of the biosimilar itself affecting e.g. performance and consistency of the manufacturing process, which must be ensured in line with current guidance (12). Therefore, we constructed a figure (see **Figure 6**) that covers all these aspects, following the key quality/CMC requirements from Bielsky et al. as a reference (28). Analytical data was extracted from the Withdrawal ARs, which contain public information that can be found on the EMA website and therefore anonymisation is not necessary (110).

Comparison of clinical biosimilarity is presented in **Table 11**, **Table *12*Table 13Table 14**, and employs the same methodology as Guillen et al. 2023 (81). For cases where discrepancies were observed in clinical attributes, it was analysed how the resulting uncertainty was resolved (**Table 15**).

3.2.4. RESULTS

3.2.4.1. MAA Evidence and Results

For 36 mAbs and fusion protein biosimilar candidates (mostly IgG1), the quality/CMC (i.e. general quality aspects and analytical comparability exercise), clinical PK/PD and clinical efficacy, safety and immunogenicity (E/S/I) aspects were analysed based on the information provided in the EPAR. Results are presented in **Figure 4** according to five possible scenarios.

For more than 80% of the biosimilar candidates analysed (29/36), the quality/CMC part of the dossier, the clinical PK/PD as well as the E/S/I results all unambiguously supported biosimilarity (**Figure 4**, Scenario 1). For two biosimilar candidates, differences in some QAs and functional assays were observed (37,82), but these differences were not seen in PK/PD and clinical E/S/I studies. One candidate had higher immunogenicity(82), later deemed irrelevant (see Discussion). All these biosimilar candidates listed for Scenario 1 obtained a MA.

Scenario 2 applies to two cases with an unsatisfactory quality/CMC package but with overall acceptable clinical trial results (**Figure 4**, Scenario 2). In these two cases(99,100), major concerns were raised regarding the biosimilarity exercise as well as regarding the comparability of the clinical batches and the commercial batches of the biosimilar. The clinical PK and efficacy trials formally met their primary endpoints. However, uncertainties remained for the clinical efficacy trial regarding secondary and subgroup analyses for the rituximab biosimilar candidate(99). Both applications were withdrawn by the companies due to major remaining uncertainties expressed in unresolved quality MO.

Scenario 3 was defined as those product candidates having an acceptable quality/CMC package but indicating differences in the clinical PK/PD profile or remaining questions regarding representativeness of test material used in the PK study, while all other clinical data demonstrated comparability (**Figure 4**, Scenario 3). Two of the biosimilar candidates analysed (43,101) had an initially failed PK study. In both instances, it was argued that the observed differences in glycan structures known to affect PK (high mannose content) were too small to explain the initially observed PK differences(62). The conduct of a second PK trial with improved design features was requested and led to successful demonstration of similar PK profiles(29). For a

third biosimilar candidate, PK results were not accepted because test product was not deemed representative of the commercial product(102).

Scenario 4 lists those cases with an acceptable quality/CMC package and successful PK trial but with issues regarding the clinical E/S/I package (**Figure 4**, Scenario 4). For both affected trastuzumabs(103,104) the primary efficacy endpoint was formally not met as the upper limit of the CI was not contained within the pre-defined equivalence margin. For both trastuzumabs, a MA was granted based on the convincing quality/CMC, PK, safety and immunogenicity data packages, despite a failed primary endpoint.

The last hypothetical scenario would be unconvincing quality/CMC data and failed clinical trials (PK and efficacy trial), which was not observed in any of the 36 cases (**Figure 4**, Scenario 5).

Figure 4. Analysis of MAA outcome. Fulfilment of EMA requirements and outcome of marketing authorisation applications (MAAs) for monoclonal antibody and fusion protein biosimilar candidates based on information provided in the European Public Assessment Reports or Withdrawal Assessment Reports. Green (no pattern) indicates fulfilment of EMA requirements. Vertical green stripes indicates some remaining uncertainties not precluding marketing authorisation (MA). Horizontal red stripes indicates failure to meet EMA requirements

Cases	IgG type	Date of MA	Quality		Clinical	Reference	
			biosimilarity	general Q	PK/PD	E/S/I	
SCENARIO 1			+	+	+	+	
Infliximab 1	IgG1	10/09/2013					(37)
Infliximab 2	IgG1	26/05/2016					(82)
Infliximab 3	IgG1	18/05/2018					(83)
Etanercept 1	Mod. IgG1	13/01/2016					(84)
Etanercept 2	Mod. IgG1	23/06/2017					(85)
Adalimumab 1	IgG1	21/03/2017					(41)
Adalimumab 2	IgG1	24/08/2017					(42)
Adalimumab 3	IgG1	17/09/2018					(44)
Adalimumab 4	IgG1	02/04/2019					(45)
Adalimumab 5	IgG1	13/02/2020					(46)
Adalimumab 6	IgG1	11/02/2021					(47)
Adalimumab 7	IgG1	15/11/2021					(86)
Rituximab 1	IgG1	15/06/2017					(87)
Rituximab 2	IgG1	13/07/2017					(88)
Rituximab 3	IgG1	01/04/2020					(89)
Bevacizumab 1	IgG1	15/01/2018					(48)
Bevacizumab 2	IgG1	14/02/2019					(49)
Bevacizumab 3	IgG1	19/08/2020					(50)
Bevacizumab 4	IgG1	24/09/2020					(90)
Bevacizumab 5	IgG1	26/03/2021					(51)
Bevacizumab 6	IgG1	21/04/2021					(52)
Bevacizumab 7	IgG1	17/08/2022					(91)
Trastuzumab 1	IgG1	09/02/2018					(92)
Trastuzumab 2	IgG1	26/07/2018					(93)
Trastuzumab 3	IgG1	12/12/2018					(94)
Trastuzumab 4	IgG1	27/07/2020					(95)
Ranibizumab 1	IgG1	18/08/2021					(96)
Ranibizumab 2	IgG1	25/08/2022					(97)
Ranibizumab 3	IgG1	09/11/2022					(98)
SCENARIO 2			-	-	+	+	
Rituximab 4	IgG1	not approved					(99)
Trastuzumab 5	IgG1	not approved					(100)
SCENARIO 3			+	+	-	+	
Adalimumab 8	IgG1	10/11/2017					(101)
Adalimumab 9	IgG1	26/07/2018					(43)
Etanercept 3	Mod. IgG1	20/05/2020					(102)
SCENARIO 4			+	+	+	-	
Trastuzumab 6	IgG1	15/11/2017					(103)
Trastuzumab 7	IgG1	16/05/2018					(104)

SCENARIO 5			-	-	-	-	
Does not apply to any of the biosimilars analyzed							

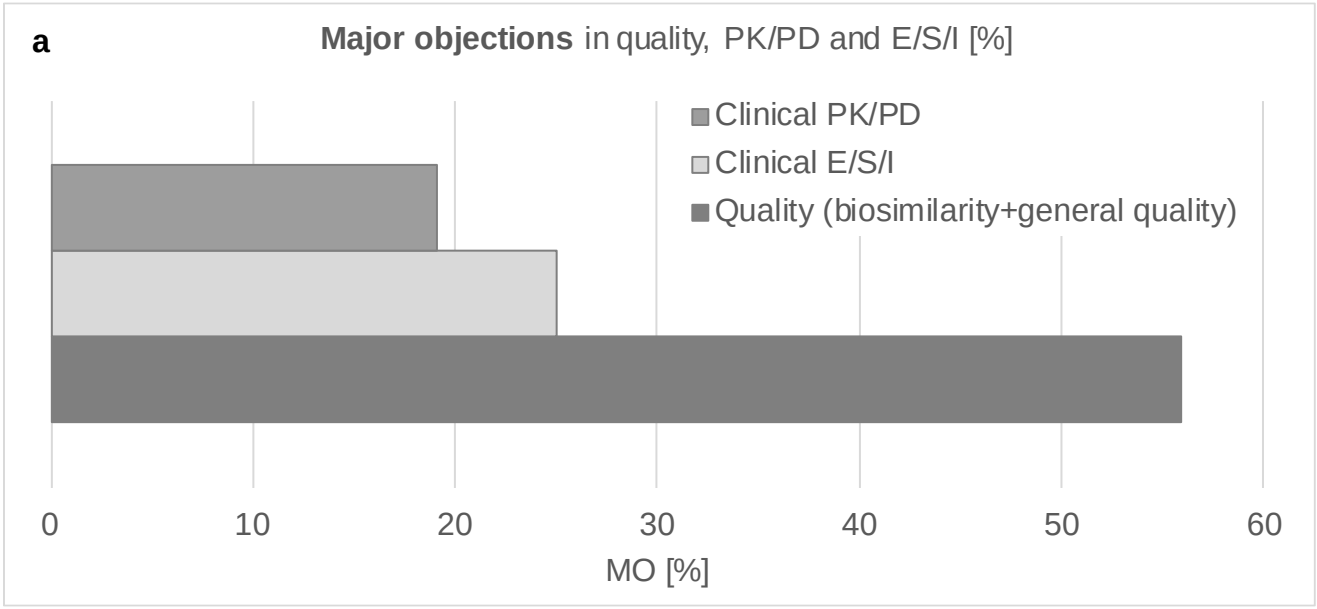
3.2.4.2. Analysis of first regulatory assessment reports

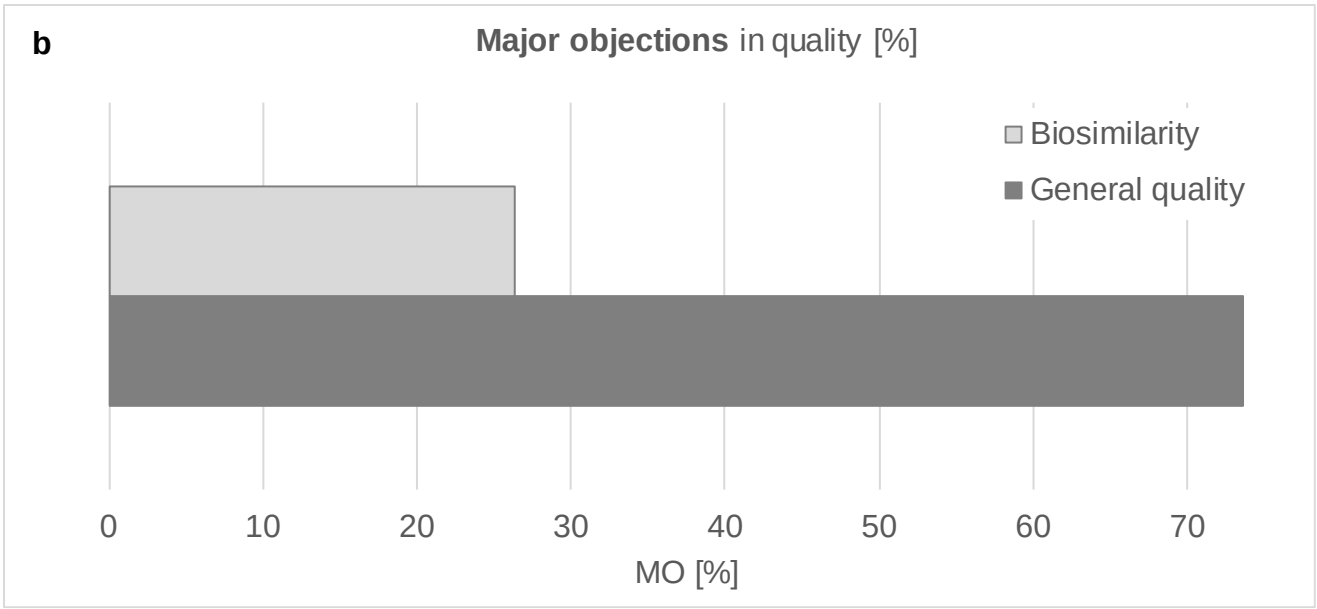
For the majority of biosimilar candidates analysed (34/36), the LoQ raised by the CHMP in the D120 AR was adequately addressed by the applicants and thus led to the final approval.

Analysing the number of MO for the 36 biosimilar candidates concerning scientific issues, indicates that 56% of MO were related to quality/CMC, 19% to clinical PK/PD and 25% to clinical E/S/I issues, respectively (**Figure 5**a). Within the quality/CMC part, the majority of MO dealt with general pharmaceutical issues rather than biosimilarity aspects (**Figure 5**b).
Analysis of OC revealed a similar distribution with 64% of OC pertaining to the quality of the biosimilar candidates, 12% to PK/PD and 24% to E/S/I (data not shown).

When categorising the 36 biosimilar candidates based on where MO were raised, i.e. quality versus PK/PD versus E/S/I, we differentiated four cases, depending on whether MO were identified and knowing that any unresolved MO would prevent approval. We differentiated case 1, when assessment of quality and clinical parts of the dossier led to no MO (positive alignment) in 42% (15/36) of the MAAs analysed, thus supporting biosimilarity. Case 2 was when the quality assessment led to MO that, if not resolved, would lead to rejection of the filing. This applies to 11% (4/36) of MAAs analysed. For case 3, which was when quality assessment supported biosimilarity but clinical queries challenge the validity of the package, 22% (8/36) of cases were identified (8% of cases with MO regarding PK/PD, 11% with MO regarding E/S/I and 3% regarding both PK/PD and E/S/I). And finally, case 4, when both quality and clinical packages raised concerns (negative alignment), with 25% (9/36) of MAAs analysed (**Figure 5**c).

Figure 5. Analysis of questions raised in the first assessment report of the MAA procedure. MO are classified in same manner as for the MAA outcome analyses. In case of MO within multidisciplinary aspects, the specific itemised questions were analysed and distributed according to their content to the quality/CMC or clinical (PK/PD, E/S/I) categories. MO regarding formal aspects were not included in the analysis because these are not of direct scientific concern. *(a) For the comparison of the percentage of MO raised with regard to quality/CMC or clinical aspects of the MAAs the sum of MO (quality/CMC, clinical PK/PD and clinical E/S/I) was calculated and normalised to the number of all MO. (b) MO related to the quality/CMC of the biosimilar candidate were analysed in more detail. Here the number of MO with regard to general quality/CMC or biosimilarity aspects was divided by the sum of all quality/CMC MO. (c) Based on the D120 AR, biosimilar candidates were categorised to four different cases with no MO (green/no pattern) or at least one MO (horizontal red stripes) in the respective area. The percentage of candidates represented by the different cases is indicated.*





Case	Quality MO	Clinical MO		% of biosimilar candidates applicable to each case*
		PK/PD	E/S/I	
1				42
2				11
3				22
4				25

*green/no pattern indicates no MO in the respective area (quality/CMC, PK/PD, E/S/I); red/striped indicates at least one MO in the respective area

The main reasons for MO are summarised in **Table 8**.

Table 8. Most frequent major objections. Major objections (MO) of the day 120 assessment reports were classified in same manner as for the MAA outcome analyses and examples for frequent questions regarding quality and clinical are given.

	MO regarding	Most frequent questions for MO
Quality	Formal aspects	• GMP certificate missing, • EU GMP inspection pending, • provision of a risk evaluation concerning the presence of nitrosamine impurities (EMA/369136/2020, EMA/409815/2020),
	Biosimilarity	• difference in critical quality attributes, • insufficiency of ADCC assays used to conclude on biosimilarity, • insufficient number of batches used for biosimilarity exercise, testing panel incomplete,
	General quality	• manufacturing process, • in-process controls, • comparability of clinical versus commercial batches of the biosimilar candidate, • consistency of the manufacturing process, • missing information or data to assess quality and comparability of the biosimilar candidate,
Clinical	PK/PD	• investigation of observed PK differences/difference in biosimilarity regarding PK, • clinical justification of the pre-specified margins of PK comparability, • PD analysis in second therapeutic area in case of extrapolation to all indications of RP, • submission of individual patient data,
	E/S/I – formal aspects	• confirmation of compliance with ethical requirements (Directive 2001/20/EC) or with the principles of GCP and of the Declaration of Helsinki, • pending GCP inspections, • one-year safety and immunogenicity data not yet submitted at timepoint of initial submission in line with EMA Guideline (EMEA/CHMP/BMWP/42832/2005 Rev. 1),
	E	• failed primary endpoint analysis, • differences observed for RP compared to published data,
	S/I	• additional safety and immunogenicity data in case of observed ADAs, • insufficient submitted data with respect to i.e. ADA and occurrence of neutralizing antibodies, • justification for observed differences in safety profile.

3.2.4.3. Evaluation of analytical biosimilarity and clinical comparability for rituximab and trastuzumab biosimilars

Rituximab and trastuzumab biosimilar products were selected for further in-depth analysis of quality/CMC (**Table 9**, **Table 10** and **Figure 6**) and clinical data (**Table 11**,

Table 12, **Table 13**, **Table 14**, and **Table 15**) as these included withdrawn applications.

Comparison of analytical biosimilarity across products

The number of biosimilar batches analysed per product varied between 3 and 40, for most QAs. The analytical comparability packages of the rituximab and trastuzumab biosimilars comprised between 35 and 85 individual assays per product. For most of the QAs, orthogonal analytical methods were used.

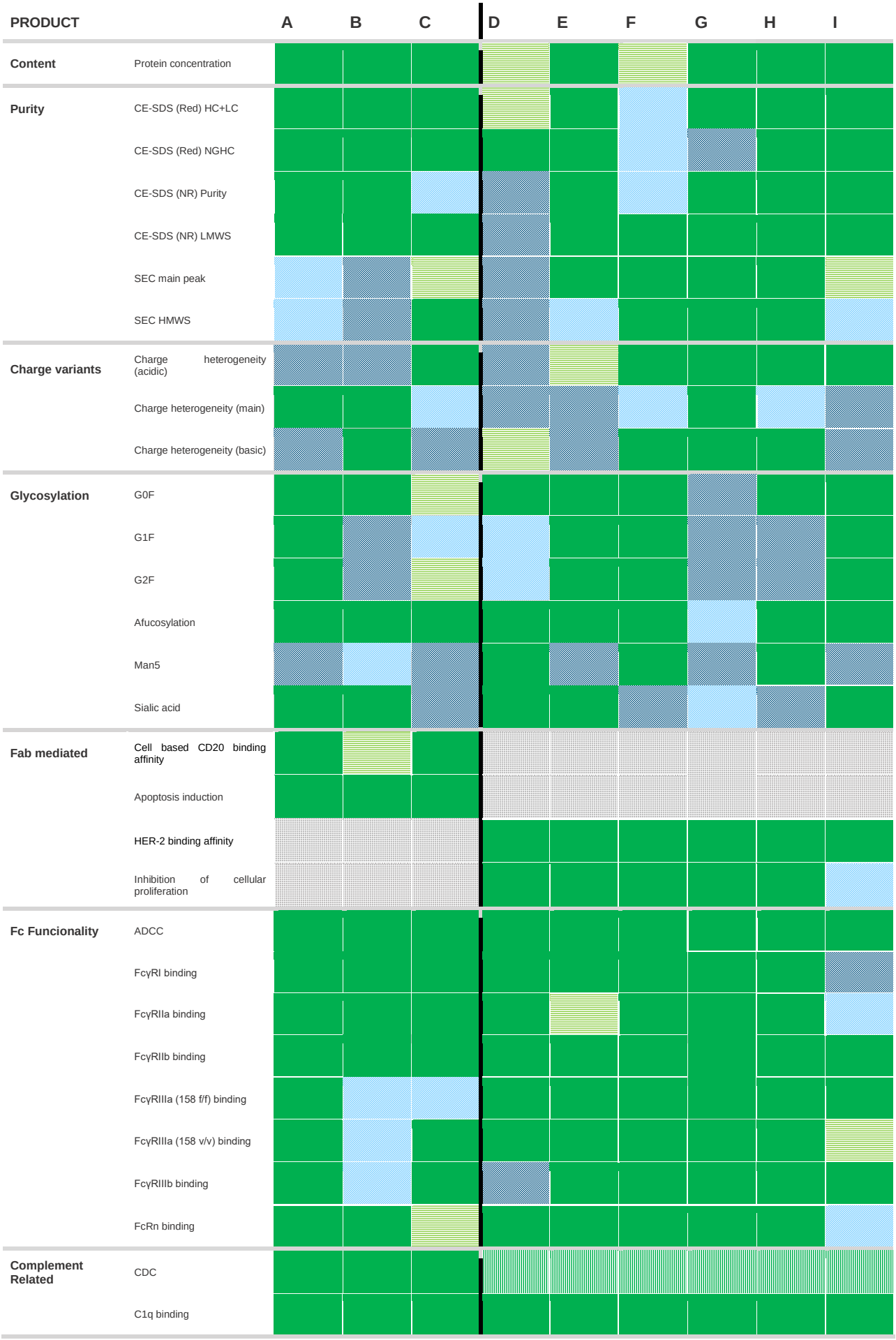
Rituximab is an IgG1 kappa type mAb directed against CD20 expressed on the surface of pre-B and mature B lymphocytes, but not on hematopoietic stem cells and terminally differentiated antibody-producing plasma cells or other tissues. Upon binding to CD20, rituximab mediates B cell lysis (leading to B cell depletion) by three distinct MoAs: CDC, ADCC and apoptosis(111). Therefore, the biological activity of rituximab is determined by a combination of CD20 binding assay and an apoptosis induction assay, together with Fc functionality. Besides activating the pathways of CDC and ADCC, binding of rituximab to its target (CD20 expressed on B cells) also triggers apoptosis via the caspase signalling pathway(112). ADCP has been further implicated as plausible MoA of rituximab in its killing of chronic lymphocytic leukaemia cells (111,113).

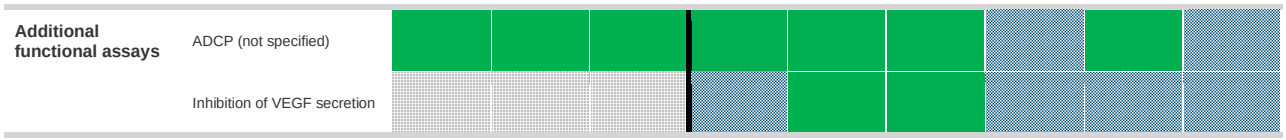
Trastuzumab is an IgG1 mAb which binds to Human Epidermal Growth Factor Receptor 2 (HER2), a transmembrane oncoprotein overexpressed in approximately 20 to 25% of invasive breast cancers(114). Binding of trastuzumab to HER2 inhibits ligand-independent HER2 signalling and prevents the proteolytic cleavage of its extracellular domain, an activation mechanism of HER2. As a result, trastuzumab inhibits the proliferation of human tumour cells that overexpress HER2. Therefore, the biological activity of trastuzumab is determined by the combination of HER2 binding assay and an inhibition of cellular proliferation assay, together with Fc functionality. However, in contrast to rituximab, CDC activation is not thought of as a MoA of trastuzumab(115).

For other Fab mediated assays, glycan and purity profile and charge variants we followed a similar categorisation as in our previous paper(81). Additional assays include for example ADCP for both rituximab and trastuzumab, and inhibition of VEGF secretion for trastuzumab.

Table 9 provides a summary of the analytical biosimilarity results for approved rituximab (products A-C) and trastuzumab (D-I) biosimilars, and **Table 10** provides a summary of the instances where less than 100% of batches were within the reference range, and how the resulting uncertainty was resolved.

Table 9. Similarity of QAs for all approved rituximab (A-C) and trastuzumab (D-I) biosimilars. Colour and patterns indicate the percentage of biosimilar batches within the similarity range derived from the EU reference product: solid dark green for 100%, horizontal light green stripes for 99-90%, diagonal light blue stripes for 89-50%, dark blue dots for <50% and also when the QA was not assessed. Gray grid represents product specific QAs which reflect the mabs main MoA. Dark green vertical stripes represent QAs that were tested but not found (in line with the mabs MoA)


PRODUCT
A
B
C
D
E
F
G
H
I
Content
Protein concentration
Purity
CE-SDS (Red) HC+LC
CE-SDS (Red) NGHC
CE-SDS (NR) Purity
CE-SDS (NR) LMWS
SEC main peak
SEC HMWS
Charge variants
Charge heterogeneity (acidic)
Charge heterogeneity (main)
Charge heterogeneity (basic)
Glycosylation
G0F
G1F
G2F
Afucosylation
Man5
Sialic acid
Fab mediated
Cell based CD20 binding affinity
Apoptosis induction
HER-2 binding affinity
Inhibition of cellular proliferation
Fc Funcionality
ADCC
FcγRI binding
FcγRIIa binding
FcγRIIb binding
FcγRIIIa (158 f/f) binding
FcγRIIIa (158 v/v) binding
FcγRIIIb binding
FcRn binding
Complement Related
CDC
C1q binding



High similarity (≥ 90% of batches within range (= solid dark and light green horizontal stripes)) was found for protein content, biological activity (CD20 binding and apoptosis induction for rituximab, and HER2 binding and inhibition of cellular proliferation assay for trastuzumab), FcγRIIIa binding, FcRn and C1q binding, ADCC and CDC for almost all rituximab and trastuzumab biosimilars. Exceptions included inhibition of cellular proliferation for one trastuzumab (product I), FcRn for one rituximab (product C) and one trastuzumab (product I) biosimilar and the high affinity FcγRIIIa v/v genotype for one rituximab (product B). However, as seen in **Table 10**, in most cases these differences were considered within the method variability or viewed as sufficiently justified based on high similarity found in other critical QAs (CQA) (i.e., ADCC for FcγRIIIa v/v), the results from PK comparability studies and regulatory experience. None of the authorised trastuzumab biosimilars displayed CDC activity (represented as dark green vertical stripes in **Table 9**), which is expected.

More variability was found for binding to other Fcγ receptors, purity and glycosylation profile, charged variants and additional assays (**Table 9**). Again, as seen in Table 2 the observed differences in Fc binding assays and the glycan profiles were accepted because similarity was confirmed in biological assays. Moreover, afucosylation was 100% within range for all except one trastuzumab biosimilar (product G). Differences in purity and charge variants were seen as negligible based on regulatory experience and product understanding, and differences in additional assays were accepted based on the totality of the evidence presented for similarity.

Comparison of analytical biosimilarity for withdrawn products

Figure 6 represents the key quality/CMC requirements and whether these were met for the two withdrawn biosimilar applications(99,100). These key requirements were categorised following the classification from Bielsky et al. (28).

Figure 6. Analysis of quality requirements for biosimilars withdrawn by the applicant during the review process. For the withdrawn biosimilar applications key relevant quality requirements were analysed and if they were met (applications withdrawn for commercial reasons not included).

Key quality requirements	Withdrawn rituximab biosimilar candidate	Withdrawn trastuzumab biosimilar candidate
In-depth knowledge of the RP		
The main MoA is known and demonstrable	✓	✓
CQA are known	✓	✓
Sufficient (representative) batches of the RP are analysed	×	×
Adequately established QTPP	×	×
Attributes of the biosimilar candidate		
The manufacturing process is well controlled. Release and stability specification limits are appropriate	×	✓
The quality profile of the batches used to generate clinical biosimilarity data is representative of the quality profile of the proposed commercial batches	×	×
Suitable and appropriately qualified analytical methods used for analytical and functional similarity assessment	✓	×
Biosimilarity exercise		
Adequate overall approach for demonstrating biosimilarity	✓	×

Of the quality/CMC requirements included, less than half were met for either of the products. Regarding the RP characterisation, both applicants failed to demonstrate two out of four of the prerequisites, demonstrating in both cases an in-depth knowledge of MoA and CQA of the RP but failing to analyse enough representative RP batches or to adequately establish the quality target product profile (QTTP). Regarding the biosimilar candidate attributes, out of the three prerequisites, only one was met for each product. The quality/CMC package included suitable and qualified analytical methods for the withdrawn rituximab and an adequate manufacturing process for the trastuzumab. However, none of the other requirements were met, including the representativeness of clinical and commercial batches or the use of additional orthogonal assays. Finally, only the withdrawn rituximab included an adequate biosimilarity exercise.

