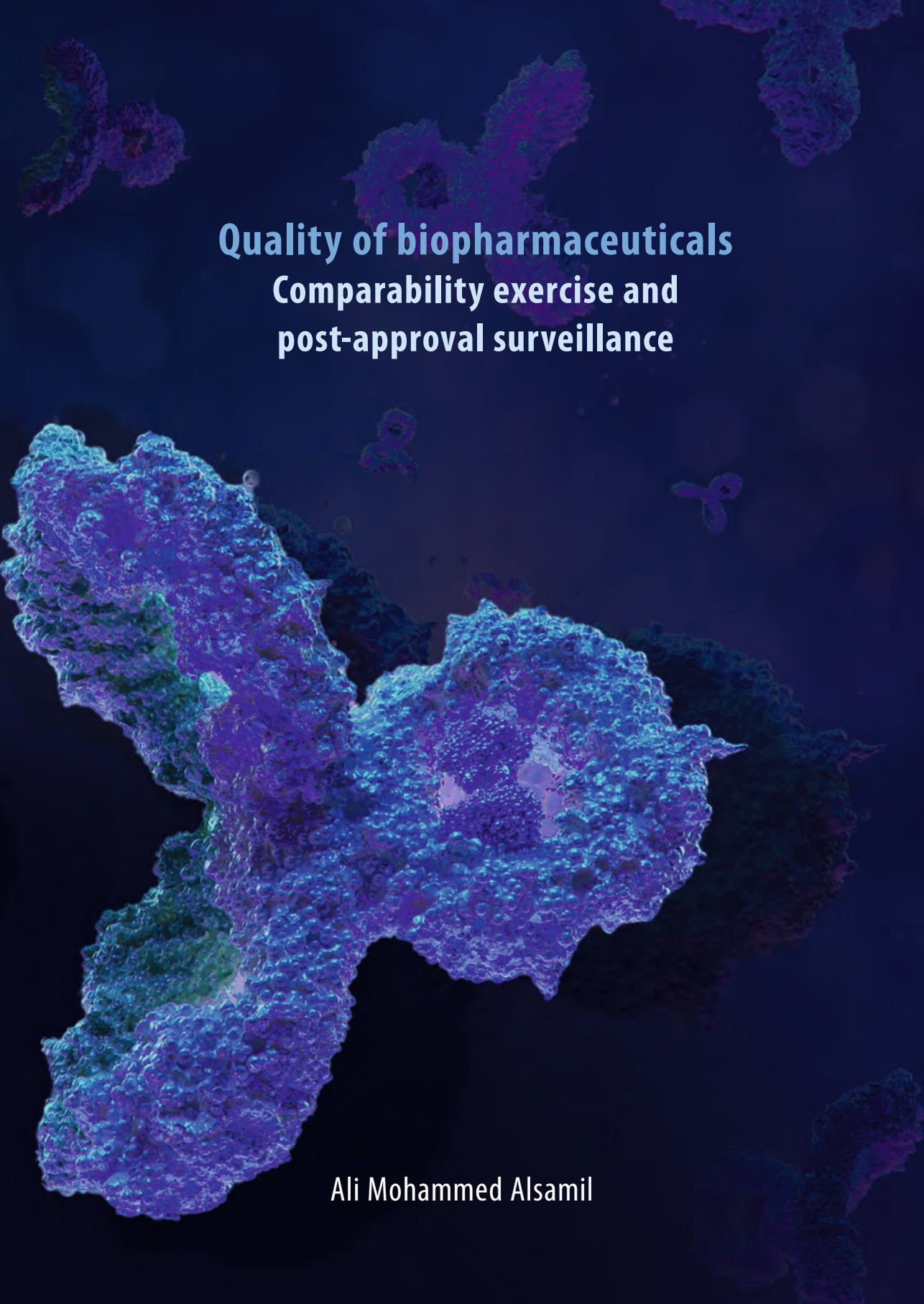
# Quality of biopharmaceuticals

## Comparability exercise and post-approval surveillance



Ali Mohammed Alsamil

# Quality of biopharmaceuticals:

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**Colophon**

The studies presented in this thesis have been conducted under the umbrella of the Regulatory Science collaboration between the Saudi Food and Drug Authority (SFDA) and the Utrecht Institute for Pharmaceutical Sciences (UIPS). The SFDA is dedicated to regulate, oversee, and monitor food, drug, medical devices, as well as to set mandatory standard specifications thereof, whether they are imported or locally manufactured. This includes ensuring the quality, safety, and efficacy of medicinal products, and that licensed medicinal products have a positive benefit-risk balance throughout their whole lifecycle. This role requires intensive collaboration with academic and clinical partners in order to develop new assessment and decision-making methods, to engage with clinic, and to strengthen regulatory science. This PhD thesis aims to go beyond its scientific merits as such by delivering science, learning and insight to promote public health.

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# Quality of biopharmaceuticals:

## Comparability exercise and post-approval surveillance

Kwaliteit van biologische geneesmiddelen: aantonen van
gelijkwaardigheid en surveillance na toelating
(met een samenvatting in het Nederlands)

**Proefschrift**

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*To my father Mohammed and my mother Sharefa,*

*To my wife Lama, and my angels Lara and Haifa.*

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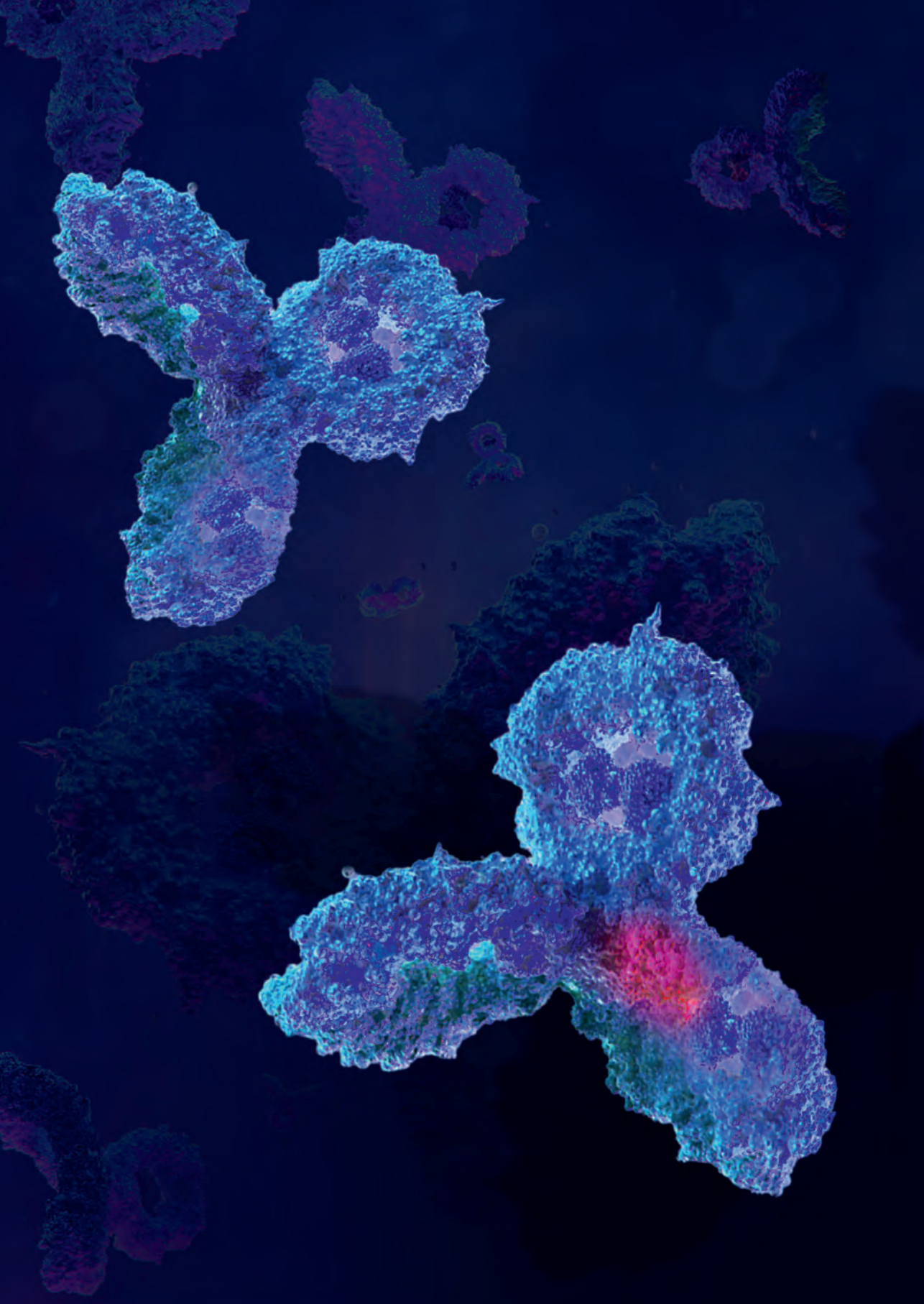