Table 10 provides a summary of the instances where less than 100% of batches were within the reference range, and how the resulting uncertainty was resolved.

Table 10. QAs with <100% of batches meeting similarity ranges and how the resulting uncertainty during MAA was resolved.

Rituximab	**QA**	**Percentage of batches within the similarity range**	**How resolved**
Product B	Cell based CD20 binding assay	≥ 90% of batches	Minor difference not expected to affect the clinical performance of the product. Slight differences explained and justified by the method variability.
Products B and C	Binding to several Fcγ-Receptors (FcγRI, FcγRIIa, FcγRIIb, FcγRIIIa-158 f/f and FcγRIIIb	Variable, see Online Resource 1	Minor differences in binding results, similarity confirmed in cell-based functional assays.
Product B	Binding to FcγRIIIa 158 v/v	80-50% of batches	Viewed as sufficient based on ADCC assay results.
Product C	Binding to FcRn	≥ 90% of batches	Based on regulatory experience and the results from the comparative PK study, the minor difference was seen as negligible.
Product A – C (all)	Glycosylation (6 attributes)	Variable, often < 90%, see Online Resource 1	Similarity confirmed in cell-based functional assays. No clinically significant difference in PK profile.
Product A – C (all)	Purity testing	Variable, often < 90%, see Online Resource 1	Based on regulatory experience, the small difference was seen as negligible. In most cases, purity of biosimilar was marginally increased.
Product A – C (all)	Charge variants	Variable, often < 90%, see Online Resource 1	Acceptable based on product understanding.
Trastuzumab	**QA**	**Percentage of batches within the similarity range**	**How resolved**
Product D and F	Protein content	≥ 90% of batches	The small difference in protein content was concluded to be of no clinical relevance. Batch-to-batch variability of the biosimilar within the expected range.
Product I	Inhibition of cellular proliferation	80-50% of batches	Slight differences explained and justified by the method variability.
Product I	Binding to FcRn	80-50% of batches	Based on regulatory experience and the results from the comparative PK study, the minor difference was seen as negligible.
Product D, E, I	Binding to several Fcγ-Receptors (FcγRI, FcγRIIa, FcγRIIb, FcγRIIIa-158 f/f and FcγRIIIb)	Variable, see Online Resource 1	Minor differences in binding results, similarity confirmed in cell-based functional assays.
Product I	Binding to FcγRIIIa 158 v/v	≥ 90% of batches	Minor differences viewed as irrelevant based on ADCC assay results.
Product D – I (all)	Glycosylation (6 attributes)	Variable, often < 90%, see Online Resource 1	Similarity confirmed in cell-based functional assays. No clinically significant difference in PK profile.
Products D – I (all)	Purity testing	Variable, often < 90%, see Online Resource 1	Based on regulatory experience, the small difference was seen as negligible.
Products D, E, F, H, I (all except G)	Charge variants	Variable, often < 90%, see Online Resource 1	Acceptable based on product understanding.

Products G, I	ADCP	< 50% of batches or not done	Acceptable based on FcγRIIa assay results, considered surrogate for ADCP function.

Results of clinical comparability studies

Clinical data is presented as raw data in **Table 11**,**Table *12*Table 13 Table 14** (the product rows are not in the same order as **Table 9** to maintain anonymity). **Table 15** provides a summary on all the uncertainties in clinical data, and how these were resolved.

PK studies

Rituximab

Table 11. Main PK results of rituximab biosimilar candidates compared to RP (published in respective EPARs or Withdrawal ARs).

		Ruxience (01/04/2020)	**Rixathon** (15/06/2017)/ **Riximyo** (15/06/2017)	**Blitzima** (13/07/2017)/ **Truxima** (17/02/2017)/ **Ritemvia** (no longer authorised)	**Rexeful/MabionCD20 (Mabion)** (MAA withdrawn)
PK STUDY	Sample size (N), Randomization	+/- 200, (1:1:1[a])	+/- 200, (1:1)	**CT-P10 1.1**: +/- 200, (2:1 - P:RP) **CT-P10 3.2:** Part 1 +/- 200 (1:1:1 [a]). Part 2 +/- 400 (2.5:1:2.5 - IP:RP:RP [a])	**MabionCD20-001RA:** (2EP) +/- 200, (1:1) **MabionCD20-002NHL:** +/-100, (5:2 – IP:RP)
	Indication	RA	RA	**CT-P10 1.1:** RA **CT-P10 3.2**: RA	**MabionCD20-001RA phase 3:** RA **MabionCD20-002NHL:** CD20-positive Diffuse Large B-cell Lymphoma
	1°EP	Cmax, AUC 0-∞	AUC (0-inf)	**CT-P10 1.1:** AUC 0-last and Cmax **CT-P10 3.2:** AUC0-last, AUC0-inf and Cmax	**MabionCD20-001RA:** AUC(0-t) and Cmax-second **MabionCD20-002NHL:** AUC(1-4) and AUC(13-26)
	2°EP	AUC 0-T, AUC 0-2wk	C max1, AUC parameters (AUC(0-14d), AUC(0-12w), and AUC(0-24w)) and Tmax	**CT-P10 1.1:** AUC0-t, AUC 0-∞, AUC t-∞**,** Cmax, Tmax, Ctrough, Vd, CL, and t1/2 **CT-P10 3.2:** AUC0-t, AUCt-inf, Cmax and Ctrough after 1st infusion	**MabionCD20-001RA:** AUC(0-inf), Cmax-first, Ctrough, T1/2, VD and CL **MabionCD20-002NHL:** AUC(1-26), Ctrough, Cmax, Kel, T1/2, CL)
	3°EP	x	x	**CT-P10 1.1:** Cmin and Ctrough following the 2nd course treatment course	x
	Prespecified equivalence margins for 1°EP	0.8 to 1.25	0.8 to 1.25	0.8 to 1.25	**MabionCD20-001RA:** 0.8 to 1.25 **MabionCD20-002NHL:** 0.70 to 1.43
	Cmax	1.06 (0.97, 1.15) 1.07 (0.98, 1.16) [a]	C max1: 1.133 (1.017, 1.262)	**CT-P10 1.1:** 0.96 (0.89, 1.03) **CT-P10 3.2:** 0.89 (0.82, 0.96) 1.01 (0.94, 1.09) [a]	**MabionCD20-001RA:** Cmax-second: PK-PP: 0.99 (0.93, 1.05) PK ITT: 0.98 (0.94, 1.04) **MabionCD20-002NHL:** Cmax_5th: PP: 1.03 (0.93, 1.13) Cmax_8th: PP: 1.00 (0.93, 1.09)

		Ruxience (01/04/2020)	**Rixathon** (15/06/2017)/ **Riximyo** (15/06/2017)	**Blitzima** (13/07/2017)/ **Truxima** (17/02/2017)/ **Ritemvia** (no longer authorised)	**Rexeful/MabionCD20 (Mabion)** (MAA withdrawn)
	AUC last	AUC 0-T: 1.03 (0.93, 1.15) AUC 0-2wk: 1.04 (0.95, 1.13) AUC 0-T:1.01 (0.91,1.13) [a] AUC 0-2wk: 1.06 (0.97, 1.15) [a]	AUC(0-24w): 1.087 (0.980, 1.206)	**CT-P10 1.1:** AUC 0-last: 0.97 (0.88, 1.07) **CT-P10 3.2:** AUC 0-last: 0.94 (0.85, 1.05) AUC 0-last: 1.02 (0.92, 1.13) [a]	**MabionCD20-001RA:** AUC(0-t) : PK-PPS: 1.02 (0.93, 1.11) PK ITT: 1.04 (0.95, 1.13) **MabionCD20-002NHL:** PPS: AUC(1-4): 1.04 (0.96, 1.13) AUC(13-26):1.06 (0.98, 1.15) AUC(1-26):1.06 (0.98, 1.14) ITT: AUC(1-4): 1.02 (0.94, 1.11) AUC(13-26): 1.05 (0.97, 1.18)
	AUC inf	AUC 0-∞: 1.04 (0.93, 1.17) AUC 0-∞: 1.00 (0.89, 1.13) [a]	AUC (0-inf): 1.064 (0.968, 1.169) Sensitivity analysis: 1.054 (0.965, 1.151)	**CT-P10 3.2:** AUC 0-inf: 0.90 (0.81, 0.99) AUC 0-inf: 0.99 (0.90, 1.09) [a]	**MabionCD20-001RA:** AUC(0-inf): PK-PPS: 1.02 (0.93, 1.11)
	Observation period (until last PK sample)	25 weeks	24 weeks	**CT-P10 1.1:** 24 weeks (for 3°EP up to 72 weeks)	**MabionCD20-001RA:** 24 weeks **MabionCD20-002NHL:** 26-Week double-blind treatment period and a follow-up period up to Week 46
	Supportive studies	Pop PK analysis in oncology indication	**Yes** In oncology indication	**Yes** In oncology indication	-

[a] US reference product

Cmax, AUC last, AUC inf, is measured in ratio of Geometric Means (90%CI). Sample size: +/- means rounded to the nearest 100.

For all rituximab biosimilars, PK studies were performed in patients with RA with supportive PK data from oncology patients as part of the efficacy studies. With regard to the withdrawn rituximab application, a comparative efficacy study (in RA) that included PK similarity as a secondary objective was conducted prior to a dedicated comparative PK study (in non-Hodgkin's lymphoma (NHL)) (99). Length of follow-up ranged from 24 to 25 weeks (26 weeks in case of the withdrawn application). Primary endpoints (AUCinf, Cmax and AUClast) were contained within the pre-specified acceptance range for all approved biosimilars and secondary endpoints supported biosimilarity. Although for the withdrawn rituximab the pre-defined equivalence margin was 70-143% in the PK

comparability study, the 90% CIs of the primary endpoints also met the standard equivalence margin of 0.8-1.25. As seen in **Table 15**, in two cases (87,88,108,109,116) results of a secondary endpoint were found outside of the standard acceptance limits, but deviations were seen as minor and not clinically relevant. Detailed information on the PK studies are available in **Table 11**.

Trastuzumab

Table 12. Main PK results of trastuzumab biosimilar candidates compared to reference product (published in respective EPARs or Withdrawal ARs).

		Ogivri (12/12/2018)	**Zercepac** (27/07/2020)	**Trazimera** (26/07/2018)	**Ontruzant** (15/11/2017)	**Herzuma** (09/02/2018)	**Kanjinti** (16/05/2018)	**Tuznue** (MAA withdrawn)
PK STUDY	Sample size (N), Randomisation	**MYL-Her-1001**: +/- 20 (1:1) **MYL-Her-1002:** +/- 100 (1:1:1 [a])	+/- 100 (1:1:1 [a])	+/- 100 (1:1:1 [a])	**SB3-G11-NHV:** +/- 100 (1:1:1 [a]) **SB3-G31-BC,** +/- 300 (1:1) in PK sub-study	**CT-P6 1.5:** +/- 70(1:1 [a]) **Pilot study CT-P6 1.4** +/- 70(1:1 [a])	+/- 200 (1:1:1 [a])	+/- 100 (1:1:1 [a])
	Indication	Healthy male subjects	Healthy Chinese male subjects	Healthy male adults	**SB3-G11-NHV:** in healthy male subjects **SB3-G31-BC:** early or locally advanced breast cancer	Healthy male subjects	Healthy male subjects	Healthy male subjects
	1°EP	**MYL-Her-1001:** Cmax, and AUC 0-∞. **MYL-Her-1002:** Cmax, AUC0-last, and AUC0-∞	AUC0-∞	AUCinf	**SB3-G11-NHV:** AUCinf, AUClast, Cmax **SB3-G31-BC:** Ctrough	**CT-P6 1.5:** AUCinf, AUClast, Cmax **CT-P6 1.4:** AUClast and Cmax	AUCinf and Cmax	AUC0-inf
	2°EP	**MYL-Her-1001:** T ½, CL, Vz, Vss **MYL-Her-1002:** T ½, Tmax	Cmax, AUC0-t, tmax, Vz, CL, and t1/2	Cmax, AUCt	**SB3-G11-NHV:** Vz, λz, CL, %AUCextrap, Tmax, and t1/2	**CT-P6 1.5:** %AUCext, Tmax Vz λz, t1/2, CL **CT-P6 1.4:** AUCinf, Tmax, Vz, λz, t1/2 and CL	AUClast	Cmax, AUC0-t, t½, CL and Vd
	Prespecified equivalence margins for 1º EP	0.80 to 1.25	0.80 to 1.25	0.80 to 1.25	0.80 to 1.25	0.80 to 1.25	0.80 to 1.25	0.80 to 1.25
	Cmax	**MYL-Her-1001** Cmax normalized: 0.92 (0.88, 0.97)	0.98 (0.92, 1.04) 0.92 (0.86, 0.99) [a]	Cmax within the pre-specified equivalence margin	**SB3-G11-NHV:** 1.00 (0.94; 1.07) 0.99 (0.92; 1.06) [a]	**CT-P6 1.5:** 0.97 (0.91, 1.03) 0.97 (0.91, 1.03) (new	0.99 (0.95, 1.03)	1.05 (0.98, 1.12) 0.99 (0.92, 1.06) [a]

		Ogivri (12/12/2018)	**Zercepac** (27/07/2020)	**Trazimera** (26/07/2018)	**Ontruzant** (15/11/2017)	**Herzuma** (09/02/2018)	**Kanjinti** (16/05/2018)	**Tuznue** (MAA withdrawn)
		Cmax native: 0.94 (0.90; 0.99) **MYL-Her-1002:** 1.04 (0.99, 1.10) 1.02 (0.96, 1.07) [a]			**SB3-G31-BC:** Ctrough at pre-dose of cycle 8 1.10 (1.02, 1.19)	ELISA method) **CT-P6 1.4:** 0.97 (0.89, 1.06)		
	AUC last	**MYL-Her-1001:** NA **MYL-Her-1002:** AUC0-last: 0.97 (0.91, 1.03) 0.96 (0.90 - 1.02) [a]	AUC 0-t: 0.86 (0.80, 0.91) 0.82 (0.83, 0.94) [a]	AUCt within the pre-specified equivalence margin	**SB3-G11-NHV:** 0.97 (0.91; 1.03) 0.93 (0.88; 0.99) [a]	**CT-P6 1.5:** AUC0-last: 0.99 (0.93, 1.06) 0.95 (0.89, 1.01) (new ELISA method) **CT-P6 1.4:** AUC0-last: 0.97. (0.89, 1.05)	1.00 (0.95, 1.06)	AUC 0-last: 1.04 (0.97, 1.11) 1.03 (0.96, 1.10) [a]
	AUC inf	**MYL-Her-1001:** AUC 0-∞ normalized: 0.94 (0.89, 0.99) AUC 0-∞ native: 0.96 (0.90, 1.01) **MYL-Her-1002:** AUC0-∞: 0.97 (0.91, 1.03) 0.96 (0.90, 1.02) [a]	AUC 0-inf: 0.86 (0.80, 0.91) 0.88 (0.83, 0.94) [a]	0.92 (0.86, 0.99)	**SB3-G11-NHV:** 0.97 (0.91; 1.03) 0.93 (0.87; 0.99) [a]	**CT-P6 1.5:** AUC0-inf: 0.99 (0.93, 1.06) 0.95 (0.89, 1.01) (new ELISA method) **CT-P6 1.4:** AUCinf similar to reference group	1.00 (0.95, 1.06)	AUC 0-inf: 1.02 (0.96, 1.09) 1.03 (0.96, 1.10) [a]
	Observation period (until last PK sample)	**MYL-Her-1001:** 99 days **MYL-Her-1002:** 71 day	57 days	NP	**SB3-G11-NHV:** 1344 hrs = 56 days **SB3-G31-BC**: 8 cycles every 3 weeks (Ctrough at pre-dose of cycle 1, 3, 5, 7 and 8)	**CT-P6 1.5:** 71 days **CT-P6 1.4:** 42 days	NP	53 days

For all trastuzumab biosimilar candidates, PK studies were performed in healthy subjects with supportive PK data obtained in clinical trials in oncology patients. For the withdrawn trastuzumab application, an additional PK similarity study in healthy subjects was submitted. Length of follow-up of the PK studies ranged from 56 to 99 days (53 days in case of the withdrawn application). In all

cases, the primary endpoints (AUCinf, Cmax and AUClast) were contained within the pre-specified acceptance range and secondary endpoints supported biosimilarity. Detailed information on the PK studies are available in **Table 12**.

Population PK

PopPK was performed for some products, using different approaches(89,93–95,104). Absence of PopPK analysis was accepted where PK similarity had been demonstrated in the dedicated PK study and PopPK was seen as supportive in the other cases.

Clinical efficacy studies

Rituximab

Table 13. Main efficacy results of rituximab biosimilar candidates compared to RP (published in respective EPARs or Withdrawal ARs).

		Ruxience (01/04/2020)	**Rixathon** (15/06/2017) / **Riximyo** (15/06/2017)	**Blitzima** (13/07/2017)/ **Truxima** (17/02/2017)/ **Ritemvia** (no longer authorised)		**Rexeful/MabionCD20 (Mabion)** (MAA withdrawn)
Efficacy Study	Sample size (N) Randomization	+/-400 (1:1)	+/-600 (1:1)	+/-400 (1:2.5:1-IP:RP:RP [a])	+/-200 (1:1 [a])	+/-600 (1:1)
	Indication	LTB-FL	FL	RA	FL (supportive, non-inferiority of efficacy)	RA
	Efficacy 1°EP	ORR (CR or PR) at w26	ORR (CR or PR) during the combination treatment phase (w24)	Change from baseline in DAS28 (CRP) at w24	ORR (CR + CRu +PR) during/over 24w	ACR20 at w24
	Efficacy 2° EP	CR at w26, PR at w26, PFS, OS, TTF, DOR	BOR, PFS, OS, CR, PR, DAS28 (CRP) at w24, ACR20 Rr at w24	DAS28 (CRP) and DAS28 (ESR) at w4, w8, w12, w16, w20, w24, w32, w40, w48; ACR20/50/70 at w4,	Bone marrow assessments and B-symptoms assessments	ACR20 response at w4, w8, w12, w16, w20, w48; ACR50 and ACR70 response at w4, w8, w12, w16, w20, w24, w48;

		Ruxience (01/04/2020)	**Rixathon** (15/06/2017) / **Riximyo** (15/06/2017)	**Blitzima** (13/07/2017)/ **Truxima** (17/02/2017)/ **Ritemvia** (no longer authorised)		**Rexeful/MabionCD20 (Mabion)** (MAA withdrawn)
				w8, w12, w16, w20, w24, w32, w40, w48		DAS28-ESR from baseline to w24; EULAR response at w24
	Prespecified equivalence margins for 1°EP	+/- 16% (FDA, EMA) (additional analysis: +/- 14.9% PMDA)	+/- 12%	pre-defined EQM of 0.6 ± 0.60	pre-defined non-inferiority margin using an absolute point estimate difference of -7% based on reference product variability	+/- 13%
	Primary analyses population for 1ºEP	ITT	PPS	efficacy population	PPS, ITT	PPS
	Results 1ºEP (95% CI); **ITT population**	RD ORR: 4.66 (-4.16, 13.47)	RD OR: -0.44 (-5,95, 5.07)	DAS28 (CRP): -0.05 (-0.29, 0.20) DAS28 (ESR): -0.06 (-0.31, 0.20)	only preliminary data ORR over 24w: 5.7%	RD ACR20: -5.1 (-11.3, 1.1)
	Results 1ºEP (95% CI); **PPS population**	RD ORR: 7.49 (-0.67, 15.80)	RD ORR -0.40 (-5.94, 5.14)	DAS28 (CRP): -0.05 (-0.29, 0.20) DAS28 (ESR): -0.06 (-0.31, 0.19)	ORR over 24w: 4.3%	RD ACR20: -5.7 (-12, 0.5)
	Results 2º EP	At week 26 CR (ITT): -2.31 (-11.09, 6.50); PR (ITT): 6.975 (-2,91, 16.71); CR (PPS): -3.28 (-12.85, 6.40); PR (PPS): 10.77 (0.13, 21.17); At week 52 ORR (ITT): 0.84 (-10.38, 8.71); CR (ITT): 0.05 (-9.07; 9,18); PR (ITT): -0.89 (-10.02, 8.24)	FAS, 90%-CI PFS Hazard Ratio: 1.33 (0.98, 1.80) OS: 1.03 (0.59, 1.80) The median PFS and OS was not reached	similar	similar	At week 24 ACR50 (PPS): -2.7% (-10.7%, 5.5%) ACR70 (PPS): -1.3% (-6.6%, 4.0%) DAS28-ESR (PPS): 0.05% (-0.077%, 0.184%) EULAR (PPS): 0.2% (-4.0, 4.4%) Equivalence margin: N/A

		Ruxience (01/04/2020)	**Rixathon** (15/06/2017) / **Riximyo** (15/06/2017)	**Blitzima** (13/07/2017)/ **Truxima** (17/02/2017)/ **Ritemvia** (no longer authorised)		**Rexeful/MabionCD20 (Mabion)** (MAA withdrawn)
		After w52 follow-up: PFS (ITT): hazard ration 1.393 (0.847, 2.291) PFS (PPS): hazard ration 1.381 (0.835, 2.284)				
	Switch (yes/no). If yes, timepoint of switch	No	No	Yes (patients having received RP in main study either stayed on RP or received IP; patients receiving RP in main study switched to IP)		Yes (open-label period, switch is possible)
	Observation period	Treatment phase: 26w Extension phase: 26w (total 52w)	Treatment phase: 24w Maintenance and/or follow up phase: 2.5 years (total up to 3 years)	Treatment phase: up to 48w Extension phase: w48/w52 - w76 (total up to 76w)	Treatment phase: 8 cycles Maintenance phase: 12 cycles Follow-up up to 3 years	Double-blind period: 24w Open-label period: 48w (Patients who experienced a clinical relapse at w24 and residual disease activity received a second open-label course)

Rituximab is currently approved in seven indications, both autoimmune and oncological (117). Two applicants, including the one for the withdrawn rituximab MAA (88,99,108,116), chose to compare efficacy in RA subjects as a model indication and the remaining two (87,89,109) chose follicular lymphoma (FL). Length of follow-up was up to three years. ORR was chosen as primary endpoint in FL and DAS28 or ACR 20 for RA. Detailed information on the efficacy studies are available in **Table 13**.

Trastuzumab

Table 14. Main efficacy results of trastuzumab biosimilar candidates compared to reference product (published in respective EPARs or Withdrawal ARs)

		Ogivri (12/12/2018)	**Zercepac** (27/07/2020)	**Trazimera** (26/07/2018)	**Ontruzant** (15/11/2017)	**Herzuma** (09/02/2018)	**Kanjinti** (16/05/2018)	**Tuznue** (MAA withdrawn)
Efficacy study	Sample size (N) Randomisation	+/-500 (1:1)	+/-600 (1:1)	+/-700 (1:1)	+/-800 (1:1)	+/-500 (1:1[a])	+/-700 (1:1)	+/-500
	Indication	MBC	MBC	MBC	EBC or LABC	EBC	EBC	EBC and LABC
	Efficacy 1°EP	Best ORR ratio (CR or PR) at w24, best ORR difference at w24	ORR up to w24 (best response of CR or PR from first assessment up to w24)	ORR (CR or PR) by w25 ±14d and confirmed on a follow-up assessment (w33 ± 14 days)	bpCR after completion of 8 cycles of neoadjuvant therapy	pCR determined following surgery (total pCR instead of bpCR)	Co-primary efficacy endpoints: RD of pCR in breast tissue and axillary lmph nodes (regardless of DCIS) RR of pCR in breast tissue and axillary lmph nodes (regardless of DCIS)	tpCR at the time of surgery
	Efficacy 2° EP	TTP, PFS, OS, DR	ORR (at w6, w12, w18 and w24), CBR, DCR, DOR, 1year PFS rate, 1/2/3 year OS rate	1-year PFS rate, DOR, 1-year survival rate	tpCR, ORR (CR or PR), best ORR, EFS, OS	ORR (BOR of CR or PR), DFS, PFS, OS, BCR, other pCRs (pCR of breast + axillary nodes with absence of DCIS, pCR of breast only (pCRB))	RD of pCR (breast tissue), RR pf pCR (breast tissue), RD of pCR in breast tissue and axillary lymph nodes and absence of DCIS, RR of pCR in breast tissue and axillary lymph nodes and absence of DCIS	bpCR, ORR (CR or PR), EFS, OS
	Prespecified equivalence margin for 1°EP	RR 0.81, 1.24 (2-sided 90% CI) sensitivity analysis: RD +/-15%° (2-sided 95% CI)	RD +/-13.5%	RR 0.80 to 1.25 RD +/- 13%	RD +/-13% RR 0.785, 1.546	RD +/-15%	RD (90 % CI) +/- 13% If auccesfull, RR (90 % CI) 0.7586, 1.3182 (=1/0.7586)	RD +/-15%
	Primary analyses population for 1ºEP	ITT	ITT	ITT	PPS	PPS	ITT	PPS

		Ogivri (12/12/2018)	**Zercepac** (27/07/2020)	**Trazimera** (26/07/2018)	**Ontruzant** (15/11/2017)	**Herzuma** (09/02/2018)	**Kanjinti** (16/05/2018)	**Tuznue** (MAA withdrawn)
	Results 1ºEP (95% CI); ITT population	RR Best ORR (ITT1): 1.09 (90% CI) (0.974, 1.211) (95% CI) (0.954, 1.237)	RD (1st interim analysis) ORR: -0.4 (-7.4, 6.6) RD (2nd interim analysis) ORR: -0.1 (-7.0, 6.9) RR ORR (90% CI): 0.999 (0.920, 1.084)	RR ORR: 0.94 (0.842, 1.049) RD CR: -0.821 (-3659, 1.929) PR: -3.158 (-10.312, 4.025) ORR: -3.979 (-11.005, 3.080)	RD bpCR (FAS): 9.86 (3.41, 16.31)	pCR after neoadjuvant therapy: -3.58 (-11.98, 4.80)	pCR in breast tissue and axillary lymph nodes (regardless of DCIS) RD (95% CI): 7.3 (0.0, 14.6) RR: 1.1877 (1.0054, 1.4031) pCR in breast tissue and axillary lymph nodes excluding subjects receiving IP lots with low ADCC (quant. by NK92 assay) (≤60%): pCR evaluation population, local lab RD: 7.3 (-0.2, 14.7) RR: 1.1879 (1.0028, 1.4073) PP population, local lab RD: 6.3 (-1.2, 13.8) RR: 1.161 (0.9776, 1.3788) pCR evaluable population, central lab RD: 5.9 (-1.6, 13.5) RR: 1.1465 (0.9678, 1.3583) PP population, central lab RD: 4.2 (-3.5, 11.8) RR: 1.101 (0.9281, 1.3062) pCR in breast tissue and axillary lymph nodes adjusting for subjects exposed to IP with low ADCC (≤65%) (quant. by PBMC assay):	RD tpCR: 1.4 (-7.7, 10.4)

		Ogivri (12/12/2018)	**Zercepac** (27/07/2020)	**Trazimera** (26/07/2018)	**Ontruzant** (15/11/2017)	**Herzuma** (09/02/2018)	**Kanjinti** (16/05/2018)	**Tuznue** (MAA withdrawn)
							pCR evaluable population, local lab RD: 4.4 (-3.4, 12.3) pCR evaluable population, central lab RD: 3.5 (-4.5, 11.5)	
	Results 1ºEP (95% CI); PPS population	RD Best ORR: 4 (-4.59, 12.61)	RD ORR: 0.8 (-6.2, 7.7)	RD ORR: -3.315 (-10.656, 4.039)	RD bpCR: 10.7 (4.13, 17.26)	RD pCR: -3.62 (-12.38, 5.16)		RD tpCR: 0.5 (-8.6, 9.6) analysis excluding specific sites, RD tpCR:0.6 (-8.6, 9.8)
	Results 2º EP	<u>ITT1, at w24 (PART1)</u> TTP HR (unstratified): 0.74 (0.477, 1.161) TTP (stratified): 0.70 (0.448, 1.106) PFS HR (unstratified): 0.80 (0.529, 1.218) PFS HR (stratified): 0.75 (0.488, 1.143) OS HR (unstratified): 0.68 (0.261, 1.799) OS HR (stratified): 0.57 (0.208, 1.584) <u>ITT1, at w48 (PART2)</u> ORR RD: 3.3 (-5.16, 11.77) TTP HR	<u>ITT (1st interim analysis)</u> RD ORR at w6: 2.6 (-5.05, 10.2) ORR at w12: 1.1 (-6.5, 8.7) ORR at w18: 1.7 (-5.8, 9.3) ORR at w24: 5.7 (-1.9, 13.3) <u>ITT (1st interim analysis)</u> RD CBR: 0.2 (-5.9, 6.4) DCR: -3.4 (-8.8, 1.9) DOR HR: 0.75 (0.55, 1.04) DOR HR (stratified): 0.75 (0.54, 1.04) PFS HR: 0.83	Analysis for 2º EP include radiology data up to w53 and data up to 378d post-randomization Derived from central radiology assessment, ITT PFS HR: 1.00 (0.80, 1.26) OS HR: 1.004 (0.655, 1.539) DOR: 0.92 (0.67, 1.27)	PPS: tpCR: 11.05 (4.44, 17.66) ORR: 5.03 (1.74, 8.31) OS HR: 0.00 (0.00, -) EFS HR: 0.86 (0.50, 1.49) FAS: tpCR: 10.23 (3.73, 16.73) ORR: 3.68 (0.32, 7.03) OS HR: 0.23 (0.03, 1.97) EFS HR: 0.94 (0.59, 1.51) FAS non-responder analysis	pCR of breast and axillary nodes with absence of DCIS, PPS -1.49 (-10.22, 7.31) pCR of breast and axillary nodes with absence of DCIS, ITT -1.58 (-9.96, 6.85)	pCR (breast only), local lab eval. RD 6.0 (-1.3, 13.4) RR 1.1463 (0.9835, 1.336) pCR (breast only), central lab eval. RD 4.1 (-3.3, 11.5) RR 1.1019 (0.9492, 1.2791) pCR (no DCIS) RD 8.0 (1.0, 15.0) RR 1.2746 (1.0316, 1.5748) pCR in breast tissue only excluding subjects receiving IP lots with low ADCC (≤60%):	RD tpCR (PPS) before re-monitoring: 0.5 (-8.6, 9.6) after re-monitoring: - 3.8 (-12.8, 5.4) RD bpCR (PPS) before re-monitoring: 1.7 (-7.5, 10.7) after re-monitoring: - 0.9 (-10, 8.2) RD bpCR (mFAS): 2.3 (-6.7, 11.4) RR bpCR (PPS) before re-monitoring: 1.031 (0.874, 1.217)

		Ogivri (12/12/2018)	**Zercepac** (27/07/2020)	**Trazimera** (26/07/2018)	**Ontruzant** (15/11/2017)	**Herzuma** (09/02/2018)	**Kanjinti** (16/05/2018)	**Tuznue** (MAA withdrawn)
		(unstratified): 0.94 (0.712, 1.254) TTP HR (stratified): 0.92 (0.692, 1.231) PFS HR (unstratified): 0.97 (0.740, 1.282) PFS HR (stratified): 0.95 (0.714, 1.251) OS HR (unstratified): 0.67 (0.402, 1.129) OS HR (stratified): 0.61 (0.360, 1.039) DR HR (unstratified): 0.96 (0.705, 1.306) DR HR (stratified): 0.97 (0.706, 1.329) <u>PPS, at w48</u> RD ORR: 1.7 (-6.77, 10.18)	(0.65, 1.06) PFS HR (stratified): 0.80 (0.63, 1.03) OS HR: 0.80 (0.48, 1.34) OS HR (stratified): 0.79 (0.47, 1.34) <u>ITT (2nd interim analysis)</u> RD CBR: 0.2 (-5.8, 6.3) DCR: -3.1 (-8.4, 2.2) DOR HR: 0.80 (0.61, 1.06) DOR HR (stratified): 0.79 (0.60, 1.05) PFS HR: 0.84 (0.68, 1.04) PFS HR (stratified): 0.83 (0.67, 1.03) OS HR: 0.87 (0.61, 1.24) OS HR (stratified): 0.85 (0.60, 1.22)		tpCR: 9.32 (3.19, 15.46) ORR: 5.94 (2.17, 9.71)		pCR evaluable population, local lab RD: 6.1 (-1.4, 13.5) RR: 1.1482 (0.9823, 1.3421) PP population, local lab RD: 5.0 (-2.6, 12.5) RR: 1.1198 (0.9561, 1.3114) pCR evaluable population, central lab RD: 3.9 (-3.6, 11.5) RR: 1.0981 (0.9432, 1.2784) PP population, central lab RD: 1.9 (-5.8, 9.5) RR: 1.0513 (0.9018, 1.2256)	At end of neoadjuvant phase PPS ORR RD: 1.3 (-7.5, 10.5) ORR RR: 1.015 (0.956, 1.078) At end of neoadjuvant phase mFAS ORR RD: 0.7 (-8.1, 9.3) ORR RR:1.008 (0.943, 1.078)
	Switch (yes/no). If yes, timepoint of switch	No	No	No	No	No	Yes, subjects receiving RP in neoadjuvant phase were randomized to either continue or switch to IP during adjuvant phase	No
	Observation period	Treatment phase: 24w (PART1) Maintenance phase: 24w (PART 2, single treatment) for those with CR, PR. Those with SD continued with combination of	Main phase: 24w (8 cycles) and continued as monotherapy for up to 17 cycles (about 12 months) Safety follow-up: 30d post-treatment Survival follow-up: 24	Main phase: 24w (8 cycles, PART1) Trastuzumab+Pacliataxel until at least w33 (weekly), after w33 regimen could be changed to every 3w. Survival follow-up: until	Main Phase: 54w (administered every 3 weeks for a total of 18 cycles (8 cycles of neoadjuvant therapy (docetaxel for 4 cycles followed by 4 cycles FEC) (24w) then	Neoadjuvant phase: 24w (8 cycles of neoadjuvant therapy (docetaxel for 4 cycles followed by 4 cycles FEC) then surgery Adjuvant phase:	Run in: Chemotherapy (epirubicin, cyclophosphamide) Neoadjuvant phase: 12w (4 cycles, every 3w) + Paclitaxel Surgery Adjuvant phase: for up to 1	Neoadjuvant phase: (8 cycles of neoadjuvant therapy (docetaxel for 4 cycles followed by 4 cycles EC) Surgery Adjuvant phase:

		Ogivri (12/12/2018)	**Zercepac** (27/07/2020)	**Trazimera** (26/07/2018)	**Ontruzant** (15/11/2017)	**Herzuma** (09/02/2018)	**Kanjinti** (16/05/2018)	**Tuznue** (MAA withdrawn)
		trastuzumab +Taxana	months	death or 1y from randomization and at least 6m following last study drug receipt	surgery and subsequently 10 cycles of adjuvant therapy (30w)) Extension phase: 4w (safety follow-up)	subsequently 10 cycles (every 3w) of adjuvant therapy (30w) for up to one year from 1st day of drug administration in neoadjuvant period total of 18 cycles/1year Follow-Up: up to 3 years from last enrolled date	year from 1st day of IMP administration in neoadjuvant phase (every 3w) Extension Phase: up to 1 year	subsequent up to 10 cycles Total 18 cycles

Trastuzumab is currently approved in three indications (118). For three biosimilars (93–95) metastatic breast cancer (MBC) was chosen as model indication in the pivotal clinical trial and for the remaining four, including the withdrawn MAA (92,100,103,104), early breast cancer (EBC) was used. Length of follow-up was up to three years.

Three applicants chose ORR and the remaining four pathologic complete response (pCR) as the primary endpoint. Pre-specified equivalence margins for RD varied even though patient populations were the same as different reference studies were used for clinical and statistical justifications (93,95,103,104). Detailed information on the efficacy studies is available in **Table 14**.

Table 15 shows those instances where some differences were found and how the remaining uncertainties were resolved. For two products (103,104), the 95% CI of the difference in the pCR rates between treatments was not fully contained within the pre-defined equivalence margin, thus superiority of the biosimilar cannot be excluded.

Safety and Immunogenicity

The overall safety and immunogenicity profiles were compared descriptively and appeared similar between the biosimilars and the RP, as reviewed in detail by Kurki et al. 2021 (68).

With regard to the withdrawn rituximab biosimilar candidate application (99), the overall safety profile appeared to be similar in patients with RA but imbalances in AEs, SAEs, severity and deaths were observed in the comparative PK study in patients with NHL. Eight patients died in the product arm versus none in the reference arm: investigators assessed the causal relationship as not (6/8) or unlikely (2/8) related to study drug for all fatal SAEs.

Table 15 provides a summary on all the uncertainties in clinical data, and how these were resolved.

Table 15. Discrepancies in clinical attributes and how the resulting uncertainty during MAA was resolved.

Rituximab	**Clinical attribute**	**Observation**	**How resolved**
Rixathon/ Riximyo	PK	Cmax1 slightly exceeded the upper limit (i.e. 126%)	1. Primary endpoint (AUC) met 2. Cmax2 was within the standard acceptance limits and therefore, the criterion of bioequivalence was met
Truxima/ Blitzima/ Ritemvia	PK	AUCt-inf outside of acceptance limits	1. Primary endpoints (AUC0-last, AUC0-inf, Cmax) met 2. Deviation seen as minor, all other secondary endpoints met standard acceptance limits
Rixathon/ Riximyo	PFS	HR of 1.33 (0.98, 1.80)	1. Primary endpoint (ORR) met 2. Study not designed to demonstrate equivalence for PFS 3. Conclusion of biosimilarity based on overall biosimilarity assessment
Rixathon/ Riximyo	OS	HR of 1.03 (0.59, 1.80)	1. Primary endpoint (ORR) met 2. Study not designed to demonstrate equivalence for PFS 3. Conclusion of biosimilarity based on the overall biosimilarity assessment
Trastuzumab	**Clinical attribute**	**Observation**	**How resolved**
Ontruzant	bpCR (RD)	95% CI not fully contained within prespecified equivalence margin	1. Justified by confounding effect of ADCC shift in reference lots 2. Conclusion of biosimilarity based on the overall biosimilarity assessment
Kanjinti	pCR in breast tissue and axilary lymph nodes (RD and RR)	95% CI not fully contained within prespecified equivalence margin	1.Justified by confounding effect of ADCC shift in reference lots 2. Additional functional analysis for ADCC performed 3. Additional analysis adjusting for subjects exposed to IMP with low ADCC 4. Conclusion of biosimilarity based on overall biosimilarity assessment
Kanjinti	pCR (only breast) (RD and RR)- except RD in PP	95% CI not fully contained within prespecified equivalence margin	1.Justified by confounding effect of ADCC shift in reference lots 2. Additional functional analysis for ADCC performed 3. Additional analysis adjusting for subjects exposed to IMP with low ADCC 4. Conclusion of biosimilarity based on overall biosimilarity assessment

Zercepac	DOR, PFS, OS	Seemingly better efficacy (HR<1)	1. Study not designed to demonstrate equivalence for PFS 2. No significant differences found in second interim analysis 3. Conclusion of biosimilarity based overall biosimilarity assessment
Withdrawn rituximab biosimilar candidate	Deaths	Eight patients died in the product arm versus none in the reference arm	1. Chance finding likely 2. Study not designed to evaluate hard clinical endpoints

3.2.5. DISCUSSION

When considering any change in the current requirements for comparative efficacy studies for biosimilar mAb and fusion protein developments, a fundamental concern of stakeholders including patients, physicians and regulators is that biosimilar product candidates with demonstrated analytical/functional comparability could nevertheless translate into failed clinical comparability. The concern is that in the absence of such clinical trial data, a biosimilar might be inappropriately approved based on quality data only.

Our study shows that this concern is not supported by data and that regulatory decision-making follows a totality of the evidence approach with the main focus on the pharmaceutical quality/CMC (biosimilarity, general quality) and PK similarity aspects. In our opinion, a clinical efficacy study may not need to be routinely requested. In the following parts, we discuss evidence obtained from different analyses performed and its implications.

3.2.5.1. Discussion of MAA Evidence and Results

We analysed the MAA reviews of approved biosimilar mAbs and fusion proteins performed by the EMA CHMP and found that in most cases (29/36 cases) good quality/CMC packages were matched with successful clinical trials leading to MA. Interestingly, good quality/CMC packages could also be paired with formally failed efficacy studies, which were evaluated to be due to reasons not related to the biosimilar candidate, thus permitting MA (see discussion of analysis of clinical comparability for rituximab and trastuzumab biosimilars including withdrawn biosimilar candidates below).

On the contrary, unconvincing quality/CMC data paired with successful clinical trials precluded MA, primarily due to the lack of demonstration of sufficient pharmaceutical quality and/or analytical/functional similarity with the RP (99,100).

In our analysis, there was only one case where clinical data analysis led to a MO with a divergent position published by the CHMP (82) which nevertheless received MA.

The issue was later resolved by more mature follow-up data, which did not confirm the objections regarding potentially increased immunogenicity of the biosimilar (119).

For three of the biosimilar candidates analysed (43,101,102) PK studies were deemed insufficient, which led to the conduct of a new, acceptable PK trial. The reasons for repeating the PK trials were due to methodological issues and differences in the formulation buffer (29) or because of representativeness of the test product used (102). For two biosimilar candidates the PK data submitted in the initial MAA raised major questions regarding biosimilarity. However, re-analysis of the data, which was already pre-specified in the statistical analysis plan, led to the conclusion that PK similarity was shown (44,85). Taking this into account, our analysis indicates that, where biosimilarity was shown at the quality/CMC level, this always translated into PK similarity of the biosimilar candidates with the RP.

3.2.5.2. Discussion of analysis of first regulatory assessment reports

We investigated whether the MAA outcome could have been predicted based on the evidence generated solely in the quality dossier. We found that the quality and clinical assessments aligned in 67% of cases i.e., both quality and clinical data packages were considered sufficient to support a MA, or both the quality and clinical data packages were not accepted. In 11% of cases, MO were identified in quality parts of the submission, whereas the clinical data supported biosimilarity.

Of particular interest are those 22% of cases (11% E/S/I only) where no MO were observed in the quality part, but MO were raised on the clinical data. Without further regulatory deliberation and additional justifications from the applicants, these cases could have resulted in false negative conclusions, i.e., a true biosimilar being rejected due to issues with clinical studies. However, even in those cases where the efficacy trials formally failed, biosimilarity was ultimately accepted by EMA based on the demonstration of analytical/functional comparability and comparable PK profiles. In each instance, identified issues in the clinical package were eventually accepted as the result of unanticipated problems such as imbalances in trial arms, immaturity of secondary endpoint data at the time of MAA submission, changes in the QA of the RP

or even chance findings. In some cases, a further in-depth sensitivity analysis improved the understanding of the clinical data and facilitated a positive conclusion. These cases highlight that for biosimilar mAbs and fusion proteins, the analytical and functional characterisation data are the most critical for decision making and regulatory approval.

In summary, our analyses of MAAs and first regulatory assessment reports show that the quality/CMC part of the dossier is predictive for the MA of a biosimilar candidate.

3.2.5.3. Discussion of analysis of analytical biosimilarity for rituximab and trastuzumab biosimilars

Since our analyses included the two withdrawn MAs, we reviewed to what degree thorough analysis of the quality/CMC package could have been predictive for the clinical outcome. These two substance classes are particularly interesting, as the two originator mAbs are used in oncology (rituximab, trastuzumab) and/or in autoimmune indications (rituximab). Based on information provided in the MAA, and following the scientific evaluation carried out by EMA CHMP, we found that over 90% (and in most cases 100%) of the biosimilar batches met the EU-RP similarity range for CQAs such as protein content, biological activity (CD20 binding and apoptosis induction for rituximab and HER2-binding and inhibition of cellular proliferation assay for trastuzumab), FcγRIIIa binding, FcRn and C1q binding, ADCC and CDC.

A lower percentage of biosimilar batches were within the similarity range for QAs which are considered less critical (glycosylation profile, charge variants or additional assays). Furthermore, as seen in **Table 10**, in all instances where uncertainties were raised, these were resolved considering the close similarity demonstrated in CQAs, the results of PK studies and overall, the totality of the evidence.

In no case were clinical trial data necessary to resolve residual uncertainties regarding the quality part. This is in line with previous findings(28,81) and further demonstrates that the array of orthogonal methods that are submitted in the quality/CMC package are robust and predictive of clinical outcome.

3.2.5.4. Discussion of analysis of clinical comparability for rituximab and trastuzumab biosimilars including withdrawn biosimilar candidates

For all rituximab and trastuzumab biosimilar candidates studied, a comprehensive clinical programme was submitted according to the relevant EMA guidelines, i.e. consisting of at least one PK study and a clinical efficacy study, which confirmed biosimilarity in all but two instances (92–95,100,103,104).

For all rituximab biosimilars included in the analysis, PK results were obtained in one therapeutic area and confirmed in a subset of patients in the second therapeutic indication either by supportive PK analysis (87,88,99,108,109,116) or by PopPK analyses (89). In no instance were deviating results observed. One applicant (87) removed PopPK in a protocol amendment which was acceptable to the regulators. In our opinion, this redundancy of PK evaluations should no longer be necessary, as besides CD20 no other antigen or target is involved in rituximab's binding and MoA. This has been demonstrated by the tissue specificity in several human tissue cross reactivity studies (117,120).

In our study, in all cases except for the withdrawn applications, remaining uncertainties regarding clinical data were resolved based on a strong quality/CMC package, together with demonstrated PK similarity and considering that the studies were not powered to demonstrate similarity with regard to secondary endpoints. Moreover, regarding safety, it should be noted that clinical trials are not powered for safety endpoints, since this is considered unnecessary according to EMA guidelines and would usually require several thousand study participants.

Therefore, the value of extensive analyses of secondary endpoints in efficacy trials remains questionable, as they were either viewed as inconclusive (88,108,116), or immature (87,109).

For both trastuzumab cases (103,104), EMA concluded that it was likely that the apparent difference was caused by a noted downward "shift" in ADCC activity in some of the RP batches and did not preclude approval. These findings have been discussed in the literature (28,121,122) and also demonstrate that physicochemical methods and functional assays are able to detect differences in functional attributes with predictive

character. With regards to the withdrawn trastuzumab biosimilar (100), a conclusion on biosimilarity from a safety point of view was precluded as the clinical batches were not considered representative for the commercial product.

Several authors (25,28–31) pointed out limitations of indiscriminative clinical efficacy studies in light of technical advances in analytical methods, which provide more discriminative research tools sparing patients from entering unnecessary and redundant clinical trials.

It is known from the literature that the recommended doses of many mAbs are in the flat part of the dose-response curve, e.g., half of the administered dose of rituximab would result in the same clinical outcome, suggesting the overall equivalence of 2 x 500 mg with the licensed dose of 2 x 1000 mg for clinical efficacy outcomes (123), thus rendering clinical trials insensitive tools to assess biosimilarity. Some authors argue that treatment response to rituximab in RA is only determined by the level of B-cell depletion, regardless of how it is achieved (123–126), yet it is important to measure binding and all functional activity. In our analysis we found that rituximab binding to the CD20 receptor plus all four important MoAs of rituximab (111) were adequately measured by appropriate analytical testing and that for the successful quality/CMC dossiers >90% of batches met the required similarity ranges.

3.2.5.5. General discussion on current flexibility in clinical trial requirements

Our analysis revealed that there is regulatory flexibility in the acceptability of clinical data packages. This is, for example, evidenced by the acceptance of different primary endpoints such as DAS28 vs. ACR20 in RA trials, different methods of analyses such as RD vs. risk ratio (RR), or different equivalence margins for the same study population depending on the reference study chosen, provided appropriate scientific justification is given (48,93,94,103).

Currently, most mAb developers are planning to evaluate S/I over one year, as advised by guidelines. However, the primary efficacy analysis is usually performed at a much earlier timepoint (87–89,93–95). Based on our analyses of the submitted clinical trial data, as well as on the raw data analysis of Kurki P. et al. (68), it is clear that most dossiers are submitted with preliminary 4-6 month S/I data, depending on the timepoint of primary efficacy analysis, while the full one-year dataset is submitted later during the evaluation period. In no instance have the conclusions reached after the initial data submission changed after completion of the study after 12 months, suggesting that such long trials are unnecessary. Other authors have concluded that pertinent comparative safety and immunogenicity data will be obtained from the PK trials, which in all instances were shown to support results of the E/S study (68).

We conclude that a sufficiently robust analytical/functional similarity package, together with a PK trial capturing data on safety and immunogenicity would be sufficient for the purpose of regulatory decision making for biosimilar mAbs and fusion proteins. In some case, if it were deemed necessary (e.g., when the MoA of the biologic is poorly understood), a shorter efficacy trial ending at or near the timepoint of primary efficacy analysis could provide additional safety and immunogenicity information.

3.2.5.6. Limitation of the study

Since our study was limited to mAb and fusion protein biosimilars of IgG class, our conclusions may not be applicable to more complex biologics. Furthermore, our analysis is restricted to products that have undergone regulatory assessment and appraisal. Whether there have been biosimilars that failed quality/CMC and/or clinical development and were therefore never submitted for regulatory evaluation or publication has not been scrutinised in our study. Also, overall the sample size of 36 was limited by the available submissions to EMA in the previous 10 years. However, to our knowledge this is the largest set of biosimilar marketing authorisations analysed to date.

3.2.6. CONCLUSIONS

The results of our analysis show that a comprehensive and convincing quality/CMC package demonstrating high analytical/functional similarity of the biosimilar with the RP is essential for MA. Since the first approval of less complex biosimilars, the analytical techniques have advanced markedly resulting in very sensitive assays for the structural and functional characterisation of even complex mAb molecules.

The concern, that in the absence of comparative efficacy and safety results, a biosimilar candidate might be inappropriately approved based on quality data only, is not supported by our findings. The analytical and biological results can be considered predictive for the clinical performance of the biosimilar candidates.

Based on the combination of modern analytics, control and pharmacovigilance systems in place, as well as requirements on comparability assessment in case of manufacturing changes, clinical performance of IgG biopharmaceuticals is ensured throughout the lifecycle of the product. As shown in our analysis, the CQA that are known to impact clinical efficacy and safety, including immunogenicity, must be closely monitored.

In the authors' opinion, these findings allow a reduction of the clinical development programme for regulatory review before MA. This conclusion is further supported by the positive experience in the market gained for biosimilar mAbs approved in the last ten years. Consequently, a revision of the respective regulatory biosimilars guidelines in Europe should be considered, in order to allow a more rational use of clinical resources and improve the access to innovative and affordable medicines for patients.

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Availability of data and material: The datasets analysed for clinical comparability during the current study are available in the EMA repository, which are publicly available at: https://www.ema.europa.eu/en/medicines/field_ema_web_categories%253Aname_field/Human. The datasets generated during and/or analysed during the current study for analytical biosimilarity, MAAs and regulatory assessment reports are not publicly available due to confidentiality issues.

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E.G. contributed equally to the manuscript. All authors read and approved the final manuscript.

Discussion

04

The limitation of patient access to biological medicines, including the challenges in biosimilar development and uptake, are becoming a concerning issue world-wide.

This thesis aims to provide deeper understanding on the current evaluation process of complex biosimilars (mAb and fusion proteins) and to analyse to what extent clinical efficacy data is relevant for the final conclusion on biosimilarity. To do so, an in-depth analysis of the MAA and the role of analytical/functional and clinical data for the conclusion on biosimilarity for a number of complex biosimilars has been performed.

In the following sections, the main conclusions and results are discussed.

4.1. ANALYTICAL BIOSIMILARITY

The analytical tools and functional methods routinely performed allow a comprehensive and in-depth characterization of mAb biosimilars.

In our analysis of 21 approved mAb biosimilars (excluding the 2 withdrawn applications), an exhaustive and thorough CMC/quality package was presented, which in combination with an adequately powered PK study, in all cases contained *per se* enough information for the final conclusion on biosimilarity. This reflects that analytical sciences and functional methods provide enough information to validate complex biosimilars' equivalence to reference biologics.

As seen in **Table 2** and **Table 3**, numerous orthogonal and state-of-the-art methods are routinely performed to analyse dozens of QAs. For example, in the analysis of adalimumab products (**Table 2**), there were applications where one sole QA could be analysed with up to 20 different assays. In line with other authors (69,70), this demonstrates that the current quality characterisation of complex biosimilars is comprehensive and robust, due to the development of numerous advanced and highly sensitive methods that can detect small differences in the attributes of both biosimilars and their RP.

Not only is this characterisation advanced and comprehensive for the comparability exercise, but also for general pharmaceutical quality. As seen in **Table 8** and **Figure 6**, the CMC/quality package must meet all regulatory requirements regarding general pharmaceutical quality (i.e., manufacturing process, comparability of clinical and

commercial batches), which is also well established and assessed during MAA. This is exemplified in **Figure 6**, which shows one withdrawn application where the overall approach for biosimilarity was considered correct. However, MA was not granted for this biosimilar candidate as other key quality requirements were not met.

These observations reflect the current maturation of analytical and functional sciences, and how detailed and comprehensive CMC/quality packages are for mAb biosimilars. Moreover, it demonstrates the meticulous evaluation of these products, ensuring they meet quality standards. Overall, this assures that no quality differences that could have an impact on the clinical outcome are found.

Comparability of biosimilar efficacy is ensured at multiple levels in the quality/CMC package.

As seen in **Table 1** and **Table 9**, the quality/CMC characterisation of mAb biosimilars ensures the demonstration of similarity through different levels of comparability with their RP.