# Chapter 1

## General introduction

Author's contribution: AMA conceived the idea and set-up of the general introduction. AMA conducted literature review, outlined and wrote down the general introduction. Throughout the process, AMA asked for and implemented input and feedback from the supervision team.

# General introduction

Biopharmaceuticals currently dominate the development and approval of new medicines, illustrated by the coronavirus disease 2019 (COVID-19) vaccines [1, 2]. The definition of biopharmaceuticals is still changing with the advancement in knowledge, science, technology, and discoveries. Although there is no consensus among the drug regulatory and health authorities, the definition of biopharmaceuticals is often deduced from the definition of biological medicine (Table 1). The definition of biological medicine covers a broad spectrum of naturally extracted and recombinant products that range from simple polysaccharides (e.g., heparin) and polypeptides (e.g., insulin) to complex monoclonal antibodies (e.g., adalimumab) and advanced gene- and cell-based therapies (e.g., genetically modified autologous T-cells).

**Table 1:** Definitions of biological medicine according to drug regulatory and health authorities.

| Authority | Region | Definitions |
|---|---|---|
| World Health Organization (WHO) | Global | "Biological therapeutics, also referred to as Biological, is that class of medicines which are grown and then purified from large-scale cell cultures of bacteria or yeast, or plant or animal cells. Biologicals are a diverse group of medicines which includes vaccines, growth factors, immune modulators, monoclonal antibodies, as well as products derived from human blood and plasma."[4] |
| European Medicines Agency (EMA) | Europe | "A medicinal product contains a biological substance that is produced by or extracted from a biological source and that needs for its characterization and the determination of its quality a combination of physicochemical-biological testing, together with the production process and its control."[5] |
| Food and Drug Administration (FDA) | USA | "Biologics can be composed of sugars, proteins, or nucleic acids or complex combinations of these substances, or may be living entities such as cells and tissues. Biologics are isolated from a variety of natural sources–human, animal, or microorganism–and may be produced by biotechnology methods and other cutting-edge technologies."[6] |
| Pharmaceuticals and Medical Devices Agency (PMDA) | Japan | "Biological products are drugs, quasi-drugs, cosmetics, or medical devices using materials manufactured from humans or other organisms (excluding plants) as raw materials or packaging materials, which are designated as requiring special precautions in terms of public health and hygiene."[7] |

However, a more specific definition of "biopharmaceuticals" has been proposed as "pharmaceuticals with active substance inherently biological in nature and manufactured using biotechnology" [3]. This definition is more specific and aligns with the regulatory definitions of biological medicine but distinguishes biopharmaceuticals produced using recombinant deoxyribonucleic acid (DNA) biotechnology from naturally extracted biologicals, such as animal- or human-derived medicine, and from small-molecule drugs produced using chemical synthesis. This biopharmaceutical definition also accommodates biosimilars developed following the expiration of a patent on a reference product for a biopharmaceutical. This thesis focuses on biopharmaceuticals used to treat and cure human diseases.

## Discovery and use of biopharmaceuticals

Before the introduction of recombinant DNA technology, biologicals used in clinical practice were extracted from biological materials, including humans (e.g., human albumin and clotting factors), animals (e.g., porcine derived heparin), plants (e.g., aspirin), yeasts (e.g., penicillin), and viruses (e.g., vaccines). Vaccines were introduced in the late eighteenth century when Edward Jenner developed and tested the first vaccine for smallpox using the relatively mild cowpox virus. The discovery and development of biopharmaceuticals could not occur without basic scientific discoveries, including the following:

- unlocking the full arrangement of the amino acid sequence of insulin (i.e., the backbone chain defining the primary structure of a biological molecule) by Frederick Sanger [8],
- the DNA structure (i.e., a molecule to provide genetic instructions for organisms and molecules) by Watson and Crick in the 1950s [9], and
- the mechanistic unraveling of many diseases.

These breakthrough scientific discoveries allow for unlocking the structure of DNA and proteins, enabling healthcare to catch up with the fruits of these discoveries. It allowed introducing a piece of DNA with appropriate elements into living cells, often referred to as recombinant DNA technology, enabling the development of protein drugs that can replace a malfunctioning endogenous counterpart [10]. The first recombinant protein was human insulin, introduced into clinical practice in the 1980s, which significantly decreased the potency variation and immunological complications associated with using animal-derived insulin [11].

Recombinant DNA technology also reduces the risk of viral transmission associated with human-derived biological materials, such as human plasma to extract coagulation

factors for bleeding disorders and human urine to extract gonadotropins for infertility treatments [12-16]. Progressive advancements in recombinant DNA technology have facilitated the production of monoclonal antibodies (mAbs), which can target-specific antigens or receptors, such as tumor necrosis factor-α (TNF-α), providing novel methods of target-specific treatments for many acute and chronic diseases [17, 18].

The development of mAbs requires a thorough understanding of the pathogenesis and biological targets. For example, TNF-α was initially recognized as a major regulator of inflammatory responses where binding to two different receptors initiates signal transduction pathways, including cell survival, differentiation, and proliferation. Excessive activation of TNF-α signaling is associated with chronic inflammation and is involved in the pathogenesis of several autoimmune diseases. Understanding the TNF-α signaling pathway led to developing several TNF-α-i products, including mAbs, such as infliximab, adalimumab, golimumab, and certolizumab and fusion proteins, such as etanercept.

In the first decade of the 2000s, new modalities to treat patients emerged from the advance therapeutic medicinal products, including modified genes and engineered cells, to intervene with human biology, providing breakthrough therapies for complex diseases with high unmet medical needs [19]. Biopharmaceutical innovation is expected to continue with the rapid advancement in science and technology [20, 21].

However, these valuable innovations are highly expensive originator biopharmaceuticals. The discovery and development of biopharmaceuticals are time-consuming and costly and could reach $2.6 billion according to the 2016 Tuft center estimation [22, 23]. According to a recent IQVIA report, biologicals, including biopharmaceuticals, accounted for $277 billion in the global pharmaceutical market sales in 2017 and are projected to reach $452 billion in sales by 2022 [25]. Global spending on medicine has been growing at 3% to 5% per year and is expected to reach around $1.6 trillion by 2025. Most of this growth derives from biopharmaceuticals representing eight of the top 10 selling medicines in 2018. Although biopharmaceuticals have found their way into clinical practice, their high cost has placed economic pressure on the healthcare budget and limits patient access, challenging the regulatory system to develop a balanced solution.

The expiration of patents and exclusivity rights for some originator biopharmaceuticals has allowed the introduction of biosimilars since 2006 in the European Union (EU), providing alternative and more affordable treatment options to alleviate the pressure on healthcare budgets and improve patient access to important biopharmaceuticals. Today, the number of approved biological medicines (primarily biopharmaceuticals) has more than doubled from less than 200 in 2000 to more than 400 in 2020. Currently, this number represents approximately half of the newly launched active (chemical and biological) entities authorized in the EU and the United States (US) [1, 24].

## Unique features and the biopharmaceutical production process

Biopharmaceuticals, whether originators or biosimilars, exhibit distinct molecular and production features compared to the chemically synthesized small-molecule pharmaceuticals (Table 2) [26]. The primary distinctions between biopharmaceuticals and small molecules are their size and the structural and functional complexity of the molecules. The size of biopharmaceuticals is defined by the molecular weight and ranges from 3.7 to 150 kDa, which is larger than small-molecule pharmaceuticals (<1 kDa). Biopharmaceuticals are often proteins made of long ribbons of amino acids (i.e., primary structure) that twist into complicated knots (i.e., higher-order structure). Knowing the shape of a protein knot can reveal how the protein works, which is crucial for understanding how diseases occur and developing new drugs. The structure of a biopharmaceutical is critical for mediating (multiple) functions, which are often triggered by replacing a malfunctioning protein or a specific or nonspecific binding to a receptor or target. Unlike small molecules, biopharmaceuticals might be immunogenic by inducing the formation of an anti-drug antibody, which often has no clinical effects but, in some cases, could lead

**Table 2:** Characteristics of biopharmaceuticals versus small-molecule pharmaceuticals [26].

| | **Biopharmaceuticals** | **Small-molecule pharmaceuticals** |
|---|---|---|
| **Size** | Large (mixture of biomolecules), high molecular weight | Small (single chemical molecule), low molecular weight |
| **Structure** | Complex (heterogeneous) structure influenced by the manufacturing process | Simple, well-defined structure, independent of the manufacturing process |
| **Function** | Complicated function, not always fully understood mechanism of action | Well-defined and understood mechanism of action |
| **Modifications** | Many options for post-translation modifications | Well-defined and controlled modifications |
| **Stability** | Unstable and highly sensitive to external conditions | Stable |
| **Immunogenicity** | Biomolecules are immunogenic | Chemical molecules are mostly nonantigenic |
| **Degradation** | Degradation can form inactive compounds | Degradation can form toxic compounds |
| **Method of synthesis** | Recombinant DNA and hybridoma technology | Chemical synthesis |
| **Production process** | Highly complex process involving unique cell lines and cultures, exact replication of identical copy is impossible | Predictable chemical process, exact replication of identical copy is possible |
| **Characterization** | Full characterization is challenging due to molecular complexity and heterogeneity | Full characterization is achievable due to molecular simplicity |

to adverse events, such as immune-mediated reactions and reduced product efficacy. Immunogenicity is the process through which a protein is recognized by the human immune system as a foreign antigen, forming an anti-drug antibody against the therapeutic protein. Several factors can induce immunogenicity, including product-related factors, such as structural attributes, formulations, impurities, administration routes, and patient-related factors [27, 28].

A paramount distinction between biopharmaceuticals and small-molecule pharmaceuticals is the production process, which is far more complicated for biopharmaceuticals. Biopharmaceuticals are produced using living systems and involve numerous biological and chemical materials and steps [29]. The typical production process for a biopharmaceutical can be divided into upstream and downstream processes to produce the drug substance (DS) and drug product (DP) [30, 31]. The upstream process starts with the cloning and expression of a cell line, followed by a cell culture under predefined growth conditions (e.g., media materials, temperature, and pH). The downstream process starts with various harvesting and purification steps involving centrifugation, chromatography, and exposure to various solution conditions and filtration to extract and purify the DS from the cell culture and remove process and product-related impurities. Then, the DS undergoes formulation (by adding excipients), concentration, and sterile filtration steps and sometimes lyophilization through freeze-drying cycles to create the liquid (and sometimes a powder) dosage form of DP that fills the primary packaging (e.g., vials and prefilled syringes) [32]. The DP is stored and transported under proper conditions to ensure product quality and stability during the entire chain from manufacturing to patient administration [33, 34].

Because biopharmaceuticals are produced using living systems and biological materials and involve a complex manufacturing process, biopharmaceuticals are inherently variable (Figure 1). In other words, the manufacturing process determines the DP, and intentional or unintentional changes in the manufacturing process can lead to changes in the DP with or without clinical consequences. This inherent variability is illustrated by post-translational modifications, such as glycosylation, which are heterogeneous and differ between a) cell lines (e.g., *Escherichia coli* and *Chinese hamster ovary*), b) different clones from the same cell line, c) biopharmaceuticals produced from the same cell line, and d) even batches from the same process.

Furthermore, biopharmaceuticals are highly susceptible to physical and chemical degradation due to environmental factors, such as temperature, humidity, light, and mechanical stress. Careful control and monitoring of the manufacturing process and post-production activities, including storage, transportation, and pharmacy and patient handling, are required to maintain the quality of biopharmaceuticals. Therefore, biopharmaceuticals are subjected to high regulatory and quality standards that are more stringent than those for most small molecules to ensure patients can use safe and effective products.