Firstly, similarity is assessed in a structural level (e.g., amino acid sequence, secondary and higher order structure). Although some of these QA were not categorised in **Table 1** and **Table 9** because in many cases the data submitted by the applicants were not entirely quantitative, those that were included (i.e., protein content) demonstrated high similarity. Moreover, although this data was not included in our tables, it was assessed by applicants and regulators, and in all cases considered similar to the RP. The second level of similarity is ensured with binding assays, which verify similarity in primary binding with target and receptors. The third level of similarity are cell-based bioassays, which ensure that this similarity is translated into an effect in living cells. As seen in **Table 1** and **Table *9*** over 90% (and in most cases 100%) of biosimilar batches met the EU-RP for binding and cell-based bioassays considered critical.

When similarity was not 100% in any of these levels, the results in the other two levels were investigated. This is exemplified in the last column of **Table 4** and **Table 10**. For instance, products A, B, C and D presented variable results for binding to several Fcy receptors (**Table 4**). However, these differences were seen as minor and not relevant because similarity was confirmed in other levels (cell-based functional assays).

If residual uncertainties remained, clinical PK data offered a fourth safety net for the demonstration of comparability.

There are a multitude of tools that characterise different structural and functional aspects of the BS candidate and that allow to discriminate minor differences compared to the RP. In case differences to the RP were detected, additional assays were performed to verify if these differences could have a clinical implication. In no case were clinical efficacy data necessary to resolve analytical/functional differences.

This raises doubts about the necessity of comparative clinical efficacy studies (with incomplete and immature data in many cases) as a fifth safety net to demonstrate similarity.

Clinical efficacy and safety data is not needed to resolve residual quality uncertainties.

As seen in **Table 4** and **Table 10**, for the set of 23 mAb biosimilars analysed, there were no instances in which clinical efficacy or safety data was necessary to resolve residual uncertainties regarding quality queries.

On the contrary, all cases were resolved based on the positive results of other QAs analysed (e.g., minor differences in binding results were accepted because similarity was confirmed in cell-based functional assays/biological assays), on product understanding, regulatory experience, and in certain instances, the results of PK comparability studies.

The quality/CMC packages of complex biosimilars are predictive of MA.

The analysis of a total of 21 approved mAbs biosimilars, which include a representative example of widely used biologicals that cover different indications and MoA (i.e, autoimmune, oncological), dual indications (i.e., rituximab), and withdrawn applications, showed that all quality/CMC packages analysed were robust and could have been predictive for the clinical outcome.

As seen in **Table 1** and **Table 9**, for all approved mAbs biosimilars analysed, over 90% (and in most cases 100%) of the biosimilar batches met the EU-RP similarity range for those QAs that are known to have a critical impact on the clinical performance of the product (i.e., CQAs such as protein content or biological activity). A more varying concordance was found for less critical QAs. Based on product understanding and regulatory experience, this variability is in certain cases already expected (i.e., charge variants or glycosylation assays), and in other instances can be viewed as acceptable based on further analysis of the quality/CMC package.

Subsequent experience with these products to date has not resulted in any safety or efficacy issues following their approval (28,72,73), others than those already know for the RP.

However, those applications where the quality package was deemed insufficient (**Figure 6**) did not receive MA, despite the clinical package being viewed as supportive for demonstration of biosimilarity (Scenario 2 of **Figure 4**).

These observations support the conclusion that the outcome of the quality dossier of biosimilars (including complex biosimilars such as mAb) is predictive of MA.

4.2. CLINICAL COMPARABILITY

Regulatory science already enables a streamlined clinical development programme today for complex biosimilars.

As seen in **Table 5**, **Table 6**, **Table 11**, **Table 12, Table 13** and **Table 14** regulatory flexibility is already accepted for clinical data. This is evidenced by the acceptance of different primary endpoints (i.e., DAS28 versus ACR20 in RA trials), different methods of analyses (i.e., RD versus RR), or different equivalence margins for the same study population depending on the reference study chosen and provided that appropriate scientific justification is given.

These findings have also been discussed by other authors (25,28). Bielsky et al, already in 2020 (28) conducted a thorough review of biosimilar applications in the EU and concluded that in-depth knowledge of the RP, allied with high-performing analytical tools, largely predicts clinical comparability, subject to confirmation by a

comparative PK trial. The authors further provided a blueprint for a biosimilar pathway that reduces the need for clinical efficacy trials.

After more than a decade of regulatory experience with complex biosimilars, this flexibility has shown no negative clinical impact, demonstrating the success and validity of clinical tailoring.

Moreover, the totality of the evidence pathway recommended for biosimilar development, has shown to be a valid approach. Further streamlining biosimilar development does not mean less evidence, but a shift away from clinical burden to more extensive quality analysis, in light of analytical advancements and regulatory experience gained in over a decade.

Relevant S/I data can be obtained from PK studies.

Comparable safety is the result of similarity of structure and functions. Comparable immunogenicity is the result of identical amino acid sequence. Both must be paired with the strict control of other relevant factors (i.e., contaminants, or process impurities), which are ensured by today's quality standards(25). Moreover, in the context of biosimilarity assessment, as current efficacy studies are not powered for safety endpoints, relevant S/I data is limited to the similarity to the S/I profile of the RP (and not the safety assessment per se).

However, one of the biggest concerns regarding clinical data tailoring is the predictiveness of quality and/or PK data for immunogenicity and safety data, which is in many cases the basis for confirmatory efficacy/safety trials.

As seen in **Table 5**, **Table 6**, **Table 13** and **Table 14**, observations regarding the comparability of immunogenicity and safety made in the PK trial were, in all instances, confirmed in the efficacy/safety study. These findings have also been observed by other authors(68). Moreover, our analysis showed that most clinical dossiers are submitted with preliminary S/I data (4-6 months depending on the timepoint of primary efficacy analysis). Although current guidelines advise one-year datasets, we observed that the conclusions reached between the initial data submission, and after 12 months, did not differ.

This supports the findings by other authors(28), that long trials are unnecessary and redundant for complex biosimilars. If a PK study already provides sufficient data on the comparability of the S/I profile, and efficacy/safety data submitted is in many cases preliminary and with no impact on MA decision, it is clear that the later does not provide additional nor critical information.

Most uncertainties regarding biosimilar clinical data are resolved considering the robustness of the quality/CMC package or justified considering the limitations of clinical data presented.

For the complete set of 23 mAbs analysed, a comprehensive clinical programme was submitted according to the relevant EMA guidelines, i.e., consisting of at least one PK study and a clinical efficacy study, which confirmed comparability in all cases except for the withdrawn products.

Again, it is most interesting how residual uncertainties in the clinical package were resolved. As seen in **Table 7** and **Table 15**, in most cases, these uncertainties were resolved based on the totality of evidence in the overall biosimilarity assessment, i.e., the presence of a strong quality/CMC package, together with demonstrated PK similarity.
Identified issues in the clinical data package were eventually accepted, seen as due to imbalances in trial arms, studies not powered to demonstrate similarity with regard to secondary endpoints, changes in the QA of the RP or chance findings.

In some cases, secondary endpoints were viewed as inconclusive or immature, which calls into question the value of secondary endpoint analyses at all.

This suggests that for the evaluation of complex biosimilars, the totality of the evidence approach is used. Where a robust quality/CMC package is available, clinical data in many cases adds very little to the evaluation process. Moreover, in certain cases, clinical data is of little value, as it is seen as incomplete or immature and, viewed as not decisive for MA.

4.3. MAA INITIAL AND FINAL OUTCOME

The quality/CMC part of the dossier appears to be predictive for the MA of a biosimilar candidate, irrespective of the outcome of the clinical trial.

In the analysis of 33 mAbs and 3 fusion proteins, in most cases (29/36 cases, scenario 1 of **Figure 4**) good quality/CMC packages were matched with successful clinical trials leading to MA.

In only one case led clinical data analysis to a MO with a divergent position published by the CHMP (82), regarding potentially increased immunogenicity of the biosimilar. The biosimilar candidate nevertheless received MA, and uncertainty was resolved by more mature follow-up data (119).

There were no cases where clinical issues precluded approval per se. When major issues were raised regarding PK, these were also resolved, seen as due to methodological issues, differences in the formulation buffer or the representativeness of the test product (scenarios 3 of **Figure 4**). Major uncertainties regarding E/S/I data were seen as due to immature and/or insensitive data (scenarios 4 of **Figure 4**).

On the contrary, unconvincing quality/CMC data, even paired with successful clinical trials (scenario 2 of **Figure 4**) were the only observed cases that precluded MA.

There are successful biosimilar programmes despite formally failed efficacy studies.

Scenario 4 of **Figure 4** shows two cases with an unsatisfactory clinical trial result(103,104). For both trastuzumab cases, the primary efficacy endpoint was formally not met as it was slightly outside the pre-specified equivalence range in the upper bound limit as defined for the European development programme. However, these findings did not preclude approval.

In both cases, MA was granted based on the convincing quality/CMC, PK, safety, and immunogenicity data packages, despite a failed primary endpoint.

Physicochemical methods and functional assays for biosimilars are able to detect differences in functional attributes with predictive character.

In both trastuzumab cases explained above, EMA concluded (103,104) that it was likely that the apparent clinical difference was caused by the confounding effect of a noted downward “shift” in ADCC activity in some of the RP batches, findings that have also been discussed in the literature (28,121,122). Overall, it was seen as doubtful that a small shift as the one observed would have any significant impact in terms of clinical outcomes although numerically it is thought to have contributed to a more extreme location of the point estimate.

Moreover, when analysing the same evaluation process following FDA standards(127,128), the approved FDA margins were missed for only one trastuzumab candidate (Kanjinti (ABP908)).

This demonstrates that cell-based bioassays are more sensitive than comparative efficacy trials, as they are able to discern variances in functional attributes with predictive properties, that clinical efficacy data cannot.

The concern, that in the absence of comparative efficacy data, a biosimilar candidate might be inappropriately approved based on quality data only, is not supported by data.

In the regulatory assessment of 33 mAbs and 3 fusion proteins evaluated by EMA, we found no instance where seemingly negative clinical data, including failed efficacy trials, led to a negative overall decision.

As seen in case 3 of **Figure 5**, in 22% of cases MO were raised in the first regulatory assessment on the clinical data package but not on the quality data. However, when analysing the MAA outcome, none of these issues precluded approval. These cases could have led to inaccurate negative conclusions and the rejection of a genuinely similar biological product due to deficiencies in clinical study outcomes.

In all cases, the failure to confirm similar clinical performance was viewed as due to reasons not related to the biosimilar candidate but seen as result of immature or insensitive data (imbalances in the trial arms, immaturity of secondary endpoint results, change in the RP, or even chance findings), thus permitting MA. Biosimilarity was ultimately accepted by EMA based on the demonstration of analytical/functional comparability and comparable PK profiles.

This is also observed in scenarios 3 and 4 of **Figure 4**.

Again, this demonstrates that regulatory decision-making follows a totality-of-the-evidence approach with the main focus on the pharmaceutical quality/CMC (biosimilarity, general quality) and PK similarity.

4.4. OBSERVED IMPACT OF THE RESULTS OF THIS THESIS

Since the publication of the first paper of this thesis, the impact of our results (together with similar results from other authors (19,28,29,68)) have been observed in updated regulatory requirements and guidelines in different healthcare systems.

The update of the Medicines and Healthcare products Regulatory Agency (MHRA) guidelines in 2022(22), contain further clarifications and some revisions to CHMP guidance documents, which take into account the scientific and regulatory experience gained since the first biosimilar product was licensed in 2004, including biosimilar mAb and fusion proteins licenced from 2013. They clearly establish that although each biosimilar development needs to be evaluated on a case-by-case basis, it is considered that, in most cases, a comparative efficacy trial may not be necessary if sound scientific rationale supports this approach. Moreover, they establish the general principles of the scientific rational: justification for comparable efficacy (i.e., although precise correlations between clinical efficacy and pharmacological effects are usually lacking, the efficacy of the RP can usually be related to the biological events triggered by the binding of the active to its known targets) and justification for comparable safety and immunogenicity (i.e., comparability of the biosimilar is informed by clinical experience and QAs of the RP, not whether the immunogenicity and safety risks are low or high).

The World Health Organization (WHO) guidelines on evaluation of biosimilars were also updated in April 2022(24). The main changes made include consideration of the amount and type of clinical data required. New guidance regarding complex biologics has been added: “The current data suggest that more-complex products such as mAbs can be sufficiently characterized by available suitable analytical methods, plus the structure–function relationships are well known and can be studied by sensitive

orthogonal functional assays", or "The current data suggest that more-complex products such as mAbs can be sufficiently characterized and also fall into this category, i.e., highly similar physicochemical characteristics and PK/PD profiles of the biosimilar and RP could provide sufficient reassurance that risks are also similar, obviating the need for further safety data", or "Conversely, for well-characterized biological substances (for example, insulin, somatropin, filgrastim, teriparatide), where an extensive literature and clinical experience indicate that immunogenicity does not impact upon product safety and efficacy, immunogenicity studies may not be necessary, provided that the biosimilar is highly similar to the RP and the risk-based evaluation indicates a low risk. This may also be applicable to other products, including mAbs".

In September 2022, the US FDA, together with the International Pharmaceutical Regulators Program (IPRP) Biosimilars Working Group (BWG) hosted a public workshop addressed to regulators, biosimilar developers, academic researchers, and other stakeholders worldwide, with the title "Increasing the Efficiency of Biosimilar Development Programs--Reevaluating the Need for Comparative Clinical(129). The public sessions of the workshop included presentations of both publications included in this thesis. A workshop summary report and a white paper/concept paper are to be published in 2024.

Moreover, starting October 2022, the FDA has started the Biosimilar User Fee Act (BsUFA) III(130), which includes a commitment for FDA to pilot a regulatory science research programme to further enhance regulatory decision-making and facilitate science-based recommendations in areas foundational to biosimilar development. This pilot programme will focus on how clinical work may be reduced.

Health Canada is also planning to publish a new draft of regulatory biosimilar guidelines in early 2024.

4.5. LIMITATIONS

The methodology employed and datasets analysed present certain limitations:

i. As this analysis is restricted to mAb and fusion protein biosimilars of IgG class, the conclusions may not be applicable to other class biologics.

ii. Due to the presence of data not entirely quantitative in most product dossiers, the different methodologies used by applicants, and moreover, confidentiality issues, the analysis of analytical/functional biosimilarity did not include full comparison of all QAs evaluated. However, the data represents those QA that are considered most critical in each case, and although some of the remaining data could not be presented, it was still initially analysed and considered correct.

iii. Additionally, this analysis is limited to products that have undergone regulatory assessment. It has not included biosimilars that were never submitted for regulatory evaluation or publication e.g., due to failure early in the development to demonstrate similarity. Nevertheless, the analysis of these products is intricate. Information is in most cases confidential, and moreover, it is not presented in a structured or even written form that can allow analysis and categorisation.

iv. The set analysed includes relatively small sample size of 36 mAb and fusion proteins. However, this set corresponds to the available submissions to EMA in the previous 10 years, representing the largest set of biosimilar mAb analysed to date.

4.6. FUTURE DURECTIONS

Based on the strategies used in the thesis to analyse quality and clinical data, as well as MA outcome, future research studies can be drawn.

However, the need of further studies will depend on the outcome of the dissemination of the current results of this thesis, and weather regulators, healthcare professionals and other stakeholders believe uncertainties remain regarding clinical tailored programmes for biosimilars.

<u>If these results are not received as sufficient for the revision of current regulatory guidelines</u>:

1. An in-depth analysis of the clinical and quality dossiers (similar to that performed in the first publication) for additional sets of biosimilar candidates that include other product classes, indications, and MoA. However, we believe the results would not provide additional information and change our conclusions, as we selected a representative set based as pre-defined criteria (e.g., different MoA, different indications and withdrawn applications)
2. The analysis of biosimilar candidate applications evaluated by other regulatory agencies worldwide.
3. The analysis of the differences and similarities of the biosimilar regulatory pathway between regulatory agencies worldwide, determining the legal framework and uptake determinants in each case.

<u>If these results truly motivate the implantation of tailored clinical programmes for all classes of biosimilars</u>:

1. The analysis of what precise changes are made, and the sequence on how these changes are implemented.
2. The analysis of the impact of biosimilar streamlining in the EU, both economical and non-economical (i.e., increased patient access, bigger development of innovative treatments)
3. The analysis of the remaining hurdles in the biosimilar framework, for healthcare professionals, patients, regulators, and other stakeholders.

Conclusions

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In summary, the work presented in this thesis has contributed to provide deeper understanding on the role of analytical/functional and clinical data for the conclusion of biosimilarity and the decision on MA for complex biosimilars. Overall, the results obtained lead to the following conclusions:

1. Regulatory science already enables a streamlined clinical development programme today for complex biosimilars.
2. The analytical tools and functional methods routinely performed allow a comprehensive and in-depth characterization of mAb biosimilars.
3. Comparability of biosimilar efficacy is ensured at multiple levels in the quality/CMC package.
4. The quality/CMC packages of complex biosimilars are predictive of MA.
5. Relevant S/I data can be obtained from PK studies.
6. Clinical efficacy and safety data is not needed to resolve residual quality uncertainties.
7. Most uncertainties regarding biosimilar clinical data are resolved considering the robustness of the quality/CMC package or justified considering the limitations of clinical data presented.
8. The quality/CMC part of the dossier appears to be predictive for the MA of a biosimilar candidate, irrespective of the outcome of the clinical trial.
9. There are successful biosimilar programmes despite formally failed efficacy studies.
10. Physicochemical methods and functional assays for biosimilars are able to detect differences in functional attributes with predictive character.
11. The concern, that in the absence of comparative efficacy data, a biosimilar candidate might be inappropriately approved based on quality data only, is not supported by data.

In conclusion, this thesis further demonstrates that clinical efficacy and safety data add very little to the overall evaluation and approval process of biosimilar mAbs and fusion proteins totality of the evidence.

In our analysis acceptable CMC/quality packages were always robust and performed with advanced methods. However, on the contrary, clinical packages in many cases

provided inconclusive and immature data, that overall add very little to the evaluation process. If this data is viewed as of such little value to the totality of the evidence, it is questionable to what extend it is needed to be performed at all. Rather than asking for almost default clinical efficacy data as currently mandated in guidelines, they should only be required in exceptional cases.

Regulatory science enables streamlined clinical biosimilar development without comparative clinical efficacy studies, based on a robust analytical package including a comprehensive panel of precise functional assays and a comparative clinical PK study. Based on the combination of modern analytics, control, and pharmacovigilance systems in place, as well as requirements on comparability assessment in case of manufacturing changes, clinical performance of IgG biopharmaceuticals is ensured throughout the lifecycle of the product.

This demonstrates that large clinical studies of biosimilars are of limited value and that comparative clinical efficacy is not a decisive criterion in biosimilar development. This follows the totality of the evidence approach, as it does not mean that less evidence is wanted, rather a shift from clinical burden more to extensive quality analysis. Clinical efficacy data should only be required in exceptional cases, if e.g., the MoA is not known.

It is important to only conduct clinical studies that provide decisive information for biosimilar evaluation, as non-decisive studies use patients whose time (and accompanying resources) would be better off participating in studies which foster progress of healthcare.

As the conduct of large and lengthy comparative efficacy/safety clinical trials are cost-intensive, streamlining of the biosimilar development would likely enable the development of more and a wider variety of biosimilars and accelerate the developmental process without impacting patient safety or effectiveness (21). This could lead to more affordable and more readily available biological drugs. Moreover, from an ethical point of view, any study involving human subjects must take particular care to contribute new knowledge not otherwise obtainable.

Consequently, a revision of the respective regulatory biosimilars guidelines in Europe should be considered, to allow a more rational use of clinical resources and improve the access to innovative and affordable medicines for patients.

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A Data Driven Approach to Support Tailored Clinical Programs for Biosimilar Monoclonal Antibodies

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Biosimilar monoclonal antibodies (mAbs) have been approved in the European Union since 2013 and have been demonstrated to reduce healthcare costs and to expand patient access. Biosimilarity is mainly established on the basis of demonstrated similarity of relevant quality attributes (QAs), determined by comprehensive physiochemical and functional analyses, and demonstration of bioequivalence. In addition, comparative efficacy/safety studies have been requested for all approved biosimilar mAbs so far, although the European Medicines Agency (EMA) Guidelines state that such confirmatory clinical trials may not be necessary in specific circumstances. In order to evaluate the degree of analytical similarity, how residual uncertainty regarding biosimilarity was resolved, and the value of clinical data, we analyzed the quality and clinical data packages for authorized adalimumab (7 products) and bevacizumab (5 products) biosimilars. The percentage of biosimilar batches meeting the similarity range for QAs, as defined by the biosimilar manufacturer based on a comprehensive characterization of the EU reference product (RP), was determined and clinical data were reviewed. Our analyses show that QAs of approved adalimumab and bevacizumab biosimilars have varying concordance with the EU-RP similarity range. In this study, we found that clinical efficacy data played a limited role in addressing quality concerns. Therefore, we encourage a regulatory review of the standards for clinical data requirements for mAb and fusion protein biosimilars. This study outlines a quality data driven approach for facilitating tailored clinical programs for biosimilars.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Biosimilarity of monoclonal biosimilar antibodies (mAbs) is established on the basis of analytical and functional (quality) similarity and demonstration of bioequivalence. In the European Union, marketing authorization approval also requires a confirmatory comparative efficacy/safety study. Whereas the European Medicines Agency (EMA) Guideline states that this confirmatory clinical trial may not be necessary in specific circumstances, to date, all approved mAbs have included one large equivalence trial.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ How similar are biosimilar mAbs compared to their respective reference products? If there is < 100% of biosimilar batches within the reference similarity range, how do regulators decide whether the product may be viewed as biosimilar? What role does data from clinical efficacy trials play in reaching conclusion on biosimilarity?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Over 90% (mostly 100%) of the batches of adalimumab and bevacizumab biosimilars met the EU reference product similarity range for critical quality attributes (QAs). For critical QAs where < 100% of batches were inside the similarity ranges, further evidence of similarity was gathered and queries resolved at the quality level.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ This study shows that comparable clinical performance, in most cases, can be predicted on the basis of quality and clinical pharmacokinetic data as analyzed for two substances (adalimumab and bevacizumab). This outlines a quality data driven approach for facilitating tailored clinical programs for biosimilars.

Biosimilars are biological medicinal products that contain a highly similar version of the active substance of an already authorized original biological product (reference product (RP)). They differ from generic drugs due to their biological source, in the size of the active substance, their complexity, and the nature of the manufacturing process. The mainstay of any biosimilar development is the comprehensive

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demonstration of close physicochemical and functional similarity as well as bioequivalence with their RP. In addition, comparative efficacy/safety studies have so far been requested for all approved biosimilar monoclonal antibodies (mAbs) to confirm the absence of clinically meaningful differences compared with the RP.[1,2] Typically, confirmation of comparable clinical efficacy in one "model" indication is required and other indications of the RP can be extrapolated,[3] which leads to reduced development costs[4] and allows for competitive price reductions for biosimilars thus facilitating patient access.[5]

In this paper, the terms comparability and (bio-)similarity exercise are used synonymously.

The biosimilar regulatory framework was initially developed with the conservative stance that one comparative efficacy study will always be required as a safeguard and precautionary measure to ensure that biosimilarity demonstrated at the analytical and functional (quality) level indeed translates into biosimilarity at the clinical level. However, in recent years, due to the advancement in the analytical sciences and the vast experience gained, the extent and usefulness of this clinical confirmation has been questioned, and regulators have started to adopt a more flexible approach where the extent of clinical data required can vary depending on the product class.[6–9] Recent guidelines[10–13] state that a pre-licensing efficacy study may be waived in case biosimilarity can be convincingly concluded based on physicochemical and functional characterization studies using sensitive, orthogonal, and state-of-the-art analytical methods, together with comparison of the pharmacokinetic (PK) and/or pharmacodynamic (PD) profiles of the biosimilar and the RP.

In the particular case of mAbs, this has been considered challenging, given their relative complexity.[14] However, since assessment and EU marketing authorization (MA) of the first biosimilar mAb in 2013,[15] the physiochemical and functional assays have continued to evolve, with greater understanding of the relevant quality attributes (QAs) and increased sensitivity of analytical methods to detect relevant differences.[16,17] Therefore, the extent of analytical data routinely provided in biosimilar dossiers currently may give sufficient assurance that a biosimilar is indeed highly similar to the RP such that no difference in clinical performance is expected. As such, the default requirement for confirmatory clinical studies could be questioned also for mAbs, and alternative regulatory pathways and/or guidance may be warranted. Streamlining developments to become more cost and time efficient and sparing patients from entering unnecessary and redundant clinical trials is of foremost importance from an ethical point of view and at a time when public and patient resources are becoming increasingly strained.[18,19]

In an effort to provide a deeper understanding of the magnitude and strength of the analytical and functional similarity data available for mAbs, data of two biosimilar mAb classes were analyzed: seven approved adalimumab and five approved bevacizumab biosimilars. In addition, clinical efficacy and safety comparability data were reviewed on a product basis by studying the European Product Assessment Reports (EPARs).

The aim of this study was to analyze whether, and to which degree, QAs were within the similarity ranges established by the biosimilar developer based on a comprehensive characterization of the RP and what role clinical data played in the final conclusion of biosimilarity.

METHODS

We reviewed, categorized, and anonymized the analytical and functional similarity data and analyzed the clinical data packages for approved adalimumab and bevacizumab biosimilars. The analysis included seven adalimumab biosimilars (Amgevita/Solymbic, Imraldi, Hyrimoz/Halimatoz/Hefiya, Hulio, Idacio/Kromeya, Amsparity, and Yuflyma)[20–26] and five bevacizumab biosimilars (Mvasi, Zirabev, Aybintio/Onbevzi, Alymsys/Oyavas, and Abevmy/Lextemy).[27–31] The data lock point for the analysis was September 2021. We only included biosimilars that were authorized at the time of study analysis. Adalimumab and bevacizumab were selected as representative examples of widely used biologicals which cover different therapeutic areas (autoimmune and oncologic indications).

Comparison of analytical biosimilarity across products

QA characterization data were extracted from raw data of the biosimilar product dossiers submitted to the European Medicines Agency (EMA) for MA approval. The data were anonymized due to confidentiality.

The comparative QA data extracted were categorized into four pattern and color-coded categories: depending on the degree of similarity with the RP (see **Table 1**). This categorization was performed considering the percentage of analyzed biosimilar batches with values within the similarity range: solid dark green for QAs with 100% biosimilar batches within the similarity range; light green horizontal stripes for QAs with 90–99%, light blue diagonal stripes for QAs with 50–89%, and dark blue dots for QAs with < 50% of the batches within the similarity range or when the data was lacking. This crude categorization was chosen by the authors to allow for meaningful differentiation of similarity ranges, without losing the anonymity of products.

Some assays are product specific (e.g., human umbilical vein endothelial cells (HUVECs) antiproliferation and human vascular endothelial growth factor (VEGF) binding for bevacizumab) and are therefore represented in the gray grid in **Table 1**.