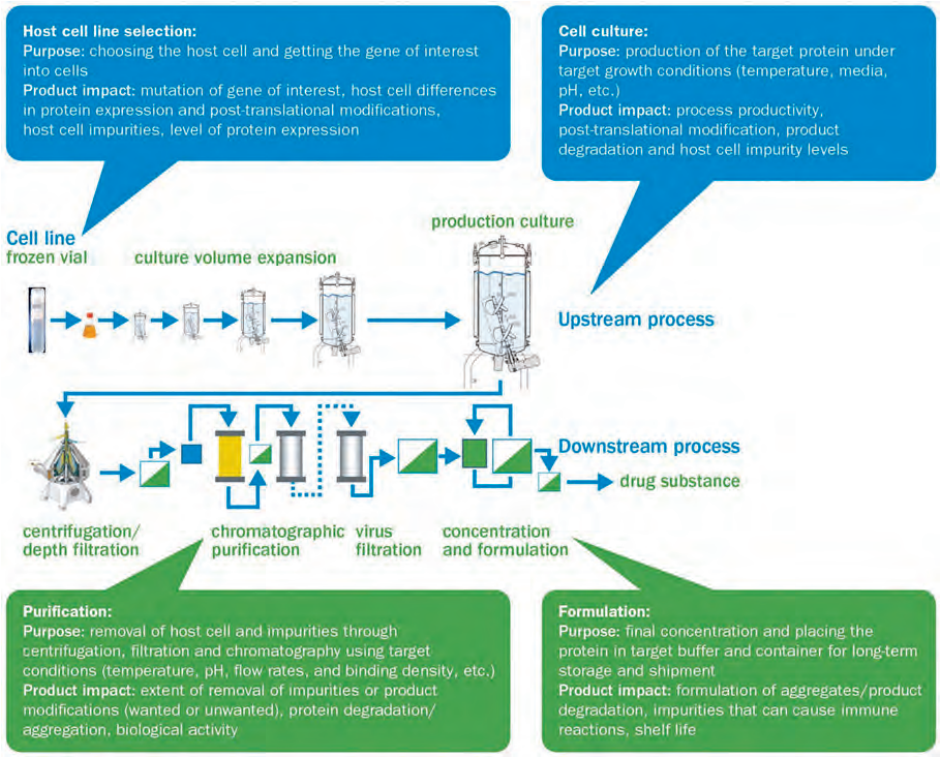



**Figure 1:** Typical manufacturing process and steps affecting biopharmaceutical quality attributes (from [32]).

## Quality of biopharmaceuticals

The quality of a biopharmaceutical can best be described by the physical, chemical, biological, and microbiological properties that define the structure and functions, known as quality attributes (QAs). The International Conference on Harmonization (ICH) defined QAs as "A molecular or product characteristic that is selected for its ability to help indicate the quality of the product. Collectively, the quality attributes define identity, purity, potency and stability of the product, and safety with respect to adventitious agents." In the same guideline, the ICH divided QAs into various types related to structural and functional attributes (Table 3). The QAs of biopharmaceuticals are more complex than the QAs of small-molecule pharmaceuticals and require a higher number of analytical tests, deploying several techniques or assays to generate complementary information on a single QA. For example, several tests may be necessary to define protein purity using different chromatography and electrophoresis techniques, including the SEC-HPLC test for detecting aggregates, a CEX-HPLC test for detecting charge variants, and RP-

HPLC or CE-SDS for detecting misfolded variants. The type and extent of QAs can vary between biopharmaceuticals and highly depend on the molecule of interest and the manufacturing process. For biopharmaceuticals, the QAs of the DS and DP are generally identical, except that QAs related to process impurities can be measured at the DS level, whereas QAs related to the final formulation (e.g., pH, appearance, volume, osmolality, particulate matter, sterility, endotoxins, microbial limits, and excipients) are typically measured at the DP level.

A subset of QAs for biopharmaceuticals is known as critical QAs (CQAs) because a slight variation in these beyond the acceptable range or limit may have direct or indirect influences on product quality and functions, including biological, immunochemical, and pharmacological activities (pharmacokinetics (PK), pharmacodynamics (PD), and clinical outcomes (i.e., safety and efficacy). The ICH defines CQAs as "physical, chemical, biological or microbiological attributes that must be within appropriate limits, range or distribution to ensure the desired product quality." Prior knowledge of the structural and functional attributes of a molecule is the foundation of identifying which QAs are critical and noncritical based on a risk assessment. Risk assessment evaluates the risk probability, severity, and potential consequences for clinical outcomes. Different quality risk assessment tools (e.g., risk ranking, primary hazard analysis, and safety assessment decision tree) can assess the criticality and determine a list of potential CQAs, which can be refined based on a continue knowledge about the QAs and understanding of the product and process.

For example, c-terminal lysine was thought to affect the bioavailability of mAbs and was considered a CQA. However, a large body of knowledge and laboratory studies have revealed that c-terminal lysine is rapidly removed after administration in human serum within 2 h with no effect on the potency and PK profiles of mAbs, indicating that c-terminal lysin does not affect bioavailability and should be considered a noncritical QA [35-41]. Knowledge of CQAs is crucial for predicting the influence on clinical outcomes. Because CQAs are potentially clinically relevant and bridge the gap between the quality and clinical outcomes, they are strictly controlled within acceptable ranges and limits to maintain the efficacy-safety profile. Although pharmacopoeias have been established to set standards for QAs to ensure medicinal quality, information on CQAs and their acceptable limits for biopharmaceuticals is often not specified in pharmacopoeias [42]. Modern and still-advancing techniques have high precision and low detection or quantification limits and can increasingly detect smaller differences. However, a considerable unknown is which (measurable) differences in CQAs are clinically meaningful.

**Table 3:** Definition of common types of quality attributes (QAs) for biopharmaceuticals.

| QA category | QA types | Definitions | Individual QA examples |
|---|---|---|---|
| **Structural attributes** | *Physiochemical properties* | Determining physical and chemical protein properties | Molecular weight, protein content, color, solubility, optical activity, and pH |
| | *Primary structure* | Linear sequence of amino acids in a polypeptide chain | Amino acid sequence, disulfide bridges, and N- and C-terminal sequences |
| | *Higher-order structure* | One or more polypeptides twisted into a three-dimensional shape forming a protein | Secondary, tertiary, and quaternary structures |
| | *Post-translation modifications* | Adding or subtracting chemical groups to or from proteins after translating from RNA via an enzymatic or chemical reaction—important for protein functions, localization, and stability | Glycosylation, deamidation, and oxidation |
| | *Purity and impurities* | Determining the absolute and relative purity of the drug substance and product and quantitively and qualitatively measuring product- and process-related impurities and contaminants | Size and charge variants, host-cell proteins, host-cell DNA, and adventitious viral and microbial species |
| **Functional attributes** | *Biological activity* | The ability or capacity of a product to perform a function to achieve the defined biological and clinical effects using various potency assays | Potency, binding activity, affinity, specificity, and molecule-specific functions (e.g., CDC and ADCC) |
| | *Immunochemical properties* | The ability and affinity of binding to specific receptors to mediate effector functions and pharmacological activities of monoclonal antibodies and fusion proteins | Binding to a) complement 1q (C1q), b) neonatal Fc receptors (FcRn), and c) Fc-gamma receptors (FcγRs) |

## Regulation of biopharmaceuticals

The establishment of regulatory authorities has been driven by several safety tragedies, including deaths associated with using contaminated diphtheria antitoxin in the first decade of the 1900s, several side effects related to using the liquid formulation of sulfanilamide elixir in the 1930s [43], and the thalidomide tragedy in 1960s, where more

than 10 thousand babies were born with phocomelia and other deformities to mothers who had taken thalidomide [44]. In the aftermath of the thalidomide tragedy, regulatory authorities have been created worldwide, and governments established regulatory systems to facilitate assessment, licensing, inspection, and post-approval surveillance and monitoring. These core regulatory activities are mandated by regional and national regulatory authorities, such as the Food and Drug Administration (FDA) in the US and the European Medicines Agency (EMA) in the EU, based on government legislation and directives. These legislations and directives were the basis for developing regulatory guidelines, which are later harmonized by the ICH to ensure that medicines are globally approved according to the same requirements. These guidelines minimized the unnecessary repetition/duplication of testing, experiments, and trials to help the industry reduce the development time and resources and, most importantly, benefit the patient.

Like all other medicines, biopharmaceuticals must obtain regulatory approval before reaching the market to ensure that patients and healthcare professionals (HCPs) can use these treatments in clinical practice with a positive benefit-risk profile. Evidence must be generated to obtain regulatory approval, comprising the three main pillars of quality, safety, and efficacy. Each pillar must be ensured at the time of approval and be monitored throughout the life cycle of (bio)pharmaceuticals.

Regulatory authorities, such as the EMA and FDA, have established several regulatory pathways for biopharmaceuticals, differentiating between reference products and biosimilars. The reference product is an originator biopharmaceutical containing a new active biological substance approved by regulatory authorities based on its stand-alone quality and nonclinical and clinical data. The biosimilar is a follow-on biopharmaceutical containing an active biological substance highly similar to an already authorized reference product and approved based on its stand-alone quality data and comparability exercises against the reference product.

For the reference product, regulators require stand-alone quality, safety, and efficacy data demonstrating that the manufacturing process (inputs) produces a product with consistent and stable QAs under predefined storage conditions (outputs) with proof that the benefits outweigh the risks (i.e., benefit-risk balance) based on clinical trials. The regulatory decision on the approval of the reference product is primarily derived from the assessment of (randomized) clinical trials (Phases I, II, and III) designed to demonstrate safety and efficacy in treating the claimed therapeutic indications in the studied population.

For biosimilars, regulators demand stand-alone quality data and comparability exercises demonstrating the biosimilarity to the reference product for QAs, safety, and efficacy. The biosimilar pathway was created because the existing generic pathway

for small molecules was considered insufficient to demonstrate the biosimilarity[1] of biopharmaceuticals. During the last decades, regulatory guidelines of biosimilars have been developed and revised, reflecting the evolution of biosimilar regulations based on scientific progress and experience with the approved biosimilars. Pioneered by the EMA (2004), regulators have issued an extensive set of guidelines for biosimilars to facilitate the development and regulatory assessment of biosimilars. These guidelines were generally adopted by other health and regulatory authorities, including the World Health Organization (WHO, 2009), Health Canada (2010), and the FDA (2015) [46-50]. The EMA has developed multidisciplinary scientific guidelines for biosimilars, ranging from addressing general principles to guidelines that cover quality, nonclinical and clinical issues, and specific product class (e.g., somatotropin, filgrastim, epoetin, insulin, follitropin, interferon alpha and beta, and monoclonal antibodies) guidelines [46].

## Comparability exercise

The comparability exercise of biopharmaceuticals aims to demonstrate that two batches (pre- versus post-change batches) from either the same manufacturer or two products (a biosimilar versus the reference product) from different manufacturers are comparable with no meaningful differences in quality, safety, and efficacy. The same principles are applied for both scenarios, which the FDA first introduced in 1996 for batches from the same manufacturer, and the EMA extended this in 1998 to cover the possibility of two versions from different manufacturers [51, 52]. Biosimilar development generally begins with an extensive characterization of multiple batches of the reference product to define QAs, determine the variability range or limits for each QA, and establish the quality-target profile, followed by reverse engineering to produce the candidate biosimilar and stepwise comparability exercises (Table 4). The comparability exercise starts by comparing the QAs of the DS between the biosimilar candidate and reference product (i.e., the DS of the reference product can be obtained by extraction, concentration, or deformulation from the reference product batches) to demonstrate high similarity and detect minor differences in QAs. The reference product is used as a comparator for biosimilars because it has been used by patients and has a well-established safety and efficacy record. Publicly available reference standards (e.g., pharmacopoeias) can be employed to calibrate the analytical procedures but cannot be used as a comparator because the reference standards were not developed for clinical use [53].

1 The term "biosimilarity" first appeared in the literature in 1977 in the *Journal of Biorheology* and was used by Hunter Roues to compare biomechanical properties between different species, becoming a principle of biosimilar development and approval decades later [45].

The variability in QAs is inherent in all biopharmaceuticals and can occur between and within batches from the same process and between versions from different manufacturers because of the complexity surrounding their molecule and manufacturing processes. However, regulators only accept minor differences if these do not alter clinical outcomes or jeopardize patient care. Schiestl et al. and Planinc et al. reported an example of acceptable minor differences in certain QAs for multiple batches of several reference products of biopharmaceuticals [30, 31]. However, a biosimilar must remain within the variability range of multiple batches of the reference product, and minor differences must not be clinically relevant. Halim et al. illustrated this small variability by analyzing multiple batches of reference products (Eprex® and NeoRecomon®) and biosimilars (Retacrit® and Binocrit®), observing minor differences in epoetin content, isoform profile, and potency between products and within batches of epoetin products. However, these minor differences were not clinically relevant [55].

Based on the outcome of comparability of QAs (Table 4, Step 3), comparative nonclinical (in vivo animal studies) and clinical exercises have been conducted to rule out the influence of minor differences in QAs on clinical outcomes, including PK/PD, safety, immunogenicity, and efficacy. Comparative nonclinical studies (Table 4, Step 4) have assessed the toxicity. However, their contribution to the comparability exercise is limited because they lack sensitivity to assess the a) influence of minor differences in QAs, b) variability of animal models and assays and c) predictability of safety and immunogenicity in humans,[56]. The later limitation is illustrated by the cytokine storm during the first human trial of anti-CD28 mAb (TGN1412), which could not be predicted from in vivo animal studies [57, 58].

Because of these limitations and to comply with the principle of the three Rs: reduce, refine, and replace, the need for comparative nonclinical exercises is limited to approving quality changes and biosimilars [56]. The comparative clinical exercises (Table 4, Step 5) have been conducted to confirm the comparability in terms of PK/PD, safety, immunogenicity, and efficacy based on various clinical trials (e.g., comparative Phase I in healthy volunteers and Phase III trials in the patient population). Regulators rarely require comparative clinical trials to support the changes in quality of the DS and DP that can be implemented after approval for biopharmaceuticals [59].

However, comparative clinical trials are required to approve biosimilars, especially those with complex and multifunctional molecules, such as mAbs and fusion proteins. Biosimilars for less complex molecules, such as insulin, (peg)filgrastim, and follitropin alpha, have been approved in recent years without the need for comparative Phase III trials because PD biomarkers are available as a surrogate for efficacy (e.g., the glucose infusion rate in a glucose clamp study for insulin and the absolute neutrophil count for (peg)filgrastim)), and the mechanism of action for these molecules is clearly understood. Furthermore, the accumulated experience with the regulatory evaluation of biosimilars

revealed that comparative phase III trials are less sensitive than comparative PK trials, preferably with a PD marker to assess the influence of minor differences in QAs identified from earlier comparability exercises [60-63]. In response to the ongoing debate on the potential reduction of unnecessary comparative Phase III trials, the EMA initiated a pilot program in 2017 to provide biosimilar developers with tailored scientific advice during biosimilar development.

**Table 4:** Overview of the stepwise approach to biosimilar development, inspired by the European Medicines Agency guidelines on biosimilars [5].

| |
|---|
| **Step 1: Quality characterization of the reference product** |
| - Multiple batches of the reference product are selected and analyzed to perform the following:<br>o identify the quality attributes of the reference product,<br>o establish the quality-target profile to guide the manufacturing process, and<br>o establish the variability range or limit for each quality attribute to assess comparability. |
| **Step 2: Knowledge transfer to develop a manufacturing process for a biosimilar** |
| - The manufacturing process is reverse engineered and optimized. |
| **Step 3: Comparability of quality attributes** |
| - Orthogonal and state-of-the-art physicochemical methods assess the comparability of structural attributes: physiochemical properties, primary structure, higher-order structures, post-translation modifications, purity, and impurities.<br>- In vitro studies assess the comparability of functional attributes: biological and pharmacological activities and immunochemical properties.* |
| **Step 4: Comparative nonclinical in vivo animal studies** |
| - In vivo animal studies assess toxicity and are only required in limited situations (e.g., if biosimilars have new quality attributes, are created using a new cell line, or use a novel excipient in the formulation). |
| **Step 5: Comparative clinical studies** |
| - Comparative Phase I trials in healthy volunteers assess the comparability of pharmacokinetics, pharmacodynamics, safety, and immunogenicity.<br>- Comparative Phase III trials for one indication in the patient population assess the comparability in safety, immunogenicity, and efficacy. These trials may be waived if a pharmacodynamic suitable as a surrogate for efficacy is available and the mechanism of action is clearly understood for the molecule of interest. |

*Assessment of immunochemical properties applies only to biosimilars containing monoclonal antibodies or fusion protein.