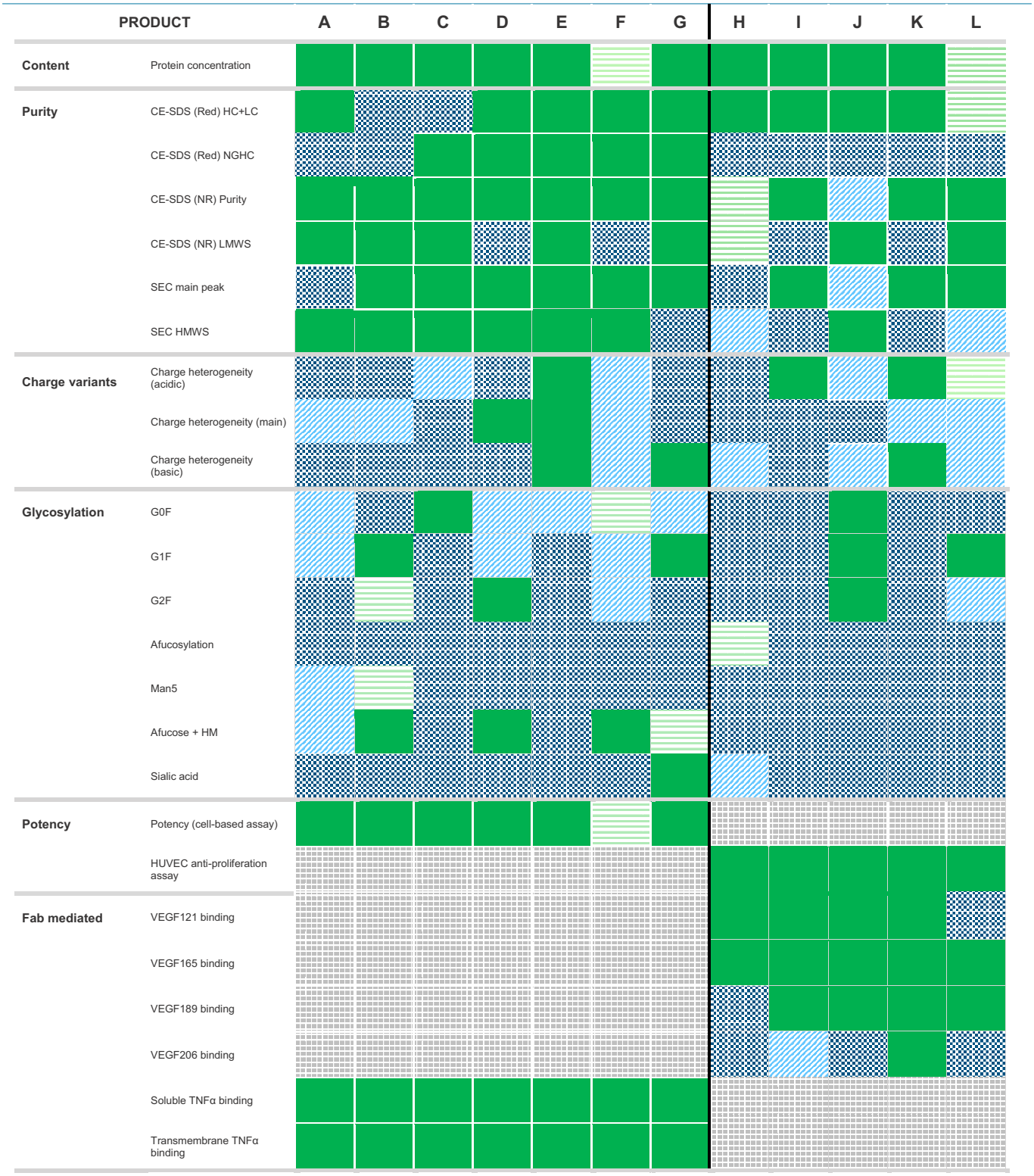
The reference (similarity) range for establishing analytical similarity is determined by the biosimilar manufacturer based on characterization data of the RP. Similarity ranges are usually calculated based on statistical analysis of the RP dataset and may be based on ranges such as mean $\pm 3 \times$ SD (standard deviation), tolerance intervals, or a minimum-maximum range.[32] The approach for setting similarity ranges may vary between products, however, all the statistical approaches used were individually justified and assessed during the respective MA procedures.

Batch results outside the similarity range were conservatively counted as being "non-similar" regardless of how far outside of the similarity range the results were. The number of biosimilar batches analyzed per product varied between 8 and 20, for most QAs. **Table 1** includes mainly the analysis of quantitative QAs. In some cases, QAs were presented graphically in the MA, if the profiles were considered to be similar (i.e., the profiles of the biosimilar and RP overlap and are visually comparable), in addition, these were categorized in solid dark green.

For purity/impurity-related QAs, one-sided similarity ranges were considered, that is, if the biosimilar exhibited higher level of purity/lower level of impurities compared to the RP, this was considered *de facto* to be comparable. In such cases, 100% biosimilar batches were considered to be within the similarity range (=solid dark green).

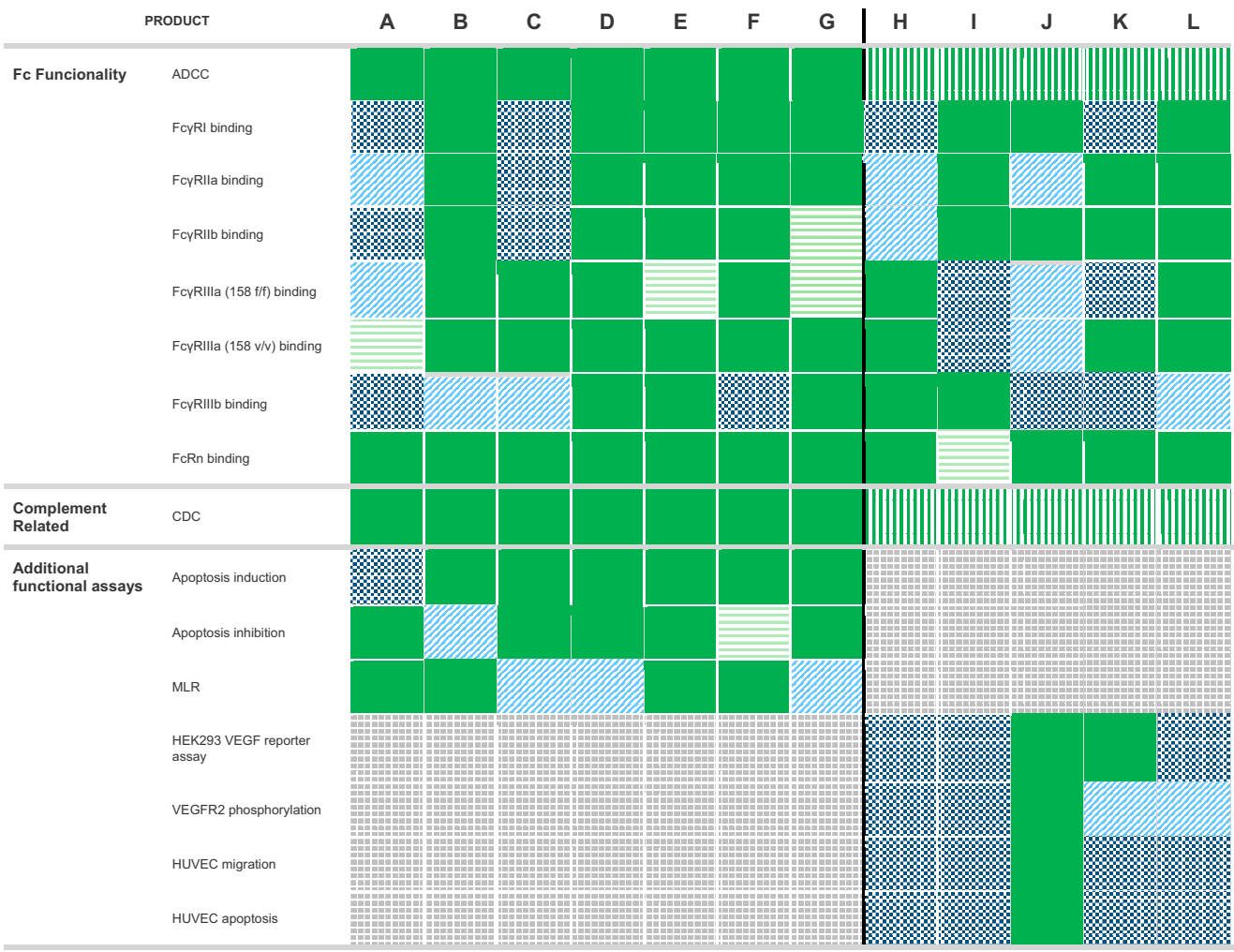
Additional QAs, for example, amino acid sequence, secondary and higher order structure, etc., are not included in this analysis because, in many cases, the data submitted were not entirely quantitative. For other QAs related to protein modifications, such as oxidation or deamidation, a quantitative comparison across products was also not possible due to different methodologies used by applicants. **Tables 2** and **3** summarize all the QAs tested, including also qualitative tests (information extracted from the EPARs).

Table 1 Similarity of QAs for all adalimumab (A–G) and bevacizumab (H–L) biosimilars. Color and patterns indicate the percentage of biosimilar batches within the similarity range derived from the EU reference product: solid dark green for 100%, horizontal light green stripes for 99–90%, diagonal light blue stripes for 89–50%, dark blue dots for < 50% and also when the QA was not assessed. Gray grid represents product specific QAs which reflect the mAbs main MoA. Green vertical stripes represent QAs that were tested but not found (in line with the mAbs MoA).



(Continued)

Table 1 (Continued)



ADCC, antibody-dependent cell-mediated cytotoxicity; CDC, complement dependent cytotoxicity; CE-SDS, capillary electrophoresis-sodium dodecyl sulfate; EU-RP, European reference product; HEK293, human embryonic kidney 293 cells; HMWS, high molecular weight species; HUVEC, Human Umbilical Vascular Endothelial Cell; Fc, fragment crystallizable region; Fab, fragment antigen-binding region; LMWS, low molecular weight species; mAb, monoclonal antibody; MLR, mixed lymphocyte reaction; MoA, mechanism of action; NR, non reduced; NGHC, non glycosylated heavy chain; QA, quality attribute; Red, reduced; SEC, size exclusion chromatography; VEGF, vascular endothelial growth factor; VEGFR2, vascular endothelial growth factor receptor 2.

Table 4 provides a summary of the instances where < 100% of batches were within the reference range (data on file at the EMA), and how the resulting uncertainty was resolved. In each case, the reason why these differences were accepted by the EMA is explained.

Comparison of clinical biosimilarity across products

Clinical data are presented as raw data in **Tables S1** and **S2** (the product columns are not in the same order as **Table 1** to maintain anonymity). Clinical data were extracted from EPARs, which contain public information that can be found on the EMA website and therefore anonymization is not necessary (European Medicines Agency. Find medicines. Available at: https://www.ema.europa.eu/en/medicines. Accessed April 2022).

For PK and efficacy parameters, acceptance ranges for comparability were defined before study start in the statistical analysis plan. Population PK (PopPK) analysis in patients was not model-based but descriptive. Safety and immunogenicity parameters are presented as raw data and were compared descriptively.

The few instances where uncertainties arose in the similarity of a specific clinical parameter are highlighted and discussed in context of other findings.

Table 5 provides a summary of all uncertainties stemming from clinical data and how they were resolved.

RESULTS

Table 1 provides a summary of the QAs considered for adalimumab (products A–G) and bevacizumab (H–L) biosimilars. For each adalimumab and bevacizumab biosimilar, the percentage of batches within the established similarity range for each individual QA is categorized in a color and pattern. The analytical similarity packages of the adalimumab and bevacizumab biosimilars comprised between 35 and 85 individual assays per product (for complete list see Tables 2 and 3). For most of the QAs, orthogonal analytical methods were used.

Protein content

Protein content is a highly critical QA which must be fully comparable between the biosimilar and the RP. For all biosimilars

Table 2 Summary of analytical assays performed for *adalimumab* biosimilars

Quality attribute (and analytical method/s) for comparative characterization	
Content	Protein content (UV-280)
Primary structure	Molecular weight/intact mass (RPLC-UV/MS)
	Amino acid sequence (peptide mapping)
	N-terminal sequencing (peptide mapping, Edman sequencing)
	C-terminal sequencing (peptide mapping)
	Peptide mapping
	Disulfide bond analyses (peptide mapping)
	Free thiols (Ellmans test)
Higher order structure	Secondary structure (FTIR)
	Secondary- and tertiary structure (far and near UV circular dichroism)
	Protein folding (Intrinsic and extrinsic fluorescence)
	Thermal stability (DSC)
	Tertiary structure (1D 1H NMR, 2D 1H-1H NOESY NMR, 2D-NMR, HDX, X-ray crystallography, antibody conformational array)
Protein modifications	N-term pyroglutamate (peptide mapping)
	C-terminal lysine (peptide mapping, CEX)
	Iso-aspartate (peptide mapping)
	Deamidation (peptide mapping)
	Oxidation (peptide mapping)
	Glycation (BAC)
	Succinimidation (peptide mapping)
	Isomerization (peptide mapping)
	Proline amide (peptide mapping)
	Thioether (peptide mapping)
	Cysteinylation (peptide mapping)
Glycosylation	N-glycan profile (LC-ESI-MS/MS, 2-AB labeling HILIC-UPLC)
	Afucosylation (LC-ESI-MS/MS, 2-AB labeling HILIC-UPLC)
	High mannose (LC-ESI-MS/MS, 2-AB labeling HILIC-UPLC)
	Sialylation (LC-ESI-MS/MS, 2-AB labeling HILIC-UPLC)
	G0F (LC-ESI-MS/MS, 2-AB labeling HILIC-UPLC)
	G1F (LC-ESI-MS/MS, 2-AB labeling HILIC-UPLC)
	G2F (LC-ESI-MS/MS, 2-AB labeling HILIC-UPLC)
	Galactosylation (LC-ESI-MS/MS, 2-AB labelling HILIC-UPLC)
Purity/ impurity profile and charged variants	Size heterogeneity (SEC, CE-SDS reducing and non-reducing, SV-AUC, SEC-MALS, DLS, FFF)
	Hydrophobic heterogeneity (HIC)
	N-linked glycosylation site (LC-ESI-MS/MS)
	Charge heterogeneity (CEX-HPLC, iCIEF, iCE, cIEF, IEC-HPLC)
Fab mediated	Soluble TNF-binding (ELISA, SPR, FRET)
	Membrane TNF-binding (cell-based assay)
	TNF-α neutralization (NF-kB reporter, viability/cell death)
Fc and complement mediated	ADCC *e.g., for one product, up to 20 assays were performed, including: • NK-PBMC ADCC using healthy and patient blood • Whole blood ADCC using healthy and patient blood • FcγRIIIa ADCC reporter • Addition of serum to these assays • Addition of IgG to these assays
	FcγRI binding (SPR)
	FcγRIIa (131H, 131R) binding (SPR)
	FcγRIIb binding (SPR)

(Continued)

Table 2 (Continued)

Quality attribute (and analytical method/s) for comparative characterization	
	FcγRIIIa (158F, 158V) binding (SPR, AlphaLISA, RGA)
	FcγRIIIb binding (SPR)
	FcRn binding (SPR)
	CDC (cell-based assay)
	C1q binding (ELISA)
Additional functional assays	Apoptosis induction, reverse signaling (cell-based assay)
	Apoptosis inhibition in intestinal epithelial cells
	MLR (T cell proliferation, regulatory macrophages (CD14/CD206))
	IL-8 release from HUVECs
	IL-8 release from PBMCs
	IL-8 release from keratinocytes
	IL-8 release from intestinal epithelial cells
	IL-6 release from synoviocytes
	sVCAM-1 release from HUVECs
	ICAM-1 expression on HUVECs
	ELAM-1 expression on HUVECs
	MIP-1β release from whole blood
	MCP-1 release from whole blood
	Lack of impact on Lymphotoxin α

ADCC, Antibody dependent cell-mediated cytotoxicity; AlphaLISA, Amplified Luminescent Proximity Homogeneous Assay; BAC, boronate affinity chromatography; CDC, complement dependent cytotoxicity; CE-SDS, capillary electrophoresis-sodium dodecyl sulfate; CEX, cation exchange chromatography; cIEF, capillary isoelectric focusing; DSC, differential scanning calorimetry; DLS, dynamic light scattering; ELAM-1, endothelial-leukocyte adhesion molecule 1; ELISA, enzyme-linked immunoassay; FcRn, neonatal Fc receptor; FcγR, fragment crystallizable gamma receptor; FFF, filed flow fractionation; FRET, Förster (flourescence) Resonance Energy Transfer; FT-IR, Fourier-transform infrared; HDX, hydrogen–deuterium exchange; HIC, hydrophobic interaction chromatography; HILIC-UPLC, hydrophilic interaction ultra performance liquid chromatography; HPLC, high-performance liquid chromatography; HUVECs, human umbilical vein endothelial cells; iCIEF, imaged capillary electrophoresis focusing; ICAM-1, intercellular adhesion molecule 1; IEC, ion exchange chromatography; IL-6, interleukin 6; IL-8, interleukin 8; LC-ESI-MS/MS, liquid chromatography electrospray ionization tandem mass spectrometric; MCP-1, monocyte chemoattractant protein-1; MIP-1-β, macrophage inflammatory protein 1-β; MLR, mixed lymphocyte reaction; NMR, nuclear magnetic resonance; NOESY, NOE correlated spectroscopy; PBMCs, peripheral blood mononuclear cells; SEC-MALS, multi-angle light scattering coupled with size exclusion chromatography; RGA, reporter gene assay; RPLC-UV/MS, reversed phase liquid chromatography-ultraviolet/mass spectrometry; SEC, size exclusion chromatography; SPR, surface plasmon resonance; SV-AUC, sedimentation velocity-analytical ultracentrifugation; sVCAM-1, soluble vascular cell adhesion molecule-1; TNF, tumor necrosis factor; UV-280, ultraviolet absorbance at 280 nm wavelength.

examined, 100% of biosimilar batches were within the reference range, except for one bevacizumab (product F) and one adalimumab (product L) biosimilar (≥ 90%).

Fragment antigen binding mediated functions

a. Binding to soluble tumor necrosis factor. Adalimumab is an IgG1 mAb that binds, via its fragment antigen binding (Fab) domain, to tumor necrosis factor α (TNFα) and prevents it from binding to its receptors TNFR1 and TNFR2, thereby blocking TNF-induced inflammation.[33,34] This is the primary mechanism of action (MoA) for adalimumab across all approved indications. The biological activity of adalimumab is determined by a combination of binding assays and a cell-based TNFα cytotoxicity inhibition assay. In addition, some applicants used a nuclear factor kappa B (NF-κB) reporter gene assay, which is viewed as supportive data (not included in **Table 1** but for those biosimilars where this assay was used, ≥ 90% of biosimilar batches were within the reference range). As shown in **Table 1**, for all 7 adalimumab biosimilars studied, 100% of the batches were within the similarity range for binding to soluble TNFα with the chosen assays.

b. Binding to transmembrane TNF and reverse signaling. In addition to binding to soluble TNFα, adalimumab can bind to membrane-associated TNFα (mTNFα) and mediate reverse (or outside-to-inside) signaling. Binding of adalimumab to mTNFα does not appear to be important for therapeutic efficacy in all indications, however, it may contribute to the clinical efficacy of adalimumab in inflammatory bowel diseases (IBDs). Several possible mechanisms explain the contribution of reverse signaling to the efficacy of adalimumab in IBD. For example, adalimumab-mediated apoptosis of lamina propria T cells may represent an additional key MoA of adalimumab in IBD indications and is thought to be mediated by reverse signaling, although it may also be mediated by binding to soluble TNF, which is in turn bound to its receptor.[35] Anti-TNF agents, such as adalimumab and infliximab, are also known to induce CD14+ CD206+ M2-type wound-healing macrophages (regulatory macrophages) which may contribute to mucosal healing in IBD.[36,37] Induction of regulatory macrophages can be assayed

Table 3 Summary of analytical assays performed for *bevacizumab* biosimilars

Quality attribute (and analytical method/s) for comparative characterization	
Content	Protein content (UV-280)
Primary structure	Molecular weight (RPLC-UV/MS)
	Intact mass/reduced mass (LC-ESI-MS)
	Isoelectric point (cIEF)
	Amino acid sequence (peptide mapping)
	N-terminal sequencing (peptide mapping, Edman sequencing)
	C-terminal sequencing (peptide mapping)
	Amino acid sequence (peptide mapping)
	Disulfide bond analyses (peptide mapping)
	Free thiols (Ellmans test)
Higher order structure	Secondary structure (FTIR, far and near UV circular dichroism)
	Tertiary structure (far and near UV circular dichroism, FL)
	Protein folding (Intrinsic and extrinsic fluorescence)
	Thermal stability (DSC)
	Epitope mapping (HDX-MS)
	Di-sulfide bridging (RP-HPLC-ESI-MS, non-reduced peptide mapping)
Protein modifications	Deamidation (peptide mapping)
	Oxidation (peptide mapping)
	Glycation (BAC)
	Aspartate Isomerization (peptide mapping)
	Thioether (peptide mapping)
	Cysteinylation (peptide mapping)
Glycosylation	N-glycan profile (peptide mapping, LC-ESI-MS/MS, HILIC-UPLC)
	O-glycosylation (peptide mapping)
	Ng-HC (CE-SDS, reduced)
	Afucosylation (NP-HPLC)
	Fucosylation (NP-HPLC)
	High mannose (NP-HPLC)
	Sialylation (NP-HPLC, UHPLC-FLR)
	G0F (LC-ESI-MS/MS, 2-AB labeling HILIC-UPLC)
	G1F (LC-ESI-MS/MS, 2-AB labeling HILIC-UPLC)
	G2F (LC-ESI-MS/MS, 2-AB labeling HILIC-UPLC)
	Galactosylation (NP-HPLC)
Purity/ impurity profile and charge variants	Size heterogeneity (SEC, CE-SDS non-reduced and reduced, CGE non-reducing and reducing, SV-AUC, SEC-MALS, DLS, FFF)
	Particles (MFI)
	Charge heterogeneity (CEX-HPLC, iCIEF, cIEF)
	Hydrophobic heterogeneity (HIC)
Fab mediated	VEGF121 binding (HUVEC-cell based assay, SPR, ELISA)
	VEGF165 binding (HUVEC-cell based assay, SPR, ELISA)
	VEGF189 binding (HUVEC-cell based assay, SPR, ELISA)
	VEGF206 binding (HUVEC-cell based assay, SPR, ELISA)
	VEGF B, C, D binding (BLI)
	HUVEC neutralization assay (cell-based assay)
	VEGFR phosphorylation inhibition (cell-based assay)
	Cell signaling assay (HEK293 RGA)
	KDR/KDR dimerization assay (cell-based assay)

(Continued)

Table 3 (Continued)

Quality attribute (and analytical method/s) for comparative characterization	
Fc and complement mediated	ADCC (cell-based assay)
	FcγRI binding (SPR)
	FcγRIIa FcγRIIa (131H, 131R) binding (SPR)
	FcγRIIIa (158F, 158V) binding (SPR, AlphaLISA)
	FcγRIIIb binding (SPR)
	FcRn binding (SPR, ELISA)
	CDC (cell-based assay)
	C1q binding (ELISA)
Off-target binding	VEGF-B (SPR)
	VEGF-C (SPR)
	VEGF-D (SPR)
	PIGF-1 (SPR)
	PIGF-2 (SPR)

BAC, boronate affinity chromatography; BLI, bioluminescence imaging; CDC, complement dependent cytotoxicity; CE-SDS, capillary electrophoresis-sodium dodecyl sulfate; CEX, cation exchange chromatography; CGE, capillary gel electrophoresis; cIEF, capillary isoelectric focusing; DSC, differential scanning calorimetry; HDX-MS, hydrogen/deuterium exchange mass spectrometric; DLS, dynamic light scattering; ELISA, enzyme-linked immunoassay; FFF, filed flow fractionation; HEK293 RGA, reporter gene assay based on the HEK-293 cell; HIC, hydrophobic interaction chromatography; HILIC-UPLC, hydrophilic interaction ultra performance liquid chromatography; HPLC, high-performance liquid chromatography; HUVECs, human umbilical vein endothelial cells; iCIEF, imaged capillary electrophoresis focusing; KDR, kinase insert domain receptor; LC-ESI-MS/MS, liquid chromatography electrospray ionization tandem mass spectrometric; MFI, micro-flow imaging; PIGF, placental growth factor; SEC-MALS, multi-angle light scattering coupled with size exclusion chromatography; RPLC-UV/MS, reversed phase liquid chromatography-ultraviolet/mass spectrometry; SEC, size exclusion chromatography; SPR, surface plasmon resonance; SV-AUC, sedimentation velocity-analytical ultracentrifugation; TNF, tumor necrosis factor; UV-280, ultraviolet absorbance at 280 mm wavelength; VEGF, vascular endothelial growth factor.

by measuring antiproliferative effects in a mixed lymphocyte reaction (MLR).

In all cases, 100% of adalimumab biosimilar batches were within the reference range for binding to mTNF.

c. Binding to VEGF. Bevacizumab is an IgG1 mAb which binds to VEGF-A and prevents the signaling of VEGF receptors.[38] VEGF comprises at least 16 different isoforms due to alternate mRNA splicing. Inhibition of VEGF-A blocks the proliferation of vascular endothelial cells and angiogenesis. Although soluble VEGF isoforms ($VEGF_{121}$ and $VEGF_{165}$) are the most predominant isoforms in tumors, cell-associated VEGF (such as $VEGF_{189}$ and $VEGF_{206}$) is also expressed in a significant number of lung and colon cancers.[38]

For all bevacizumab biosimilars, 100% of batches were within the reference range for at least 2 of 3 VEGF isoforms. Binding to $VEGF_{165}$ could be demonstrated for all batches and binding to $VEGF_{121}$ and $VEGF_{189}$ was observed for all but one product (product L and product H, respectively). Binding to $VEGF_{206}$ was frequently not performed by applicants which was accepted because this isoform is seen as less important.[39]

Cell-based assays. Cell-based potency assays are considered highly important for determination of biosimilarity. In the absence of comparable biological activity, a product cannot be approved as a biosimilar. For adalimumab biosimilars, the functional cell-based assays were based on measuring adalimumab inhibition of TNFα mediated cell death. In 6 out of 7 adalimumab biosimilars, all 100% of batches were within the similarity range and for one product (product F), ≥ 90% of batches were within the similarity range. For bevacizumab biosimilars, an HUVEC-based antiproliferation assay was used, and, in all cases, 100% of batches were within the similarity range.

Fc-related assays. Adalimumab is known to induce antibody-dependent cellular cytotoxicity (ADCC) through the binding of the Fab region to mTNFα and the Fc region to FcγRIIIa, which is expressed on effector cells, such as NK cells (mainly via high affinity receptor genotype 158 v/v).[40] It is well-known that the binding of IgGs to FcγRIIIa is influenced by the glycan profile of the antibody. For example, levels of afucosylated glycans are generally correlated with ADCC activity. All seven adalimumab biosimilar applicants performed one or more comparative ADCC assays, which usually included peripheral blood mononuclear cells (PBMCs) or natural killer (NK) effector cells. As shown in **Table 1**, 100% of biosimilar batches were within the reference range for ADCC for all products. Although most applicants used 1 or 2 ADCC assay formats, for 1 biosimilar product, ADCC activity was measured using more than 20 different ADCC assay setups (see **Table 2**). This is an example of the large variety of assays that can be used to study a single QA.