This initiative has shifted the attention of stakeholders involved in biosimilar regulation, development, and use in clinical practice toward the comparability of QAs, which can detect and assess the influence of minor differences and determine the need for comparative nonclinical and clinical exercises and provide the basis for extrapolating indications. The extrapolation of indications is a well-established scientific and regulatory concept. For a reference product with multiple indications, the biosimilar can be granted all indications based on the outcome of QAs and a comparative clinical trial in one therapeutic indication. This concept has also been applied for long-term reference

products when quality changes are introduced after approval, allowing for a reduction or elimination of duplicative and unnecessary clinical trials, which is the main reason biosimilars are cheaper and more accessible than reference products and have a potentially significant effect on patient care.

To date (May 2022), 105 biosimilars of 17 different reference products have obtained regulatory approval from the EMA and FDA. The first wave of approved biosimilars comprised relatively simple proteins that replaced a malfunction of the body, such as growth hormones (e.g., somatotropin, follitropin alfa, and insulin), or enhanced an existing pathway, such as growth factors (e.g., epoetin and filgrastim). The second wave of approved biosimilars included more complex and multifunctional monoclonal antibodies. The first mAb biosimilar to be approved was the TNF-a-i infliximab in 2013, followed by adalimumab and etanercept. Later, the anticancer drugs rituximab, trastuzumab, and bevacizumab entered the market and, most recently (2021), a biosimilar for ranibizumab for eye diseases received market approval. Currently, biosimilars of mAbs and fusion proteins represent half of the approved biosimilars in the EU and US. The number of biosimilars is expected to increase, given that more than 15 candidate biosimilars are currently under evaluation by the EMA and FDA. Furthermore, the imminent expiration of patent and exclusivity rights for several best-selling biopharmaceuticals in the following years could pave the way for other waves of biosimilars [64].

Although biosimilars have reached the EU and US markets, the uptake and acceptance of biosimilars in clinical practice varies within the European countries and is still low in the US. This variance has been attributed to, among others, budget and reimbursement factors and a lack of understanding, acceptance, or trust in the science behind the biosimilar approval that heavily relies on the comparability of QAs [65, 66]. Previous research on the comparability of QAs by Halim et al. focused on comparing filgrastim and epoetin products and found that certain QAs for products (copies) from less regulated markets differed significantly from the reference products and biosimilars available on the EU market. Although Halim et al. provided insight into the consistency and variability between products and batches, their investigation covers less complex proteins than those available today and did not reflect on the comparability of QAs that should be assessed to support biosimilar approval.

Information on the comparability of QAs for (un)approved biopharmaceuticals is shared through various information sources. These sources include the European Public Assessment Report (EPAR) published by the EMA after the final decision on the marketing authorization application is made by the EC and scientific publications in peer-reviewed journals that can be communicated before or after obtaining regulatory approval. While the EPAR reflects the regulatory assessment, scientific publications communicate what researchers find interesting to share with the scientific community. Previous research has focused on navigating scientific publications to assess the availability of comparability

exercises for (intended) biosimilars and found variations in the number of publications per molecule across different therapeutic areas [67-69]. Although these studies demonstrated that comparability exercises might be available in the literature, information on the comparability of QAs for intended biosimilars is scarce.

Furthermore, studies that assessed information on comparability exercises in EPARs are limited to the comparative nonclinical and clinical exercises, which found a substantial variation in the extent of details and type of (non)clinical data between the EPARs on biosimilars [70, 71]. Whether this is also the case for the comparability of QAs is entirely unknown and is addressed in this thesis. Studies that compared how the regulatory and scientific communities share information on biopharmaceuticals are limited to safety and efficacy and report a substantial discord between the two sources, necessitating consulting both sources to obtain a complete picture and make informed clinical decisions. Although it is acknowledged that the two sources have different objectives, little is known about the consistency and complementarity of information on the comparability of QAs described in the regulatory reports and scientific publications for biosimilars.

## Post-approval quality surveillance and potential implications for patient care

Drug regulations must ensure the quality, safety, and efficacy of (bio)pharmaceuticals at the time of approval and continuously control and monitor these throughout their life cycle through post-marketing surveillance and pharmacovigilance systems [33, 34]. The establishment of post-approval surveillance and pharmacovigilance systems was prompted by the thalidomide tragedy in the 1960s, resulting in developing pharmacovigilance to further characterize and monitor the safety profile of a (bio)pharmaceutical when knowledge is limited at the time of approval [72]. Today, post-approval surveillance of safety and efficacy is a fundamental part of the drug regulation. The WHO defined pharmacovigilance as "the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other medicine-related problem."

Post-approval surveillance comprises various pharmacovigilance activities and tools, including routine activities, such as the spontaneous reporting of adverse drug reactions and periodic safety update reports, and proactive activities, such as the risk management plan (RMP) describing additional post-authorization safety or efficacy studies (Table 5). The marketing authorization holder (MAH) provides a periodic safety update report to regulatory authorities at defined time points after approval, which includes further characterizations of all adverse drug reactions reported during the period and a critical assessment of the benefit-risk balance of the product. The MAH mandates submittal of

the RMP document, which includes a list of safety concerns for which a distinction is made in the "important identified risks," "important potential risks," and "missing information." The RMP is updated throughout the life cycle to reflect new safety information for a (bio)pharmaceutical. New safety information is assessed, and if considered relevant, risk minimization measures are taken (e.g., via direct healthcare professional communication or letters such as DHPC and DHPLs, black-box warnings, product or batch recalls, and marketing withdrawals issued to inform HCPs and patients).

The mainstays of post-approval quality surveillance are good manufacturing practice inspections and mandatory lot-release testing, where each manufactured lot is independently tested by the manufacturer and regulators [73, 74]. Lot-release testing is important to ensure the acceptable quality and safety of each lot before reaching the market and patients to obtain confidence in the potency and strength assigned to each lot and assess the validity and accuracy of QA testing performed on that lot by the manufacturer. Thus, the lot-release is a gate-keeper step to ensure the quality and safety of biopharmaceuticals before they reach patients.

**Table 5:** Post-approval surveillance tools for biopharmaceuticals.

| | Quality | Safety | Efficacy |
|---|---|---|---|
| Spontaneous reporting of adverse reactions | + | + | + |
| Periodic safety update report | + | + | + |
| Post-authorization safety or efficacy studies | - | + | + |
| Risk management plan | - | + | + |
| Good manufacturing practice inspections | + | + | - |
| Lot-release testing | + | + | + |

The post-approval surveillance tool covers (+) or does not cover (-) monitoring quality, safety, and efficacy aspects.

However, quality aspects can occur after approval for approved medicines (including biopharmaceuticals), including post-approval changes and defects in the quality of the DS and DP. Post-approval quality changes require regulatory approval or notification through submission of the variation of terms of marketing authorization, whereas post-approval quality defects require regulatory action. Companies can implement changes for many reasons: compliance with regulatory commitments and standards; maintaining product quality and consistency between batches; and increasing the manufacturing scale, robustness, efficiency, and reliability [75-77]. The regulatory approval of changes in the QAs of the DS and DP can be accessible in post-approval regulatory reports, such EPARs on the EMA public website. Regulatory actions due to quality defects can be communicated through DHPCs and DHPLs, recalls, or marketing withdrawals. Quality-driven tools are generally not publicly communicated, and pharmacovigilance

tools are safety and efficacy focused; thus, little is known about the quality aspects of biopharmaceuticals after approval. Therefore, investigating these quality aspects is of primary interest because they could potentially affect clinical outcomes and patient care.

Moreover, biopharmaceuticals are vulnerable molecules and can be affected by manufacturing, storage, and transportation changes. Vlieland et al. demonstrated this vulnerability by investigating how inadequate compliance with storage recommendations by patients at home could influence certain QAs, such as the formation of aggregates and particles, where the potential risk for clinical outcomes could not be estimated [78-81]. Post-approval changes in the quality of the DS and DP can occur concerning changes in manufacturing, quality control, formulation, packaging, and stability. Such changes can affect CQAs, potentially influencing clinical outcomes and patient care [54, 82].

There are at least two examples of a link between approval changes in the quality and potential implications for clinical outcomes and patient care. The first example is an unpredictable increased rate of pure red cell aplasia in patients treated with Eprex®, which was associated with a post-approval formulation change in 1998, widely known as the Eprex® tragedy [83]. The company replaced human albumin with polysorbate 80 and glycine to decrease the risk of contamination with viral infections associated with using human plasma. Pure red cell aplasia was attributed to an immunogenic reaction toward some level of protein aggregation in the new formulation, induced by eliciting the formation of epoetin-containing micelles or interacting with leachates released by the uncoated rubber stoppers of prefilled syringes. Since then, protein aggregation has been considered a CQA and must be within the acceptable limits set by regulators. The second example is the shift and drift in glycosylation and potency for several batches of Herceptin®, which was associated with a post-approval change in the manufacturing site and process. Glycosylation and potency are considered CQAs; hence, it raises the question about the potential implications of the shift and drift for clinical outcomes and patient care.

Eprex and Herceptin shifted attention to understanding and exploring post-approval changes in quality, which have been explored in previous studies focused on quantifying and assessing the risk level of the changes in the QAs of biopharmaceuticals [33, 34]. These studies reported a substantial number of changes in quality, often rated at a low or medium risk level (95%), reflecting that the regulatory system has gained experience in how to evaluate post-approval changes and the influence that these changes may have on the quality in general and the CQAs for biopharmaceuticals. Previous studies have reported on post-approval changes in quality focused on reference products of biopharmaceuticals, but information on post-approval changes for biosimilars is lacking in the literature.

When biosimilars are approved, they are considered stand-alone products, which means comparison against the reference product to redemonstrate the biosimilarity is

no longer required for biosimilars [84]. Furthermore, little is known about the type of post-approval changes in quality of the DS and DP and whether patterns exist in the timing of implementing post-approval changes. Therefore, the characterization of the nature, including the type, risk level, and timing of post-approval changes in quality, for biopharmaceuticals is relevant to complement the current evidence. The TNF-α-i products, including the reference products and biosimilars of infliximab, adalimumab, and etanercept, were selected as case examples because these biosimilars account for more than half of those approved by the EMA for mAbs and have the longest post-approval history on the EU market [85-88].

Biopharmaceuticals with quality defects can have potential implications for clinical outcomes and pose a risk for patient care. Post-approval quality defects can occur due to unintentional or inattentive errors during manufacturing, storage, transportation, or at any moment throughout the life cycle. When a (new) safety concern or a defect in quality aspects is identified by a manufacturer or reported by HCPs or patients, it is the company's responsibility to inform regulators as soon as it occurs. In response, regulators investigate the issue and take certain actions to address the potential implications for clinical outcomes that pose a risk to patient care. Regulatory actions often include summary information about the issue (whether safety or efficacy concerns or quality defects) and instructions for HCPs to deal with the issue to protect patients from potential clinical consequences.

An example of regulatory action is the recall of several heparin lots in 2007 because of quality defects concerning contamination with a semi-synthetic over-sulfated chondroitin sulfate, which was associated with serious acute hypersensitivity reactions in patients treated with contaminated batches [89]. As per the regulatory investigation, the over-sulfated chondroitin sulfate is chemically synthesized and similar to heparin in structure and was used to reduce the production process cost. This heparin crisis led the US regulators to require a batch release test for each heparin batch and to revise relevant pharmacopoeia standards to new tests for over-sulfated chondroitin sulfate as an impurity.

Previous studies have focused mostly on regulatory actions issued due to safety and efficacy concerns regarding (bio)pharmaceuticals, revealing that knowledge of the clinical risks and benefits of (bio)pharmaceuticals expands after approval [90-97]. However, studies that have explored regulatory actions due to the quality defects of biopharmaceuticals are scarce. Most previous studies have focused on analyzing recalls for medicines in general, which demonstrated that the number of and reasons for recalls varied between countries and occurred in both less and highly regulated markets [98-104].

Of these studies, only Ebbers et al. reported information on the number and nature of recalls issued in the US between 2003 and 2013 for biopharmaceuticals. Ebbers et al. found that a small fraction of recalls were issued for biopharmaceuticals compared to

small-molecule drugs. Recalls of biopharmaceuticals were related to defective devices or containers and packaging or labeling errors, which were unrelated to the complexity of the molecule and manufacturing process. Although the study indicated that none of the recalls for biopharmaceuticals were associated with unexpected risks for clinical outcomes and patient care, the study focused only on a single regulatory action (recalls) and might underestimate the quality defects that could be communicated through different types of regulatory action. Furthermore, the study provided no insight into the product associated with quality defects and, most importantly, how HCPs should act to counter the potential implications of the quality defects for clinical outcomes and patient care.

A few studies have assessed the quality and applicability of instructions for HCPs on clinical and biomarkers monitoring patients in clinical practice, which were often found to be of insufficient quality regarding both the regulatory letters sent to HCPs and the summary of product characteristics [105-107]. However, little is known about the actions required to be taken by HCPs when regulatory actions are issued due to quality defects, which we address in this thesis.

## Knowledge gap and the rationale behind this PhD thesis

Biopharmaceuticals are produced through complex processes using living systems, resulting in molecules with inherent variability and minor differences in QAs even between batches from the same process. Biopharmaceuticals, whether reference products or biosimilars, must have consistent and comparable QAs throughout their life cycle to ensure that patients can use safe and effective treatments. Previous research and PhD theses on biosimilars have focused on requirements for developing regulatory guidelines for biosimilar approval [108], analysis of a selection of QAs to compare the reference product and biosimilars of filgrastim and epoetin obtained from the EU market with copies from emerging markets [109], market access to biosimilars [65], barriers to sustainable biosimilar competition and uptake in clinical practice [66], scientific, legal, and regulatory hurdles for biosimilar development, and interchangeability of biosimilars [110].

In recent years, the comparability of QAs with more emphasis on CQAs has played a primary role in biosimilar regulation and could dominate biosimilar approval in the future. Knowledge of the comparability of QAs has become increasingly relevant because it is the basis for regulatory decisions for biosimilars and quality changes that can be implemented for biopharmaceuticals after approval.

The quality of biopharmaceuticals must be ensured and maintained throughout the medicine life cycle, which is a prerequisite for safe and effective treatment. Previous research, including PhD theses from our group, has focused on post-marketing regulatory learning for biopharmaceuticals after approval by the characterization of the post-mar-

keting safety and efficacy concerns and the evaluation of regulatory tools available to assess these concerns [108, 111-114]. This research has resulted in several studies that assessed various post-approval regulatory actions and activities related to safety and efficacy for (bio)pharmaceuticals after approval [90-97].

Studies that have assessed the quality of biopharmaceuticals after approval are limited and have focused on the quality of biopharmaceuticals after dispensing to assess patient compliance with storage recommendations and the effect of noncompliance on the quality of the biopharmaceuticals [79-81]. However, specific quality aspects have yet to be explored, including changes and defects that may occur for biopharmaceuticals before dispensing them to patients. These quality aspects require regulatory approval for quality changes and regulatory actions to mitigate the potential risk of quality defects. Insight into these post-approval quality aspects and their potential implications for clinical outcomes and patient care is still lacking.