Activation of complement-dependent cytotoxicity (CDC) is also viewed as a relevant MoA for adalimumab. For all adalimumab biosimilars, 100% of batches were within the reference range for CDC activity.

Bevacizumab is theoretically capable of mediating Fc-related effector functions. However, none of the authorized bevacizumab biosimilars displayed ADCC or CDC activity (represented as green vertical stripes in **Table 1**, meaning it was tested but not found), which is in line with previously published results for originator bevacizumab.

Fc binding assays. Neonatal Fc receptor (FcRn) has been shown to play a role in regulating IgG levels in the serum through recycling of bound antibodies, with an impact on the serum levels of therapeutic mAbs.[41] For this reason, binding to FcRn is considered as a critical QA.[42] In all cases except one bevacizumab biosimilar (product I), 100% of batches were found to be within the similarity range.

The results of binding assays for five other FcγR (FcγRIa, FcγRIIa, FcγRIIb, FcγRIIIa (158 f/f), and FcγRIIIb) showed a variable percentage of batches lying within the reference range. Only 3 of 7 adalimumab products had ≥ 90% or 100% of batches within the similarity range for all 5 FcR binding assays (products D, E, and G). Binding to the FcγRIIIa by therapeutic mAbs is known to enhance ADCC activity (relevant MoA for adalimumab). For the high affinity FcγRIIIa 158 v/v genotype, 6 out of 7 adalimumab biosimilars had 100% of batches within the similarity range and for one product ≥ 90% of batches were within the similarity range (product A). For bevacizumab biosimilars, the results were more variable, with between one (product L) and 4 (products J and K) FcγR binding assays having < 90% batches within similarity range. However, as discussed above, bevacizumab does not exhibit effector function, and therefore binding to FcγR is not considered a critical aspect of biosmiliarity.[30,31]

Glycosylation profile. Tests for glycosylation profile included as a minimum: G0F, G1F, G2F, afucosylation, sialylation, and high mannose content. It can affect the immunogenicity and, in some cases, (adalimumab) also the functionality of the mAb. In most cases, < 90% of batches tested were within the similarity range or the assay was not performed. This is not unexpected, because it is known that the glycoprofile is highly dependent on the cell line that is used as expression system, media, and several growth conditions.[43,44] Although differences in glycoprofile could impact the ADCC activity, in all cases for adalimumab, the ADCC activity was shown to be highly similar.

Purity testing. The purity/impurity profile is viewed as critical by the EMA as certain impurities may impact on safety and immunogenicity. Protein impurities can be measured by size exclusion chromatography (SEC) and capillary electrophoresis-sodium dodecyl sulfate (CE-SDS) under reduced and non-reduced conditions to detect several relevant impurities, such as fragmentation, truncation, and aggregation. For both the adalimumab and bevacizumab biosimilars, there were several instances where < 50% of the batches were inside of the similarity range. However, during the assessment process these differences were judged to be irrelevant in terms of safety and efficacy. In most cases, this was due to the fact that the absolute difference in impurity levels was so small as to not to be clinically meaningful. Furthermore, the purity is controlled by the release specification.

Charge variants. Due to the complex contribution of numerous QAs to the overall charge profile of mAbs, charge variations may quantitatively differ between biosimilars and their RP. As can be seen in **Table 1**, in most cases, the charge profile differed, with only one adalimumab biosimilar (product E) with 100% batches within similarity range. Differences in charge profile can generally be accepted, provided applicants justify why any observed charge differences would not have an adverse clinical impact.

Additional assays. Additional assays include, for example, inhibition of TNFα-induced apoptosis and inhibition of release of IL-8 or sVCAM-1 in cell culture (not included in **Table 1**) for adalimumab, and induction of HUVEC migration or apoptosis, site-specific phosphorylation of VEGFR2, and HEK293 VEGF reporter assay for bevacizumab biosimilars. These assays are not considered mandatory but can be useful to strengthen the claim of biosimilarity.

For the majority of adalimumab biosimilars, 100% of batches were within the reference range when measuring induction of apoptosis. For one biosimilar (product A), this function was not addressed or outside the similarity range. Data from MLR studies were provided for all adalimumab biosimilars, with 3 products (C, D, and G) showing < 90% batches within range. Only one bevacizumab biosimilar (product J) had all additional functions assessed and with 100% batches within range.

However, due to the inherent variability of these assays and the low numbers of batches tested, the evidence provided by these assays was considered supportive only.

Table 4 provides a summary of the instances where < 100% of batches were within the reference range. In each case, the reason why these differences were accepted by the EMA is explained.

RESULTS OF CLINICAL COMPARABILITY STUDIES

The clinical results obtained for each adalimumab biosimilar are provided in **Table S1**.

The clinical results obtained for each bevacizumab biosimilar are provided in **Table S2**.

In the following section, results of PK analyses (obtained in healthy volunteers (HVs) and patients), efficacy analyses of clinical trials in patients and safety, and immunogenicity evaluation (obtained from PK and efficacy studies) are presented.

PK studies

PK in healthy volunteers. In **Tables S1** and **S2**, the primary end points with prespecified margins and all secondary end points, including safety and immunogenicity, are presented.

Observation period. For adalimumab biosimilars, the length of follow-up ranged from 62 to 71 days, and for bevacizumab biosimilars from 85 to 100 days, which represents ~ 5 half-lives.

Primary end point. In all instances the primary end points (area under the curve to infinity (AUC_{inf}), maximum concentration (C_{max}), and AUC from time of administration up to the time of the last quantifiable concentration (AUC_{last})) were contained within the prespecified acceptance range of 0.8–1.25. For three adalimumab products (Hyrimoz/Halimatoz/Hefiya, Hulio, and Amsparity) and one bevacizumab (Alymsys/Oyavas) the end points were such that unity was not included in the 90%

Table 4 QAs with <100% of batches meeting similarity ranges and how the resulting uncertainty during MAA and how this was resolved

Adalimumab	QA	Percentage of batches within the similarity range	How resolved
Product F	Protein content	≥90% of batches	The small difference in protein content was concluded to be of no clinical relevance. Batch-to-batch variability of the biosimilar within the expected range.
Product F	Cell based potency assay	≥90% of batches	Minor difference not expected to affect the clinical performance of the product.
Products A,B,C,F	Binding to several FcγR receptors (FcγRIa, FcγRIIa, FcγRIIb, FcγRIIIa-158 f/f and FcγRIIIb)	Variable, see **Table 1**	Minor differences in binding results, similarity confirmed in cell-based functional assays.
Product A	Binding to FcγRIIIa 158v/v	≥90% of batches	Viewed as sufficient based on ADCC assay results
Product A – G (all)	Glycosylation (7 attributes)	Variable, often <90%, see **Table 1**	Similarity confirmed in cell-based functional assays. No clinically significant difference in PK profile.
Products A, B, C, D, and F (all except E)	Purity testing	Variable, often <90%, see **Table 1**	Based on regulatory experience, the small difference was seen as negligible. In most cases purity of biosimilar was marginally increased.
Products A, B, C, D, and F (all except E)	Charge variants	Variable, often <90%, see **Table 1**	Acceptable based on product understanding.
Product A	Apoptosis induction	<50% of batches or not done	The assay is not considered as highly critical, accepted based on high similarity for binding to transmembrane TNFα. Alternative assay used as a functional readout of transmembrane TNF binding, e.g., MLR.
Product B	Apoptosis inhibition	Variable, <90% in one case, see **Table 1**	Additional orthogonal assays supported biosimilarity. Accepted based on the totality of evidence.
Bevacizumab	**QA**	**Percentage of batches within the similarity range**	**How resolved**
Product L	Protein content	≥90% of batches	The small difference in protein content was concluded be of no clinical relevance. Batch-to-batch variability of the biosimilar within the expected range.
Product L	Binding to VEGF 121	<50% of batches or not done	High similarity for binding to other VEGF isoforms confirmed using orthogonal methods
Product H	Binding to VEGF 189	<50% of batches or not done	High similarity for binding to other VEGF isoforms confirmed using orthogonal methods
Products H, I, J, and L (all except K)	Binding to VEGF 206	Variable, often <90%, see **Table 1**	High similarity for binding to other VEGF isoforms confirmed using orthogonal methods VEGF 206 is a less frequent isoform in human tissues[39]
Product I	Binding to FcRn	≥90% of batches	Based on regulatory experience and the results from the comparative PK study, the minor difference was seen as negligible.
Products H–L (all)	Binding to several FcγR receptors	Variable, see **Table 1**	Binding to Fcγ receptors are not considered critical for the mode of action of bevacizumab.
Products H–L (all)	Glycosylation (7 attributes)	Variable, often <90%, see **Table 1**	Due to the lack of Fc functions for bevacizumab, glycosylation pattern is not critical for bevacizumab. The PK profiles demonstrated similarity.
Products H–L (all)	Purity testing	Variable, often <90%, see **Table 1**	Based on regulatory experience, the small difference was seen as negligible.
Products H–L (all)	Charge variants	Variable, often <90%, see **Table 1**	Acceptable based on product understanding.
Products H, I, K, and L (all except J)	Additional functional assays	Variable, often <90%, see **Table 1**	The assays are not considered as highly critical, differences accepted based on the totality of evidence presented for similarity.

ADCC, antibody-dependent cell-mediated cytotoxicity; QAs, quality attributes; MAA, marketing authorization assessment; MLR, mixed lymphocyte reaction; PK, pharmacokinetics; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

confidence intervals (CIs), which may be permissible.[45] Root cause analysis for not being included in the 90% CI was performed by additional supplementary analyses on the primary end point and scrutinizing relevant QAs (for example, high mannose and sialic acid) with no negative signals.

In addition, for two adalimumab products (Hyrimoz/Halimatoz/Hefiya, and Hulio), initially failed and subsequently successful PK studies were submitted. Root cause analysis was performed, without finding analytical dissimilarities that could have explained the initial failure to show bioequivalence. Further, in both instances, a second, more strictly standardized PK study was conducted with reduced intersubject variability, and PK similarity was shown.[6,14]

Population PK in patients. For some products, PopPK data were collected in a subset of patients as part of the clinical efficacy/safety study, with trough plasma concentration (C_{trough}) as an end point, as recommended in the mAb guideline.[10]

For adalimumab biosimilars, samples were typically collected at 5 timepoints (sparse sampling) for all patients in the initial 6 months study period and, in some instances, until week 50 or even 60. In all instances, PopPK results were considered comparable.

For bevacizumab biosimilars, C_{trough} was typically collected at time zero (baseline), and at weeks 4, 7, 13, and 19. Acceptable PopPK data were provided in four of six cases; for Alymsys, PopPK analysis was not carried out, and for Abevmy, the data set was viewed as insufficient after assessment. In both cases, the insufficiency of comparative PopPK data in patients was justified by proven PK similarity in HVs and totality of evidence from other parts of the similarity exercise.

Clinical efficacy studies. Adalimumab is currently approved in 13 autoimmune indications.[46] Four applicants chose to compare efficacy in subjects with rheumatoid arthritis (RA) as a model indication in the clinical trial. Three applicants chose chronic plaque-type psoriasis as the model indication. Both indications are viewed as sufficiently sensitive by the EMA to detect potential clinically relevant differences between the biosimilar and the RP due to the large treatment effect.

Bevacizumab is currently approved in six indications in the European Union.[47] All applicants chose newly diagnosed or recurrent stage (IIIB)/IV nonsquamous non-small cell lung cancer (NSCLC) as the most sensitive model indication due to the large treatment effect.

Observation period. The length of follow-up was typically 1 year for all adalimumab and bevacizumab biosimilars (for Hulio, follow-up was 24 weeks with an extension trial proceeding up to 1 year).

Primary end point. For adalimumab biosimilars, American College of Rheumatology Response (ACR 20) and Psoriasis Area and Severity Index (PASI) score were chosen as primary end points for RA and psoriasis, respectively. The equivalence margins for the risk difference varied between ± 10% and ± 15% for RA and ± 15% and ± 18% for psoriasis, depending on the number and nature of trials performed with the RP that were included in the meta-analysis to derive the equivalence margin. In all instances, the 95% CI for the primary end points were within the prespecified equivalence margins and all other secondary end points also supported similar clinical performance. Results obtained in both analysis sets (intention to treat (ITT); per protocol set (PPS)) were concordant in all instances. Secondary end points in the trials were ACR 50 and ACR 70 scores, and Disease Activity Score (DAS) 28 in RA, and PASI 50, 75, and 90 scores in psoriasis, as well as additional efficacy measurements at different timepoints.

For bevacizumab products, the predefined equivalence margin for the risk difference of overall response rate (ORR) varied between ± 12 and ± 13% depending on the chosen reference studies. The 95% CI for the ORR was fully contained within the prespecified acceptance range for all five substances. Results of secondary end points of progression-free survival (PFS) and duration of response (DOR) generally provided further support for biosimilarity. However, for Alymsys, PFS was seemingly worse for the biosimilar with a hazard ratio (HR) of 1.2 (0.98, 1.46); median (weeks): 36.0 (33.57–36.86) vs. 43.0 (36.14 to 45.14). Nevertheless, this finding was not viewed as critical as the primary end point was met and the study was not designed to demonstrate equivalence for PFS.

Secondary end points. Time-dependent end points were included as secondary end points, but are less sensitive and informative for conclusions on biosimilarity than end points reflecting the MoA, because they are likely influenced by patient-related factors, such as general health status. For bevacizumab biosimilars, median overall survival (OS) could not be estimated for either group in all instances due to limited observation time and due to the fact that > 50% of patients were still alive at the cutoff. In instances where the HR for OS was > 1.0 (e.g., Abevmy and Alymsys), suggesting higher mortality in the biosimilar group, the OS results were viewed with caution by the EMA because the studies were neither adequately powered to demonstrate equivalence, nor to detect differences in OS, and no type 1 error control was included.

Clinical safety data. Safety parameters, such as treatment emergent adverse events (TEAEs), adverse events of special interest (AESi), serious adverse events (SAEs), deaths, and TEAEs leading to discontinuation were comparable between groups in most cases, as seen in **Tables S1** and **S2**. Adverse events (AEs) were mild to moderate and the adverse episodes resolved in all instances with no deaths reported.

It should be noted that clinical trials are not powered for safety end points, because this is considered unnecessary and would usually require several thousand study participants.

Clinical immunogenicity data. Adalimumab is a highly immunogenic product and antidrug antibodies (ADAs) were detected in 30–88% of subjects across all trials. The variability may be explained by differences in study populations and sensitivity of antibody

Table 5 Discrepancies in clinical attributes and how they were resolved

Adalimumab	Clinical attribute	Observation	How resolved
Hyrimoz/Halimatoz/ Hefiya, Hulio, and Amsparity	PK	Unity was not included in the 90% CI	1. Permissable[44] 2. Relevant QAs (high mannose, sialic acid) showed close similarity
Hyrimoz/Halimatoz/ Hefiya and Hulio)	PK	Initial study failed to meet predefined acceptance range	1. Root cause analysis 2. Subsequently, successful PK studies were submitted
Bevacizumab	**Clinical attribute**	**Observation**	**How resolved**
Alymsys/Oyavas	PK	Unity was not included in the 90% CI	1. Permissable[44] 2. Relevant QAs (high mannose, sialic acid) showed close similarity
Alymsys/Oyavas	Pop PK	Not carried out	1. Pop PK only supportive 2. PK similarity proven in HV
Abevmy	Pop PK	Insufficient	1. Pop PK only supportive 2. PK similarity proven in HV
Alymsys/Oyavas	PFS	HR of 1.2 (0.98, 1.46); median (weeks): 36.0 (33.57–36.86) vs 43.0 (36.14 to 45.14).	1. Primary endpoint (ORR) met 2. Study not designed to demonstrate equivalence for PFS 3. Totality of evidence in overall biosimilarity assessment
Abevmy, Alymsys/ Oyavas	OS	HR for OS > 1.0; suggesting higher mortality in the biosimilar group	1. Primary endpoint (ORR) met 2. Study not designed to demonstrate equivalence for OS 3. Totality of evidence in overall biosimilarity assessment

CI, confidence interval; HV, healthy volunteers; HR, hazard ratio; ORR, overall response rate; OS, overall survival; PK, pharmacokinetic; PFS, progression-free survival; PopPK, population PK.

assays used. Importantly, patients with ADA-positive samples at any time were similar between treatment arms for all biosimilar products.

Bevacizumab is a low immunogenic product and bevacizumab ADAs and neutralizing antibodies were rarely detected, except for higher percentages observed for Alymsys/Oyavas and Aybintio/ Onbevzi; however, they were similar between treatments arms and almost all ADAs were transient and appeared not to have effects on PKs or safety.

A summary of all observations with clinically deviating results is provided in **Table 5**.

DISCUSSION

In the early years of biosimilar development, it was considered that even with a convincing quality and PK package, there would always be some "residual uncertainty" which in most cases could only be addressed by a sufficiently powered efficacy study in patients. However, since then, the discriminatory power of the analytical methods used has vastly increased. EU-regulators have gained a large body of knowledge on the quality profile of several mAbs, adalimumab and bevacizumab being just two examples. As shown in **Tables 2** and **3**, the panel of analytical testing for biosimilars is very comprehensive, with numerous orthogonal methods used to analyze dozens of QAs. Therefore, every relevant aspect of the mAb structure and activity is interrogated to ensure that it is sufficiently aligned with the RP in order to guarantee comparable clinical efficacy and safety.

This is the first study that performs an in-depth analysis of all quality and clinical data for currently authorized biosimilars of two originator mAbs used in either oncologic (5 products) or in autoimmune indications (7 products).

Based on information provided to the EMA in the MA submissions, and following the scientific evaluation carried out by the Agency, we found that over 90% (and in most cases 100%) of the biosimilar batches met the EU-RP similarity range for critical QAs. A lower percentage of biosimilar batches were within the similarity range for QAs which may be considered less critical to safety and efficacy, such as glycosylation profile or charge variants (see **Tables 1, 4, 5**).

The most critical QAs for the determination of biosimilarity are those that could have an impact on the PK profile, on safety (including immunogenicity) and efficacy. Therefore, for high criticality QAs, a high degree of similarity to the RP is expected. Of the numerous QAs studied, the ones which as part of the assessment process were considered by the EMA to be of high criticality for determination of adalimumab biosimilarity, and which have demonstrated high concordance (marked solid green) are: protein content, soluble TNFα binding, transmembrane TNFα binding, FcγRIIIa binding, FcRn, biological activity (as measured in cell-based TNFα neutralization assay), ADCC, CDC, and a functional read out of reverse signaling (e.g., apoptosis induction). C1q binding is also considered critical, but these data were not presented in our analyses due to problems with anonymity. Similarly, high criticality for determination

of bevacizumab biosimilarity (as considered by the EMA and marked solid green) are: protein content, biological activity (as measured in cell-based antiproliferation assay), binding to main VEGF isoforms, and FcRn binding.

If < 100% of batches were within the similarity range for these highly critical QAs, data from relevant additional analytical and functional assays were reviewed in order to establish that the variations will not lead to differences in clinical performance of the biosimilar (see **Table 4**).

For all adalimumab products (A–G in **Table 1**) > 90% of batches were within the similarity range for highly critical QAs: protein content, potency, ADCC, CDC, and binding to soluble TNFa, mTNFa, FcγRIIIa (158 v/v), and FcRn. Regarding less critical QAs, < 90% of biosimilar batches were within the similarity range (for instance, products A and E, indicated as dark blue dots or light blue diagonal stripes). This was considered acceptable and the PK trial demonstrated no impact on safety/immunogenicity. In addition, the clinical data were supportive of biosimilarity.

Regarding the differences found in glycosylation, although high similarity was observed for all adalimumabs compared with the RP Humira with regard to FcγRIIIa (158 v/v) binding, as well as for ADCC and CDC activity, none of the adalimumab biosimilars had a fully comparable glycoprofile to the RP Humira. The specific glycoprofile is highly dependent on the manufacturing process, including the cell line and growth conditions used, therefore manufacturing an mAb with a highly similar glycoprofile is challenging. In all cases, the minor differences in glycoprofile were justified not to have a functional impact through orthogonal methods, for example, leading to a difference in ADCC activity. Therefore, any observed differences in the glycoprofile between the biosimilar and the originator were justified not to affect the clinical performance of the biosimilar.

For bevacizumab products (H–L), again, for highly critical QAs, ≥ 90% of batches were within the similarity range (i.e., protein content, HUVEC antiproliferation assay, and binding to FcRn and VEGF165). For products H and L, < 90% of biosimilar batches were within the similarity range for binding to VEGF189 and VEGF121, but this was accepted given as binding to other VEGF isoforms was highly similar. For products I and K, differences were apparent in several purity and glycosylation attributes, but again this was accepted by the EMA during the MA evaluation as Fc functionality (glycosylation profile) is not critical for bevacizumab.

Assessing the PK trial demonstrated no impact on safety/immunogenicity of patients and further clinical data were also supportive of biosimilarity.

Regarding the differences found in purity, these need to be justified or appropriately clinically qualified as they may affect efficacy and safety, including immunogenicity. In some instances, there were minor differences in impurity levels between the biosimilars and RP, with some biosimilars showing slightly higher purity levels and some slightly lower. However, in all cases, differences were considered minimal in absolute numbers and were justified to have no impact on safety or efficacy. Further, no immunogenicity signals were observed in the clinical PK or efficacy trials.

As a general comment, the list of critical QAs known to be of high importance for determination of biosimilarity should not be interpreted in such a way that these are the only QAs of interest and other QAs do not need to be studied. Rather, if differences between the biosimilar and the RP are detected, the biosimilar applicant needs to justify the impact of the difference. Moreover, although we did not include them in our analysis because the data could not be categorized in a quantitative way, the amino acid sequence secondary and higher order structure of a biosimilar is expected to be the same or highly similar to the RP and therefore these are also considered critical QAs. Information presented on the number and type of conducted assays for all products should not be leveraged by future developers as the presented results are reviewed by regulators on a case-by-case basis.

Several scientists[6,14,16,48,49,50] pointed out limitations of indiscriminative efficacy and safety studies in light of technical advances in analytical methods which provide more discriminative research tools. Even large molecules can currently be thoroughly characterized using state-of-the-art analytical and *in vitro* functional testing. This thorough characterization is also routinely applied as part of comparability studies conducted for biological medicinal products following introduction of manufacturing process changes.[51–53] The recently established EMA tailored scientific advice pathway for biosimilars acknowledges these scientific advances.[54]

For all adalimumab and bevacizumab biosimilars studied, a comprehensive clinical program was submitted consisting of a PK trial and a clinical efficacy study, which confirmed biosimilarity.

Secondary efficacy end points, safety, PopPK, and immunogenicity end points were always descriptive in nature and results generally concordant with those of the primary end points. In those cases where a trend toward a possible difference was observed, it was judged to be negligible and/or likely due to immaturity of the data. In some cases, certain data were not obtained or incomplete. These deviating or missing results did not preclude approval, as similarity was shown for the relevant QAs and in dedicated PK studies and confirmed by clinical data in the efficacy study. As this paper analysed already approved biosimilar products, and subsequent experience with these products to date has not resulted in any safety or efficacy issues following their approval,[16,55,56] it can be concluded that the regulatory decisions taken were correct.

Our study supports previous observations[48] that adequately powered PK trials, provide sufficient clinical safety and immunogenicity data, especially when close similarity in analytical and functional parameters together with comparable PK and impurity profiles can already largely predict similar safety and immunogenicity of the biosimilar and the RP.

As stated by Kurki *et al.*[48] the intrinsic immunogenicity observed for each RP was also observed for the respective biosimilars. In no instance did RPs with high immunogenicity have a biosimilar with low immunogenicity, or vice versa. Observations regarding comparability of immunogenicity made in the PK trial were, in all instances, confirmed in the efficacy/safety study. Similar observations were made with regard to safety parameters.

Furthermore, the EU pharmacovigilance systems and risk management planning are sufficiently robust[16,55,56] to detect safety signals in postmarketing use. However, safety signals (including reports on reduced efficacy) are not anticipated, because more than

a decade of clinical experience indicates that a new safety signal solely identified with a biosimilar is extremely unlikely.[55,56]

For the biosimilars included in this study, differences in several QAs were found. As part of the EMA approval process, applicants were challenged to justify that the observed differences would have no impact on the clinical performance of the biosimilar. Importantly, in all cases, these questions were answered by applicants based either on quality data alone or on a combination of quality, PK, and immunogenicity data (**Table 4**). In no instance were data from the clinical efficacy, safety, and immunogenicity study required to justify the differences at the quality level. On this basis, we argue that for the adalimumab and bevacizumab biosimilars, clinical efficacy, safety, and immunogenicity data were not needed to address residual uncertainty remaining from the quality and PK studies.

This analysis adds to the ongoing debate about the role of clinical studies for biosimilars.

In the authors' opinion, the usefulness of clinical efficacy, safety, and immunogenicity data for the purposes of regulatory decision could be questioned. Where the quality, PK, and immunogenicity data are sufficiently robust and convincing for regulatory decision making, as in the case of the adalimumab and bevacizumab examples cited in this paper, then it is our contention that the current expectations for clinical efficacy, safety, and immunogenicity could be re-examined. Therefore, we encourage a regulatory review of the standards for clinical data requirements for biosimilars, and propose that clinical data requirements should be further tailored.

Given the 10 years of regulatory experience in assessing and approving biosimilars, and the positive performance of approved biosimilar mAbs on the market,[6,14,48,57] the authors suggest to move to a concept of "tailored evidence," depending on the nature of the product and the available orthogonal assays for quality similarity. For example, this could include removing the standard requirement for equivalence trials, accepting wider equivalence margins, omitting PopPK studies, and/or reducing secondary clinical end points.

Such tailored approaches may prove particularly useful in the case of biosimilars for orphan medicines or other treatments where there is a small patient population or products with a narrow treatment effect where a comparative efficacy study may not be feasible due to the inability to recruit a sufficient number of subjects for any meaningful statistical analysis.

In the authors' opinion, if the efficacy study is omitted, sponsors may consider expanding their PK studies with regard to study size or observation period to gather additional safety/immunogenicity data. In other instances, a clinical study generating some limited safety and immunogenicity data in patients may be beneficial.

Where the quality package or the PK data are not sufficiently convincing, a root cause analysis would be necessary, potentially requiring changes to the manufacturing process of the biosimilar candidate or an improved design/power of the PK study, as was, for example, observed during biosimilar adalimumab development.[14] Alternatively, a stand-alone application could be pursued. Whereas the analysis in our study is based on adalimumab and bevacizumab as representative examples, the principle could be generalized to mAbs as a class.

In conclusion, in the author's opinion, a tailored evidence approach for all biosimilars including mAbs and fusion proteins, where a robust and convincing analytical biosimilarity package is available in conjunction with an appropriately powered PK study that also provides safety and immunogenicity data, the extent of the clinical trial requirements can be further reduced, or such trials even omitted. This would allow for more rational use of clinical resources, reduce the type of clinical data analyzed or number of clinical trials, and streamline the development of biosimilar mAbs and fusion proteins to the benefit of patients and healthcare stakeholders which is also in line with the strategic priorities of the EMA.[58]

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

E.G., N.E., S.B., M.W., and E.W.-H. wrote the manuscript. N.E., S.B., and E.W.-H. designed the research. E.G., N.E., S.B., and E.W.-H. performed the research. N.E., S.B., E.G., and E.W.-H. analyzed the data.

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ORIGINAL RESEARCH ARTICLE

Do the Outcomes of Clinical Efficacy Trials Matter in Regulatory Decision-Making for Biosimilars?

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Abstract

Background There is an increasing body of evidence supporting a more flexible approach in clinical data requirements for the approval of more complex biosimilar substances such as monoclonal antibodies (mAbs).

Objective The aim of this paper is to further analyse the role of quality/chemistry, manufacturing and controls (CMC) and clinical data for the conclusion on biosimilarity and the decision on marketing authorisation (MA).

Methods In the present study, we analysed the MA applications (MAAs) of all 33 mAbs and three fusion proteins evaluated by the European Medicines Agency (EMA) between July 2012 and November 2022 with special emphasis on all submitted rituximab (four products) and trastuzumab (seven products) biosimilar candidates, including withdrawn applications. For the two withdrawn applications, the comparative efficacy trials suggested biosimilarity, but the quality/CMC package was not accepted by EMA. We therefore investigated whether a negative MAA outcome could have been predicted based on the evidence generated in the quality/CMC packages, regardless of clinical trial data. For this purpose, we reviewed the respective European Public Assessment Reports (EPARs) or withdrawal assessment reports, and the first regulatory assessments for all these 36 MAAs (i.e. day 120 of the centralized procedure), which are not publicly available. During EMA review, where significant issues are identified which would preclude a marketing authorisation, these issues are raised as questions to the applicant and are classified as major objections (MO).

Results In 67% of cases, the outcome of the quality and clinical assessment was the same, i.e. both the quality and clinical assessments either supported approval or did not support approval. In 11% of cases, MO were identified in the quality part of the submission but not in the clinical data. In 22% of cases, MO were raised on the clinical data package but not on the quality data. However, we found no instance where seemingly negative clinical data, including failed efficacy trials, led to a negative overall decision. In each instance, the failure to confirm similar clinical performance in all investigated aspects was eventually viewed as not being related to the biosimilar per se but as being due to imbalances in the trial arms, immaturity of secondary endpoint results, change in the reference product, or even chance findings. Furthermore, when performing an in-depth analysis of the quality and clinical packages of trastuzumab and rituximab biosimilars, we found that in no case were clinical trial data necessary to resolve residual uncertainties regarding the quality part.

Conclusion The results further support the argument that sufficient evidence for biosimilarity can be obtained from a combination of analytical and functional testing and pharmacokinetic studies which may also generate immunogenicity data. This calls into question the usefulness of comparative efficacy studies for the purposes of regulatory decision-making when approving biosimilar mAbs and fusion proteins.

1 Introduction

Biologic medicines are effective treatment options for complex conditions such as cancer and an increasingly important component of health care solutions.

However, patient access to highly effective biological medicines is still unequally distributed across countries, often due to the high cost of these medicines.

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The work contributed by EW-H has been carried out while she was an employee of PEI, member of the Scientific Advice Working Party and chair of the Biosimilar Medicinal Products Working Party.

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Key Points

In the regulatory assessment of 33 mAbs and three fusion proteins evaluated by EMA, we found no instance where seemingly negative clinical data, including failed efficacy trials, led to a negative overall decision.

In the analysis of quality and clinical packages of trastuzumab and rituximab biosimilar candidates, in no case were clinical trial data necessary to resolve residual uncertainties regarding the quality part.

Our analyses suggest that the quality/CMC part of the dossier appears to be predictive for the marketing authorisation of a biosimilar mAb or fusion protein candidate, irrespective of the outcome of the clinical trial.

In the authors' opinion, the findings of this study may allow a reduction of the clinical development program for regulatory review before marketing authorisation.

Biologics represent 35% of medicine spending in Europe at list prices and have been growing at an 11.3% compound annual growth rate over the past 5 years [1]. The expenditure on cancer medicines is growing at rates higher than the growth rates of the patient population and overall health expenditure [2].

Biosimilar competition, i.e. having multiple suppliers of the same active substance, is necessary to curtail overall healthcare costs and to avoid supply shortages. Although the savings from current biosimilar competition in the European Union (EU) market and patient access are improving, a growing disparity is occurring across countries [1].

The past 5 years have shown a maturation of the biosimilars market. However, it is estimated that in the period 2023–2027, 55% of biologics with loss of market exclusivity will be without competitors [1]. Products with low sales value are unattractive to biosimilar manufacturers due to clinical development costs, largely driven by large and lengthy clinical trials and procurement costs of reference product (RP) comparator batches. Cost estimates for developing a biosimilar range from 100 to 300 million US dollars [3], compared with 1–5 million US dollars for a small molecule generic, largely due to clinical development costs [4–6]. This cannot be in the interest of stakeholders, including regulators, and contradicts the strategic priorities of the European Medicines Agency (EMA) [7], which has set out to allow for more rational use of clinical resources.

Recent efforts have resulted in alternatives to costly comparative clinical studies for certain biosimilars, where the extent of clinical data required can vary depending on the complexity and characterisation of the molecule [8–10]. Also, several initiatives have been launched that could impact regulatory decision making and lead to revised guidelines such as omitting or reducing the size of studies involving human subjects for a larger set of biosimilar products [11, 12].

In a recent publication [13], the degree of analytical similarity and the role of clinical data were analysed for authorised adalimumab and bevacizumab biosimilars. It was argued that clinical trial requirements for monoclonal antibody (mAb)-biosimilars can be further reduced, or such trials even omitted, where a robust and convincing analytical biosimilarity package is available in conjunction with an appropriately powered pharmacokinetic (PK) study that also provides safety and immunogenicity data.

The remaining question is whether product candidates with good or promising quality data could nevertheless translate into poor (i.e. those with high uncertainty) or failed clinical trials, which may prevent approval of the product where otherwise it would have been approved.

The aim of this paper is to further analyse the role of quality/chemistry, manufacturing and controls (CMC) and clinical data for the conclusion on biosimilarity in a broader setting and in more depth, including all classes of approved biosimilar mAbs and refused or withdrawn biosimilar candidates, which likely would have failed marketing authorisation (MA).

We therefore analysed the final outcome of the submitted marketing authorisation applications (MAAs) of all 36 mAbs and fusion protein biosimilar candidates evaluated by EMA up to November 2022, including those for which a withdrawal/refusal assessment report (AR) is available on the EMA website, i.e. one trastuzumab and one rituximab biosimilar candidate, and contextualised these findings by analyses of all approved rituximab and trastuzumab biosimilar products.

We also reviewed the regulatory assessment during the first phase of these MAAs [i.e. the day 120 assessment reports (D120 AR) of the centralised procedure], which are not publicly available, to analyse whether quality data are predictive for clinical outcome and how clinical assessment impacts the decision on MA.

2 Methods

2.1 Analysis of MAA Outcome

We analysed the outcome of the MAAs, as well as the available regulatory ARs of all biosimilar mAbs and fusion proteins, i.e. adalimumab, bevacizumab, etanercept, infliximab, ranibizumab, rituximab, and trastuzumab, which included 33 mAbs

and three fusion proteins evaluated by the EMA between July 2012 and November 2022. This included biosimilar candidates, which received a MA or a negative opinion by the Committee for Medicinal Products for Human Use (CHMP) or which were withdrawn by applicants prior to a CHMP opinion; a European Public Assessment Report (EPAR) or a withdrawal AR for these candidates is available on the EMA website [14–49]. Concerns regarding quality/CMC (biosimilarity, general quality) or clinical aspects [PK/pharmacodynamic (PD), efficacy (E)/safety (S)/immunogenicity (I)], as indicated in the EPAR or withdrawal AR, were analysed. We categorised the 36 mAbs and fusion protein biosimilar candidates according to five possible scenarios (Fig. 1) indicating whether the quality/CMC, PK and clinical aspects of the biosimilar MAA dossier were acceptable to the EMA (shown in green/no pattern) or not acceptable (shown as horizontal red stripes). Vertical green stripes indicate some remaining uncertainties that were discussed within the EMA scientific committees and working parties, but not severe enough to prevent MA. Furthermore, the outcome of the MAA is indicated as well as the active substance and the IgG class.

2.2 Analysis of First Regulatory Assessment Reports

We analysed the list of questions (LoQ) raised by the CHMP in the D120 ARs of all above-mentioned 36 mAbs and fusion protein biosimilar candidates. D120 ARs are discussed and agreed by the CHMP during the first phase of the centralised MA procedure. They are shared with the applicant but are usually not publicly available [50].

They include formal regulatory aspects (Table 1) and the scientific evaluation of all quality/CMC, non-clinical and clinical data and the risk management plan that led to a LoQ regarding concerns and uncertainties that must be addressed by applicants before MA [51]. These questions are classified as major objections (MO) and other concerns (OC). MO are defined as critical issues that will preclude authorisation if not resolved [52], while OC should be resolved but are not severe enough to preclude authorisation per se.

We counted the number of MO and OC and classified those as either related to quality or clinical aspects, in the same manner as for the MAA outcome analyses (Fig. 2). Formal regulatory aspects were not included in the analysis because these are not of direct scientific concern in the context of this study.

2.3 Evaluation of Analytical and Clinical Biosimilarity for Rituximab and Trastuzumab Biosimilars

We analysed the quality/CMC and clinical data packages for all submitted rituximab and trastuzumab biosimilars to contextualise the results observed for the two withdrawn products [43, 44]. The analysis included four rituximab biosimilars [26–28, 43, 53, 54] and seven trastuzumab biosimilars [36–39, 44, 48, 49]. The data lock point for the analysis was February 2023.

Comparison of analytical biosimilarity [quality attributes (QA)] for approved biosimilars was performed using the methodology of our previous paper. Briefly, we extracted raw data from the biosimilar product dossiers, anonymised and colour-categorised them depending on the percentage of analysed biosimilar batches with values within the similarity range of the RP (Online Resource 1). For cases where less than 100% of batches were within the reference range it was analysed how the resulting uncertainty was resolved (Table 2).

For full details, see Guillen et al. [13].

For withdrawn applications, we also looked at quality issues of the biosimilar itself affecting performance and consistency of the manufacturing process, which must be ensured in line with current guidance [55]. Therefore, we constructed a figure (Fig. 3) that covers all these aspects, following the key quality/CMC requirements from Bielsky et al. as a reference [56]. Analytical data were extracted from the withdrawal ARs, which contain public information that can be found on the EMA website and therefore anonymisation is not necessary [57].

Comparison of clinical biosimilarity is presented in Online Resources 2–5 and employs the same methodology as Guillen et al. [13]. For cases where discrepancies were observed in clinical attributes, it was analysed how the resulting uncertainty was resolved (Table 3).

3 Results

3.1 MAA Evidence and Results

For 36 mAbs and fusion protein biosimilar candidates (mostly IgG1), the quality/CMC (i.e. general quality aspects and analytical comparability exercise), clinical PK/PD and clinical efficacy, safety and immunogenicity (E/S/I) aspects were analysed based on the information provided in the EPAR. Results are shown in Fig. 1 according to five possible scenarios.

For more than 80% of the biosimilar candidates analysed (29/36), the quality/CMC part of the dossier, the clinical PK/PD as well as the E/S/I results all unambiguously supported biosimilarity (Fig. 1, Scenario 1). For two biosimilar candidates, differences in some QAs and functional assays were observed [14, 15], but these differences were not seen in PK/PD and clinical E/S/I studies. One candidate had higher immunogenicity [15], later deemed irrelevant (see Discussion). All these biosimilar candidates listed for Scenario 1 obtained a MA.

	Cases	IgG type	Date of MA	Quality		Clinical		Reference
				biosimilarity	general Q	PK/PD	E/S/I	
	SCENARIO 1			+	+	+	+	
1	Infliximab 1	IgG1	10-09-2013					[14]
2	Infliximab 2	IgG1	26-05-2016					[15]
3	Infliximab 3	IgG1	18-05-2018					[16]
4	Etanercept 1	Mod. IgG1	13-01-2016					[17]
5	Etanercept 2	Mod. IgG1	23-06-2017					[18]
6	Adalimumab 1	IgG1	21-03-2017					[19]
7	Adalimumab 2	IgG1	24-08-2017					[20]
8	Adalimumab 3	IgG1	17-09-2018					[21]
9	Adalimumab 4	IgG1	02-04-2019					[22]
10	Adalimumab 5	IgG1	13-02-2020					[23]
11	Adalimumab 6	IgG1	11-02-2021					[24]
12	Adalimumab 7	IgG1	15-11-2021					[25]
13	Rituximab 1	IgG1	15-06-2017					[26]
14	Rituximab 2	IgG1	13-07-2017					[27]
15	Rituximab 3	IgG1	01-04-2020					[28]
16	Bevacizumab 1	IgG1	15-01-2018					[29]
17	Bevacizumab 2	IgG1	14-02-2019					[30]
18	Bevacizumab 3	IgG1	19-08-2020					[31]
19	Bevacizumab 4	IgG1	24-09-2020					[32]
20	Bevacizumab 5	IgG1	26-03-2021					[33]
21	Bevacizumab 6	IgG1	21-04-2021					[34]
22	Bevacizumab 7	IgG1	17-08-2022					[35]
23	Trastuzumab 1	IgG1	09-02-2018					[36]
24	Trastuzumab 2	IgG1	26-07-2018					[37]
25	Trastuzumab 3	IgG1	12-12-2018					[38]
26	Trastuzumab 4	IgG1	27-07-2020					[39]
27	Ranibizumab 1	IgG1	18-08-2021					[40]
28	Ranibizumab 2	IgG1	25-08-2022					[41]
29	Ranibizumab 3	IgG1	09-11-2022					[42]
	SCENARIO 2			-	-	+	+	
30	Rituximab 4	IgG1	not approved					[43]
31	Trastuzumab 5	IgG1	not approved					[44]
	SCENARIO 3			+	+	-	+	
32	Adalimumab 8	IgG1	10-11-2017					[45]
33	Adalimumab 9	IgG1	26-07-2018					[46]
34	Etanercept 3	Mod. IgG1	20-05-2020					[47]
	SCENARIO 4			+	+	+	-	
35	Trastuzumab 6	IgG1	15-11-2017					[48]
36	Trastuzumab 7	IgG1	16-05-2018					[49]
	SCENARIO 5			-	-	-	-	
	Does not apply to any of the biosimilars analyzed							

Fig. 1 Analysis of MAA outcome. Fulfilment of EMA requirements and outcome of marketing authorisation applications (MAAs) for monoclonal antibody and fusion protein biosimilar candidates based on information provided in the European Public Assessment Reports or Withdrawal Assessment Reports. Green (no pattern) indicates fulfilment of EMA requirements. Vertical green stripes indicate some remaining uncertainties not precluding marketing authorisation (MA). Horizontal red stripes indicate failure to meet EMA requirements. *EMA* European Medicines Agency, *E/S/I* efficacy, safety and immunogenicity, *IgG* Immunoglobulin G, *MA* marketing authorisation, *PD* pharmacodynamics, *PK* pharmacokinetics, *Q* quality

Table 1 Most frequent major objections

	MO regarding	Most frequent questions for MO
Quality	Formal aspects	GMP certificate missing EU GMP inspection pending Provision of a risk evaluation concerning the presence of nitrosamine impurities (EMA/369136/2020, EMA/409815/2020)
	Biosimilarity	Difference in critical quality attributes Insufficiency of ADCC assays used to conclude on biosimilarity Insufficient number of batches used for biosimilarity exercise, testing panel incomplete
	General quality	Manufacturing process In-process controls Comparability of clinical versus commercial batches of the biosimilar candidate Consistency of the manufacturing process Missing information or data to assess quality and comparability of the biosimilar candidate
Clinical	PK/PD	Investigation of observed PK differences/difference in biosimilarity regarding PK Clinical justification of the pre-specified margins of PK comparability PD analysis in second therapeutic area in case of extrapolation to all indications of RP Submission of individual patient data
	E/S/I—formal aspects	Confirmation of compliance with ethical requirements (directive 2001/20/EC) or with the principles of GCP and of the Declaration of Helsinki Pending GCP inspections 1-year safety and immunogenicity data not yet submitted at timepoint of initial submission in line with EMA Guideline (EMEA/CHMP/BMWP/42832/2005 Rev. 1)
	E	Failed primary endpoint analysis Differences observed for RP compared to published data
	S/I	Additional safety and immunogenicity data in case of observed ADAs Insufficient submitted data with respect to i.e. ADA and occurrence of neutralizing antibodies Justification for observed differences in safety profile

Major objections (MO) of the day 120 assessment reports were classified in same manner as for the MAA outcome analyses and examples for frequent questions regarding quality and clinical are given

ADA anti-drug antibodies, *ADCC* antibody dependent cellular cytotoxicity, *E* efficacy, *EMA* European Medicines Agency, *EU* European Union, *GCP* good clinical practice, *GMP* good manufacturing practice, *I* immunogenicity, *MAA* marketing authorization application, *MO* major objections, *PD* pharmacodynamics, *PK* pharmacokinetics, *RP* reference product, *S* safety

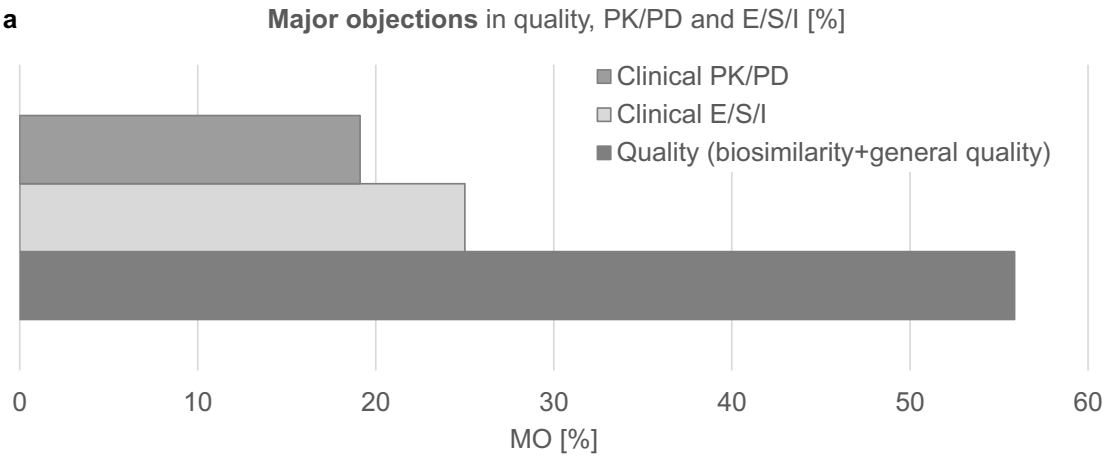
Scenario 2 applies to two cases with an unsatisfactory quality/CMC package but with overall acceptable clinical trial results (Fig. 1, Scenario 2). In these two cases [43, 44], major concerns were raised regarding the biosimilarity exercise as well as regarding the comparability of the clinical batches and the commercial batches of the biosimilar. The clinical PK and efficacy trials formally met their primary endpoints. However, uncertainties remained for the clinical efficacy trial regarding secondary and subgroup analyses for the rituximab biosimilar candidate [43]. Both applications were withdrawn by the companies owing to major remaining uncertainties expressed in unresolved quality MO.

Scenario 3 was defined as those product candidates having an acceptable quality/CMC package but indicating differences in the clinical PK/PD profile or remaining questions regarding representativeness of test material used in the PK study, while all other clinical data demonstrated comparability (Fig. 1, Scenario 3). Two of the biosimilar candidates analysed [45, 46] had an initially failed PK study. In both instances, it was argued that the observed differences in glycan structures known to affect PK (high mannose content) were too small to explain the initially observed PK differences [58]. The conduct of a second PK trial with improved design features was requested and led to successful demonstration of similar PK profiles [59]. For a third biosimilar candidate, PK results were not accepted because the test product was not deemed representative of the commercial product [47].

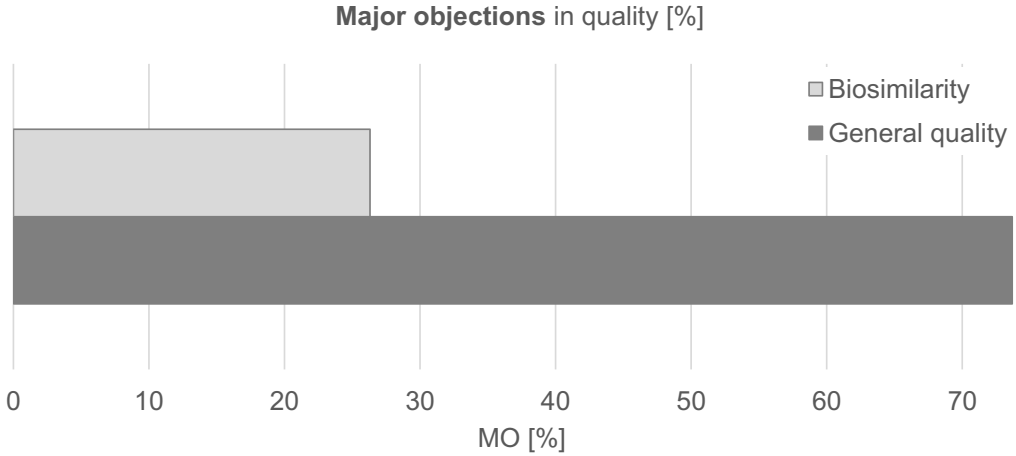
Scenario 4 lists those cases with an acceptable quality/CMC package and successful PK trial but with issues regarding the clinical E/S/I package (Fig. 1, Scenario 4). For both affected trastuzumabs [48, 49] the primary efficacy endpoint was formally not met as the upper limit of the confidence interval (CI) was not contained within the pre-defined equivalence margin. For both trastuzumabs, a MA was granted based on the convincing quality/CMC, PK, safety and immunogenicity data packages, despite a failed primary endpoint.

The last hypothetical scenario would be unconvincing quality/CMC data and failed clinical trials (PK and efficacy trial), which was not observed in any of the 36 cases (Fig. 1, Scenario 5).

a



b



c

Case	Quality MO	Clinical MO		% of biosimilar candidates applicable to each case*
		PK/PD	E/S/I	
1				42
2				11
3				22
4				25

*green/no pattern indicates no MO in the respective area (quality/CMC, PK/PD, E/S/I); red/striped indicates at least one MO in the respective area

3.2 Analysis of First Regulatory Assessment Reports

For the majority of biosimilar candidates analysed (34/36), the LoQ raised by the CHMP in the D120 AR was adequately addressed by the applicants and thus led to the final approval.

Analysing the number of MO for the 36 biosimilar candidates concerning scientific issues indicates that 56% of MO were related to quality/CMC, 19% to clinical PK/PD and 25% to clinical E/S/I issues, respectively (Fig. 2a). Within the quality/CMC part, the majority of MO dealt with general

◂**Fig. 2** Analysis of questions raised in the first assessment report of the MAA procedure. MO are classified in same manner as for the MAA outcome analyses. In case of MO within multidisciplinary aspects, the specific itemised questions were analysed and distributed according to their content to the quality/CMC or clinical (PK/PD, E/S/I) categories. MO regarding formal aspects were not included in the analysis because these are not of direct scientific concern. **a** For the comparison of the percentage of MO raised with regard to quality/CMC or clinical aspects of the MAAs, the sum of MO (quality/CMC, clinical PK/PD and clinical E/S/I) was calculated and normalised to the number of all MO. **b** MO related to the quality/CMC of the biosimilar candidate were analysed in more detail. Here the number of MO with regard to general quality/CMC or biosimilarity aspects was divided by the sum of all quality/CMC MO. **c** Based on the D120 AR, biosimilar candidates were categorised to four different cases with no MO (green/no pattern) or at least one MO (horizontal red stripes) in the respective area. The percentage of candidates represented by the different cases is indicated. *CMC* chemistry manufacturing and control, *D120 AR* day 120 assessment report, *E/S/I* efficacy, safety, immunogenicity, *MAA* marketing authorisation application, *MO* major objections, *PD* pharmacodynamics, *PK* pharmacokinetics

pharmaceutical issues rather than biosimilarity aspects (Fig. 2b).

Analysis of OC revealed a similar distribution with 64% of OC pertaining to the quality of the biosimilar candidates, 12% to PK/PD and 24% to E/S/I (data not shown).

When categorising the 36 biosimilar candidates based on where MO were raised, i.e. quality versus PK/PD versus E/S/I, we differentiated four cases, depending on whether MO were identified and knowing that any unresolved MO would prevent approval. We differentiated case 1 when assessment of quality and clinical parts of the dossier led to no MO (positive alignment) in 42% (15/36) of the MAAs analysed, thus supporting biosimilarity. Case 2 was when the quality assessment led to MO that, if not resolved, would lead to rejection of the filing. This applies to 11% (4/36) of MAAs analysed. For case 3, when quality assessment supported biosimilarity but clinical queries challenged the validity of the package, 22% (8/36) of cases were identified (8% of cases with MO regarding PK/PD, 11% with MO regarding E/S/I and 3% regarding both PK/PD and E/S/I). And finally, case 4, when both quality and clinical packages raised concerns (negative alignment), with 25% (9/36) of MAAs analysed (Fig. 2c).

The main reasons for MO are summarised in Table 1.

3.3 Evaluation of Analytical Biosimilarity and Clinical Comparability for Rituximab and Trastuzumab Biosimilars

Rituximab and trastuzumab biosimilar products were selected for further in-depth analysis of quality/CMC (Online Resource 1, Table 2; Fig. 3) and clinical data (Online Resource 2–5, Table 3) as these included withdrawn applications.

3.3.1 Comparison of Analytical Biosimilarity Across Products

The number of biosimilar batches analysed per product varied between 3 and 40, for most QAs. The analytical comparability packages of the rituximab and trastuzumab biosimilars comprised between 35 and 85 individual assays per product. For most of the QAs, orthogonal analytical methods were used.

Rituximab is an IgG1 kappa type mAb directed against CD20 expressed on the surface of pre-B and mature B lymphocytes, but not on hematopoietic stem cells and terminally differentiated antibody-producing plasma cells or other tissues. Upon binding to CD20, rituximab mediates B cell lysis (leading to B cell depletion) by three distinct mechanisms of action (MoAs): complement dependent cytotoxicity (CDC), antibody dependent cellular cytotoxicity (ADCC) and apoptosis [60]. Therefore, the biological activity of rituximab is determined by a combination of CD20 binding assay and an apoptosis induction assay, together with fragment crystallisable (Fc) functionality. Besides activating the pathways of CDC and ADCC, binding of rituximab to its target (CD20 expressed on B cells) also triggers apoptosis via the caspase signalling pathway [61]. Antibody-dependent cellular phagocytosis (ADCP) has been further implicated as plausible MoA of rituximab in its killing of chronic lymphocytic leukaemia cells [60, 62].

Trastuzumab is an IgG1 mAb which binds to human epidermal growth factor receptor 2 (HER2), a transmembrane oncoprotein overexpressed in approximately 20–25% of invasive breast cancers [63]. Binding of trastuzumab to HER2 inhibits ligand-independent HER2 signalling and prevents the proteolytic cleavage of its extracellular domain, an activation mechanism of HER2. As a result, trastuzumab inhibits the proliferation of human tumour cells that overexpress HER2. Therefore, the biological activity of trastuzumab is determined by the combination of HER2 binding assay and an inhibition of cellular proliferation assay, together with Fc functionality. However, in contrast to rituximab, CDC activation is not thought of as a MoA of trastuzumab [64].

For other fragment antigen binding (Fab) mediated assays, glycan and purity profile and charge variants we followed a similar categorisation as in our previous paper [13]. Additional assays include, for example, ADCP for both rituximab and trastuzumab, and inhibition of vascular endothelial growth factor (VEGF) secretion for trastuzumab.

Online Resource 1 provides a summary of the analytical biosimilarity results for approved rituximab (products A–C) and trastuzumab (D–I) biosimilars, and Table 2 provides a summary of the instances where less than 100% of batches were within the reference range and how the resulting uncertainty was resolved.

Table 2 QAs with < 100% of batches meeting similarity ranges and how the resulting uncertainty during MAA was resolved

	QA	Percentage of batches within the similarity range	How resolved
Rituximab			
Product B	Cell based CD20 binding assay	≥ 90% of batches	Minor difference not expected to affect the clinical performance of the product Slight differences explained and justified by the method variability
Products B and C	Binding to several Fcγ-Receptors (FcγRI, FcγRIIa, FcγRIIb, FcγRIIIa-158 f/f and FcγRIIIb	Variable, see Online Resource 1	Minor differences in binding results, similarity confirmed in cell-based functional assays
Product B	Binding to FcγRIIIa 158 v/v	80–50% of batches	Viewed as sufficient based on ADCC assay results
Product C	Binding to FcRn	≥ 90% of batches	Based on regulatory experience and the results from the comparative PK study, the minor difference was seen as negligible
Product A–C (all)	Glycosylation (6 attributes)	Variable, often < 90%, see Online Resource 1	Similarity confirmed in cell-based functional assays No clinically significant difference in PK profile
Product A–C (all)	Purity testing	Variable, often < 90%, see Online Resource 1	Based on regulatory experience, the small difference was seen as negligible. In most cases, purity of biosimilar was marginally increased
Product A–C (all)	Charge variants	Variable, often < 90%, see Online Resource 1	Acceptable based on product understanding
Trastuzumab			
Product D and F	Protein content	≥ 90% of batches	The small difference in protein content was concluded to be of no clinical relevance. Batch-to-batch variability of the biosimilar within the expected range
Product I	Inhibition of cellular proliferation	80–50% of batches	Slight differences explained and justified by the method variability
Product I	Binding to FcRn	80–50% of batches	Based on regulatory experience and the results from the comparative PK study, the minor difference was seen as negligible
Product D, E, I	Binding to several Fcγ-Receptors (FcγRI, FcγRIIa, FcγRIIb, FcγRIIIa-158 f/f and FcγRIIIb)	Variable, see Online Resource 1	Minor differences in binding results, similarity confirmed in cell-based functional assays
Product I	Binding to FcγRIIIa 158 v/v	≥ 90% of batches	Minor differences viewed as irrelevant based on ADCC assay results
Product D–I (all)	Glycosylation (6 attributes)	Variable, often < 90%, see Online Resource 1	Similarity confirmed in cell-based functional assays No clinically significant difference in PK profile
Products D–I (all)	Purity testing	Variable, often < 90%, see Online Resource 1	Based on regulatory experience, the small difference was seen as negligible
Products D, E, F, H, I (all except G)	Charge variants	Variable, often < 90%, see Online Resource 1	Acceptable based on product understanding
Products G, I	ADCP	< 50% of batches or not done	Acceptable based on FcγRIIa assay results, considered surrogate for ADCP function
Products D, G, H, I	Inhibition of VEGF secretion	< 50% of batches or not done	The assays are not considered as critical, differences accepted based on the totality of evidence presented for similarity

ADCC antibody dependent cellular cytotoxicity, *ADCP* antibody dependent cellular phagocytosis, *FcRn* neonatal Fc receptor, *QAs* quality attributes, *MAA* marketing authorisation application, *PK* pharmacokinetics, *VEGF* vascular endothelial growth factor

Key quality requirements	Withdrawn rituximab biosimilar candidate	Withdrawn trastuzumab biosimilar candidate
In-depth knowledge of the RP		
The main MoA is known and demonstrable	✓	✓
CQA are known	✓	✓
Sufficient (representative) batches of the RP are analysed	×	×
Adequately established QTPP	×	×
Attributes of the biosimilar candidate		
The manufacturing process is well controlled. Release and stability specification limits are appropriate	×	✓
The quality profile of the batches used to generate clinical biosimilarity data is representative of the quality profile of the proposed commercial batches	×	×
Suitable and appropriately qualified analytical methods used for analytical and functional similarity assessment	✓	×
Biosimilarity exercise		
Adequate overall approach for demonstrating biosimilarity	✓	×

Fig. 3 Analysis of quality requirements for biosimilars withdrawn by the applicant during the review process. For the withdrawn biosimilar applications key relevant quality requirements (and if they were met) were analysed (applications withdrawn for commercial reasons not included). *BS* biosimilar candidate, *CQA* critical quality attributes, *MoA* mechanism of action, *QTPP* quality target product profile, *RP* reference product

High similarity [≥ 90% of batches within range (solid dark and light-green horizontal stripes)] was found for protein content, biological activity (CD20 binding and apoptosis induction for rituximab, and HER2 binding and inhibition of cellular proliferation assay for trastuzumab), FcγRIIIa binding, neonatal Fc Receptor (FcRn) and C1q binding, ADCC and CDC for almost all rituximab and trastuzumab biosimilars. Exceptions included inhibition of cellular proliferation for one trastuzumab (product I), FcRn for one rituximab (product C) and one trastuzumab (product I) biosimilar and the high affinity FcγRIIIa v/v genotype for one rituximab (product B). However, as seen in Table 2, in most cases these differences were considered within the method variability or viewed as sufficiently justified based on high similarity found in other critical QAs (CQA) (i.e., ADCC for FcγRIIIa v/v), the results from PK comparability studies and regulatory experience. None of the authorised trastuzumab biosimilars displayed CDC activity (represented as dark-green vertical stripes in Online Resource 1), which is expected.

More variability was found for binding to other Fcγ receptors, purity and glycosylation profile, charged variants and additional assays (Online Resource 1). Again, as seen in Table 2 the observed differences in Fc binding assays and the glycan profiles were accepted because similarity was confirmed in biological assays. Moreover, afucosylation was 100% within range for all except one trastuzumab biosimilar (product G). Differences in purity and charge variants were seen as negligible based on regulatory experience and product understanding, and differences in additional assays were accepted based on the totality of the evidence presented for similarity.

3.3.2 Comparison of Analytical Biosimilarity for Withdrawn Products

Figure 3 represents the key quality/CMC requirements and whether these were met for the two withdrawn biosimilar applications [43, 44]. These key requirements were categorised following the classification from Bielsky et al. [56].

Of the quality/CMC requirements included, less than half were met for either of the products. Regarding the RP characterisation, both applicants failed to demonstrate two out of four of the prerequisites, demonstrating in both cases an

Table 3 Discrepancies in clinical attributes and how the resulting uncertainty during MAA was resolved

	Clinical attribute	Observation	How resolved
Rituximab			
Rixathon/Riximyo	PK	Cmax1 slightly exceeded the upper limit (i.e. 126%)	1. Primary endpoint (AUC) met 2. Cmax2 was within the standard acceptance limits and therefore, the criterion of bioequivalence was met
Truxima/Blitzima/Ritemvia	PK	AUCt-inf outside of acceptance limits	1. Primary endpoints (AUC0-last, AUC0-inf, Cmax) met 2. Deviation seen as minor; all other secondary endpoints met standard acceptance limits
Rixathon/Riximyo	PFS	HR of 1.33 (0.98, 1.80)	1. Primary endpoint (ORR) met 2. Study not designed to demonstrate equivalence for PFS 3. Conclusion of biosimilarity based on overall biosimilarity assessment
Rixathon/Riximyo	OS	HR of 1.03 (0.59, 1.80)	1. Primary endpoint (ORR) met 2. Study not designed to demonstrate equivalence for PFS 3. Conclusion of biosimilarity based on the overall biosimilarity assessment
Trastuzumab			
Ontruzant	bpCR (RD)	95% CI not fully contained within prespecified equivalence margin	1. Justified by confounding effect of ADCC shift in reference lots 2. Conclusion of biosimilarity based on the overall biosimilarity assessment
Kanjinti	pCR in breast tissue and axilary lymph nodes (RD and RR)	95% CI not fully contained within prespecified equivalence margin	1. Justified by confounding effect of ADCC shift in reference lots 2. Additional functional analysis for ADCC performed 3. Additional analysis adjusting for subjects exposed to IMP with low ADCC 4. Conclusion of biosimilarity based on overall biosimilarity assessment
Kanjinti	pCR (only breast) (RD and RR)-except RD in PP	95% CI not fully contained within prespecified equivalence margin	1. Justified by confounding effect of ADCC shift in reference lots 2. Additional functional analysis for ADCC performed 3. Additional analysis adjusting for subjects exposed to IMP with low ADCC 4. Conclusion of biosimilarity based on overall biosimilarity assessment
Zercepac	DOR, PFS, OS	Seemingly better efficacy (HR < 1)	1. Study not designed to demonstrate equivalence for PFS 2. No significant differences found in second interim analysis 3. Conclusion of biosimilarity based overall biosimilarity assessment
Withdrawn rituximab biosimilar candidate	Deaths	Eight patients died in the product arm versus none in the reference arm	1. Chance finding likely 2. Study not designed to evaluate hard clinical endpoints

ADCC antibody dependent cell-mediated cytotoxicity, *AUC* area under the curve, *AUC-inf* AUC to infinity, *Cmax* maximum concentration, *pCR* pathologic complete response, *bpCR* breast pathologic complete response, *CI* confidence interval, *Cmax* peak concentration, *DOR* duration of response, *HV* healthy volunteers, *HR* hazard ratio, *IMP* investigational medicinal product, *MAA* marketing authorisation application, *ORR* overall response rate, *OS* overall survival, *PFS* progression-free survival, *PK* pharmacokinetic, *PP* per protocol, *RA* rheumatoid arthritis, *RD* risk difference, *RR* risk ratio

in-depth knowledge of MoA and CQA of the RP but failing to analyse enough representative RP batches or to adequately establish the quality target product profile (QTTP). Regarding the biosimilar candidate attributes, out of the three prerequisites, only one was met for each product. The quality/CMC package included suitable and qualified analytical methods for the withdrawn rituximab and an adequate manufacturing process for the trastuzumab. However, none of the other requirements were met, including the representativeness of clinical and commercial batches or the use of additional orthogonal assays. Finally, only the withdrawn rituximab included an adequate overall approach for demonstrating biosimilarity.

3.4 Results of Clinical Comparability Studies

Clinical data are presented as raw data in Online Resource 2–5 (the product rows are not in the same order as Online Resource 1 to maintain anonymity). Table 3 provides a summary on all the uncertainties in clinical data, and how these were resolved.

3.4.1 PK Studies

3.4.1.1 Rituximab For all rituximab biosimilars, PK studies were performed in patients with rheumatoid arthritis (RA) with supportive PK data from oncology patients as part of the efficacy studies. With regard to the withdrawn rituximab application, a comparative efficacy study (in RA) that included PK similarity as a secondary objective was conducted prior to a dedicated comparative PK study [in non-Hodgkin's lymphoma (NHL)] [43]. Length of follow-up ranged from 24 to 25 weeks (26 weeks in case of the withdrawn application). Primary endpoints [area under the curve to infinity (AUCinf), maximum concentration (Cmax) and AUC from time of administration up to the time of the last quantifiable concentration (AUClast) were contained within the pre-specified acceptance range for all approved biosimilars and secondary endpoints supported biosimilarity. Although for the withdrawn rituximab the pre-defined equivalence margin was 70-143% in the PK comparability study, the 90% CIs of the primary endpoints also met the standard equivalence margin of 0.8–1.25. As seen in Table 3, in two cases [26, 27, 53, 54, 65], results of a secondary endpoint were found outside of the standard acceptance limits, but deviations were seen as minor and not clinically relevant. Detailed information on the PK studies is available in Online Resource 2.

3.4.1.2 Trastuzumab For all trastuzumab biosimilar candidates, PK studies were performed in healthy subjects with supportive PK data obtained in clinical trials in oncology patients. For the withdrawn trastuzumab application, an additional PK similarity study in healthy subjects was submitted. Length of follow-up of the PK studies ranged from 56 to 99 days (53 days in case of the withdrawn application). In all cases, the primary endpoints (AUCinf, Cmax and AUClast) were contained within the pre-specified acceptance range and secondary endpoints supported biosimilarity. Detailed information on the PK studies is available in Online Resource 4.

3.4.1.3 Population PK Population PK (PopPK) was performed for some products, using different approaches [28, 37–39, 49]. Absence of PopPK analysis was accepted where PK similarity had been demonstrated in the dedicated PK study and PopPK was seen as supportive in the other cases.

3.4.2 Clinical Efficacy Studies

3.4.2.1 Rituximab Rituximab is currently approved in seven indications, both autoimmune and oncological [66]. Two applicants, including the one for the withdrawn rituximab MAA [27, 43, 53, 65], chose to compare efficacy in RA subjects as a model indication and the remaining two [26, 28, 54] chose follicular lymphoma (FL). Length of follow-up was up to 3 years. Overall response rate (ORR) was chosen as primary endpoint in FL and disease activity score using 28 joint counts (DAS28) or American College of Rheumatology Response (ACR 20) for RA. Detailed information on the efficacy studies is available in Online Resource 3.

3.4.2.2 Trastuzumab Trastuzumab is currently approved in three indications [67]. For three biosimilars [37–39] metastatic breast cancer (MBC) was chosen as model indication in the pivotal clinical trial and for the remaining four, including the withdrawn MAA [36, 44, 48, 49], early breast cancer (EBC) was used. Length of follow-up was up to 3 years.

Three applicants chose ORR and the remaining four pathologic complete response (pCR) as the primary endpoint. Pre-specified equivalence margins for risk difference (RD) varied even though patient populations were the same as different reference studies were used for clinical and statistical justifications [37, 39, 48, 49]. Detailed information on the efficacy studies is available in Online Resource 5.

Table 3 shows those instances where some differences were found and how the remaining uncertainties were resolved. For two products [48, 49], the 95% CI of the difference in the pCR rates between treatments was not fully contained within the pre-defined equivalence margin, thus superiority of the biosimilar cannot be excluded.

3.4.2.3 Safety and Immunogenicity The overall safety and immunogenicity profiles were compared descriptively and

appeared similar between the biosimilars and the RP, as reviewed in detail by Kurki et al. [68].

With regard to the withdrawn rituximab biosimilar candidate application [43], the overall safety profile appeared to be similar in patients with RA but imbalances in adverse events (AEs), serious adverse events (SAEs), severity and deaths were observed in the comparative PK study in patients with NHL. Eight patients died in the product arm versus none in the reference arm; investigators assessed the causal relationship as not (6/8) or unlikely (2/8) related to study drug for all fatal SAEs.

4 Discussion

When considering any change in the current requirements for comparative efficacy studies for biosimilar mAb and fusion protein developments, a fundamental concern of stakeholders including patients, physicians and regulators is that biosimilar product candidates with demonstrated analytical/functional comparability could nevertheless translate into failed clinical comparability. The concern is that in the absence of such clinical trial data, a biosimilar might be inappropriately approved based on quality data only.

Our study shows that this concern is not supported by data and that regulatory decision-making follows a totality-of-the-evidence approach with the main focus on the pharmaceutical quality/CMC (biosimilarity, general quality) and PK similarity aspects. In our opinion, a clinical efficacy study may not need to be routinely requested. In the following parts, we discuss evidence obtained from different analyses performed and its implications.

4.1 Discussion of MAA Evidence and Results

We analysed the MAA reviews of biosimilar mAbs and fusion proteins performed by the EMA CHMP and found that in most cases (29/36 cases) good quality/CMC packages were matched with successful clinical trials leading to MA. Interestingly, good quality/CMC packages could also be paired with formally failed efficacy studies, which were evaluated to be due to reasons not related to the biosimilar candidate, thus permitting MA (see discussion of analysis of clinical comparability for rituximab and trastuzumab biosimilars including withdrawn biosimilar candidates below).

On the contrary, unconvincing quality/CMC data paired with successful clinical trials precluded MA, primarily due to the lack of demonstration of sufficient pharmaceutical quality and/or analytical/functional similarity with the RP [43, 44].

In our analysis, there was only one case where clinical data analysis led to a MO with a divergent position published by the CHMP [15] (Annex) which nevertheless received MA. The issue was later resolved by more mature follow-up data, which did not confirm the objections regarding potentially increased immunogenicity of the biosimilar [69].

For three of the biosimilar candidates analysed [45–47] PK studies were deemed insufficient, which led to the conduct of a new, acceptable PK trial. The reasons for repeating the PK trials were due to methodological issues and differences in the formulation buffer [59] or because of representativeness of the test product used [47]. For two biosimilar candidates the PK data submitted in the initial MAA raised major questions regarding biosimilarity. However, re-analysis of the data, which was already pre-specified in the statistical analysis plan, led to the conclusion that PK similarity was shown [18, 21]. Taking this into account, our analysis indicates that, where biosimilarity was shown at the quality/CMC level, this always translated into PK similarity of the biosimilar candidates with the RP.

4.2 Discussion of Analysis of First Regulatory Assessment Reports

We investigated whether the MAA outcome could have been predicted based on the evidence generated solely in the quality dossier. We found that the quality and clinical assessments aligned in 67% of cases, i.e. both quality and clinical data packages, were considered sufficient to support a MA, or both the quality and clinical data packages were not accepted. In 11% of cases, MO were identified in quality parts of the submission, whereas the clinical data supported biosimilarity.

Of particular interest are those 22% of cases (11% E/S/I only) where no MO were observed in the quality part, but MO were raised on the clinical data. Without further regulatory deliberation and additional justifications from the applicants, these cases could have resulted in false negative conclusions, i.e. a true biosimilar being rejected due to issues with clinical studies. However, even in those cases where the efficacy trials formally failed, biosimilarity was ultimately accepted by EMA based on the demonstration of analytical/functional comparability and comparable PK profiles. In each instance, identified issues in the clinical package were eventually accepted as the result of unanticipated problems such as imbalances in trial arms, immaturity of secondary endpoint data at the time of MAA submission, changes in the QA of the RP or even chance findings. In some cases, a further in-depth sensitivity analysis improved the understanding of the clinical data and facilitated a positive conclusion. These cases highlight that for biosimilar mAbs and fusion proteins, the analytical and functional characterisation data are the most critical for decision making and regulatory approval.

In summary, our analyses of MAAs and first regulatory assessment reports show that the quality/CMC part of the dossier is predictive for the MA of a biosimilar candidate.

4.3 Discussion of Analysis of Analytical Biosimilarity for Rituximab and Trastuzumab Biosimilars

Since our analyses included the two withdrawn MAs, we reviewed to what degree thorough analysis of the quality/CMC package could have been predictive for the clinical outcome. These two substance classes are particularly interesting, as the two originator mAbs are used in oncology (rituximab, trastuzumab) and/or in autoimmune indications (rituximab). Based on information provided in the MAA, and following the scientific evaluation carried out by EMA CHMP, for approved rituximab and trastuzumab biosimilars, we found that over 90% (and in most cases 100%) of the biosimilar batches met the EU reference product similarity range for CQAs such as protein content, biological activity (CD20 binding and apoptosis induction for rituximab and HER2-binding and inhibition of cellular proliferation assay for trastuzumab), FcγRIIIa binding, FcRn and C1q binding, ADCC and CDC.

A lower percentage of biosimilar batches were within the similarity range for QAs which are considered less critical (glycosylation profile, charge variants or additional assays). Furthermore, as seen in Table 2, in all instances where uncertainties were raised, these were resolved considering the close similarity demonstrated in CQAs, the results of PK studies and overall, the totality of the evidence.

In no case were clinical trial data necessary to resolve residual uncertainties regarding the quality part. As seen in Fig. 1 (Scenario 4), for the withdrawn MAs, clinical data was also not sufficient to justify the differences in the quality/CMC package. This is in line with previous findings [13, 56] and further demonstrates that the array of orthogonal methods that are submitted in the quality/CMC package are robust and predictive of clinical outcome.

4.4 Discussion of Analysis of Clinical Comparability for Rituximab and Trastuzumab Biosimilars Including Withdrawn Biosimilar Candidates

For all rituximab and trastuzumab biosimilar candidates studied, a comprehensive clinical program was submitted according to the relevant EMA guidelines, i.e. consisting of at least one PK study and a clinical efficacy study, which confirmed biosimilarity in all but two instances [36–39, 44, 48, 49].

For all rituximab biosimilars included in the analysis, PK results were obtained in one therapeutic area and confirmed in a subset of patients in the second therapeutic indication either by supportive PK analysis [26, 27, 43, 53, 54, 65] or by PopPK analyses [28]. In no instance were deviating results observed. One applicant [26] removed PopPK in a protocol amendment which was acceptable to the regulators. In our opinion, this redundancy of PK evaluations should no longer be necessary, as besides CD20 no other antigen or target is involved in rituximab's binding and MoA. This has been demonstrated by the tissue specificity in several human tissue cross reactivity studies [66, 70].

In our study, in all cases except for the withdrawn applications, remaining uncertainties regarding clinical data were resolved based on a strong quality/CMC package, together with demonstrated PK similarity and considering that the studies were not powered to demonstrate similarity with regard to secondary endpoints. Moreover, regarding safety, it should be noted that clinical trials are not powered for safety endpoints, since this is considered unnecessary according to EMA guidelines and would usually require several thousand study participants.

Therefore, the value of extensive analyses of secondary endpoints in efficacy trials remains questionable, as they were either viewed as inconclusive [27, 53, 65], or immature [26, 54].

For both trastuzumab cases [48, 49], EMA concluded that it was likely that the apparent difference was caused by a noted downward "shift" in ADCC activity in some of the RP batches and did not preclude approval. These findings have been discussed in the literature [56, 71, 72] and also demonstrate that physicochemical methods and functional assays are able to detect differences in functional attributes with predictive character. With regards to the withdrawn trastuzumab biosimilar [44], a conclusion on biosimilarity from a safety point of view was precluded as the clinical batches were not considered representative for the commercial product.

Several authors [56, 59, 73–75] pointed out limitations of indiscriminative clinical efficacy studies in light of technical advances in analytical methods, which provide more discriminative research tools sparing patients from entering unnecessary and redundant clinical trials.

It is known from the literature that the recommended doses of many mAbs are in the flat part of the dose-response curve, e.g. half of the administered dose of rituximab would result in the same clinical outcome, suggesting the overall equivalence of 2 × 500 mg with the licensed dose of 2 × 1000 mg for clinical efficacy outcomes [76], thus rendering clinical trials insensitive tools to assess biosimilarity. Some authors argue that treatment response to rituximab in RA is only determined by the level of B cell depletion, regardless of how it is achieved [76–79], yet it is important

to measure binding and all functional activity. In our analysis we found that rituximab binding to the CD20 receptor plus all four important MoAs of rituximab [60] were adequately measured by appropriate analytical testing and that for the successful quality/CMC dossiers > 90% of batches met the required similarity ranges.

4.5 General Discussion on Current Flexibility in Clinical Trial Requirements

Our analysis revealed that there is regulatory flexibility in the acceptability of clinical data packages. This is, for example, evidenced by the acceptance of different primary endpoints such as DAS28 versus ACR20 in RA trials, different methods of analyses such as RD versus risk ratio (RR), or different equivalence margins for the same study population depending on the reference study chosen, provided appropriate scientific justification is given [29, 37, 38, 48].

Currently, most mAb developers are planning to evaluate S/I over one year, as advised by guidelines. However, the primary efficacy analysis is usually performed at a much earlier timepoint [26–28, 37–39]. Based on our analyses of the submitted clinical trial data, as well as on the raw data analysis of Kurki et al. [68], it is clear that most dossiers are submitted with preliminary 4–6 months S/I data, depending on the timepoint of primary efficacy analysis, while the full one-year dataset is submitted later during the evaluation period. In no instance have the conclusions reached after the initial data submission changed after completion of the study after 12 months, suggesting that such long trials are unnecessary. Other authors have concluded that pertinent comparative safety and immunogenicity data will be obtained from the PK trials, which in all instances were shown to support results of the E/S study [68].

We conclude that a sufficiently robust analytical/functional similarity package, together with a PK trial capturing data on safety and immunogenicity would be sufficient for the purpose of regulatory decision making for biosimilar mAbs and fusion proteins. In some case, if it were deemed necessary (e.g. when the MoA of the biologic is poorly understood), a shorter efficacy trial ending at or near the timepoint of primary efficacy analysis could provide additional safety and immunogenicity information.

4.6 Limitation of the Study

Since our study was limited to mAb and fusion protein biosimilars of IgG class, our conclusions may not be applicable to more complex biologics. Furthermore, our analysis is restricted to products that have undergone regulatory assessment and appraisal. Whether there have been biosimilars that failed quality/CMC and/or clinical development and were therefore never submitted for regulatory evaluation or publication has not been scrutinised in our study. Also, overall, the sample size of 36 was limited by the available submissions to EMA in the previous 10 years. However, to our knowledge this is the largest set of biosimilar MAs analysed to date.

5 Conclusions of Our Study

The results of our analysis show that a comprehensive and convincing quality/CMC package demonstrating high analytical/functional similarity of the biosimilar with the RP is essential for MA. Since the first approval of less complex biosimilars, the analytical techniques have advanced markedly resulting in very sensitive assays for the structural and functional characterisation of even complex mAb molecules.

The concern, that in the absence of comparative efficacy and safety results, a biosimilar candidate might be inappropriately approved based on quality data only, is not supported by our findings. The analytical and biological results can be considered predictive for the clinical performance of the biosimilar candidates.

Based on the combination of modern analytics, control and pharmacovigilance systems in place, as well as requirements on comparability assessment in case of manufacturing changes, clinical performance of IgG biopharmaceuticals is ensured throughout the lifecycle of the product. As shown in our analysis, the CQA that are known to impact clinical efficacy and safety, including immunogenicity, must be closely monitored.

In the authors' opinion, these findings allow a reduction of the clinical development program for regulatory review before MA. This conclusion is further supported by the positive experience in the market gained for biosimilar mAbs approved in the last ten years. Consequently, a revision of the respective regulatory biosimilars guidelines in Europe should be considered, to allow a more rational use of clinical resources and improve the access to innovative and affordable medicines for patients.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40259-023-00631-4.

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Declarations

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Availability of data and material The datasets analysed for clinical comparability during the current study are available in the EMA repository, which are publicly available at: https://www.ema.europa.eu/en/medicines/field_ema_web_categories%253Aname_field/Human. The datasets generated and/or analysed during the current study for analytical biosimilarity, marketing authorization applications and regulatory assessment reports are not publicly available due to confidentiality issues.

Code availability Not applicable.

Author contributions NK-S, EG, NE, SB, VK, SK, MW, and EW-H wrote the manuscript. NK-S, EG, NE, and EW-H designed the research. NK-S, EG, NE, VK, and EW-H performed the research and analysed the data. NK-S and EG contributed equally to the manuscript. All authors read and approved the final manuscript.

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