## Objectives of the thesis

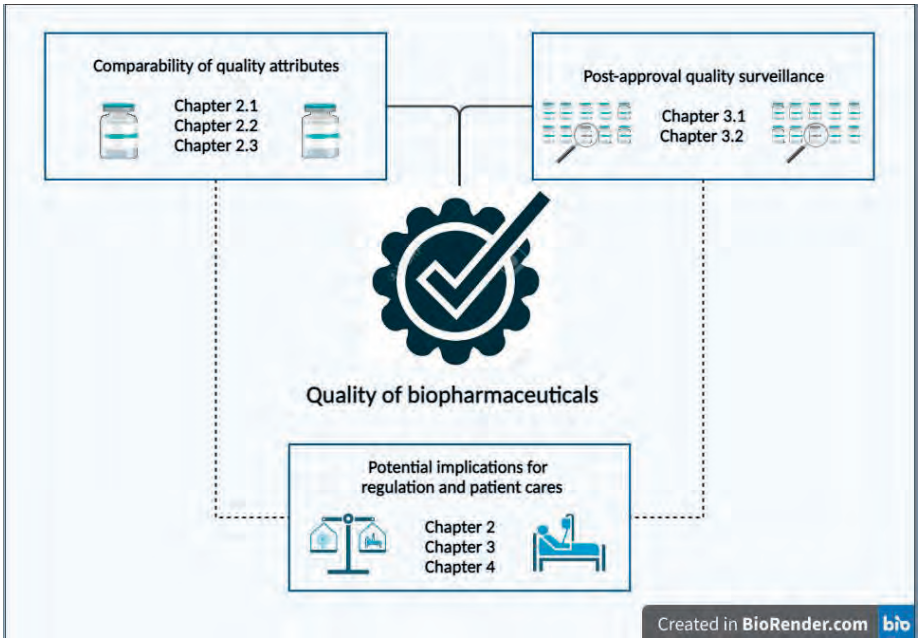
The thesis aims to study the quality of biopharmaceuticals by providing insight into (1) the comparability of QAs with emphasis on the CQAs, and (2) post-approval quality-related surveillance and regulatory actions of biopharmaceuticals. Moreover, this thesis aims to provide learning regarding post-approval changes and defects in quality of biopharmaceuticals that could potentially influence patient care.

## Outline of the thesis

Apart from this introductory **chapter 1,** this thesis includes five studies divided into two chapters, followed by a general discussion. **Chapter 2** focuses on the comparability exercises of QAs with more emphasis on CQAs. **Chapter 2.1** explores the availability of and reporting on comparability assessments of QAs for (intended) biosimilars in scientific publications. This study is the first step in studying the role of potential CQAs in the comparability exercise for a specific monoclonal antibody. **Chapter 2.2** compares the consistency and complementarity of reporting biosimilar QAs between regulatory reports and scientific publications using adalimumab as a case study. **Chapter 2.3** focuses on (potentially critical) QAs and assesses how EU regulators reflect the assessment of the comparability exercise of QAs in the European regulatory assessment reports on adalimumab biosimilars. **Chapter 3** addresses post-approval quality aspects of biopharmaceuticals, including quality changes and defects that could have potential implications for patient care. **Chapter 3.1** evaluates the nature and timing of post-approval manu-

facturing changes for TNF-α inhibitor products during more than 20 years of follow-up. The type and risk level of manufacturing changes were compared for originators and biosimilars to assess whether differences exist between the two groups. **Chapter 3.2** assesses the type, content, frequency, timing of post-approval regulatory actions due to quality defects of biopharmaceuticals approved in the EU and US between 1995 and 2019. This study provides insight into the underlying nature of the quality defects and the actions and recommendations required by HCPs. **Chapter 4** provides a general discussion of the results of the previous chapters from a broader perspective and highlights quality-related aspects for future consideration.

## Thesis outline infographic



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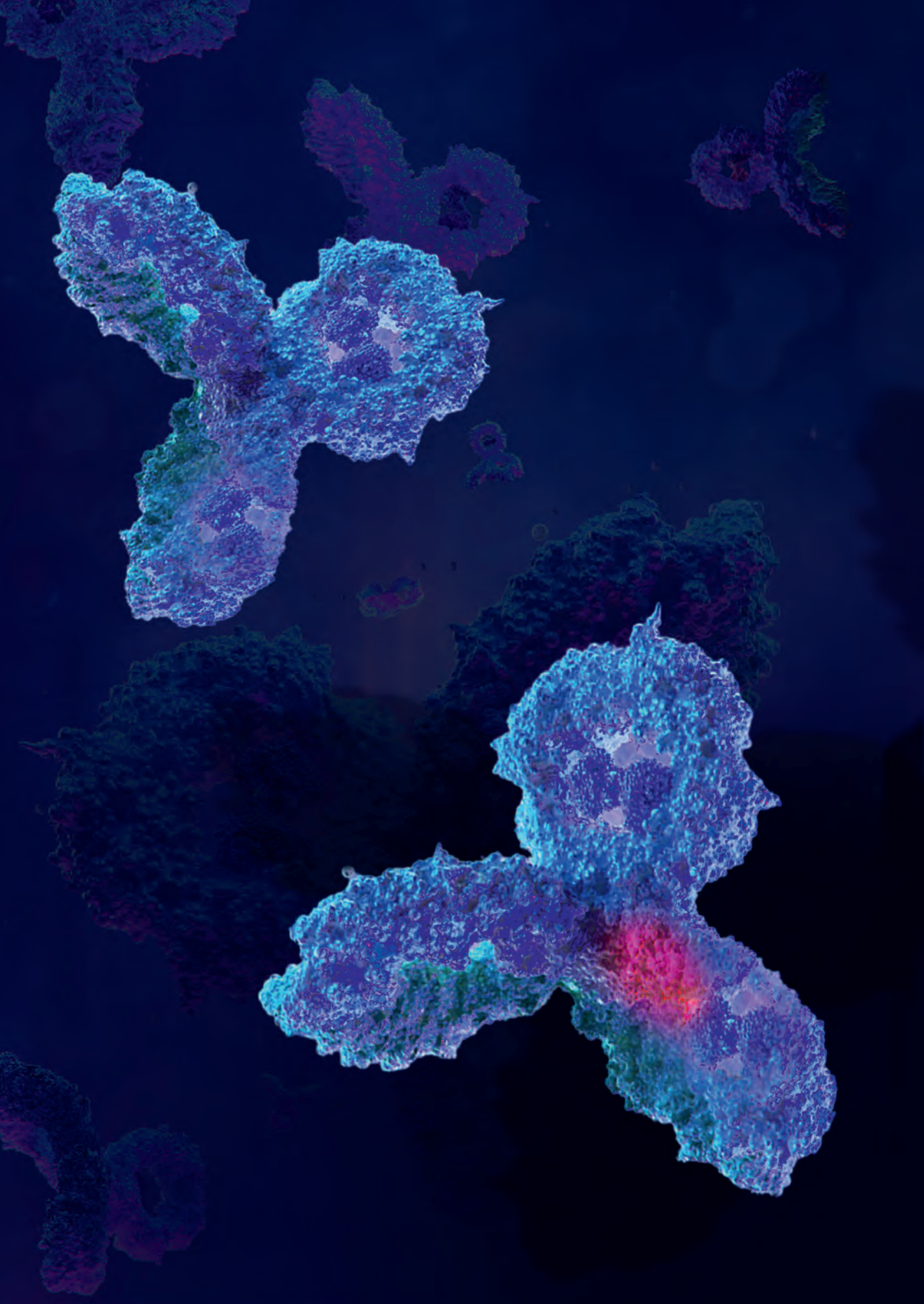
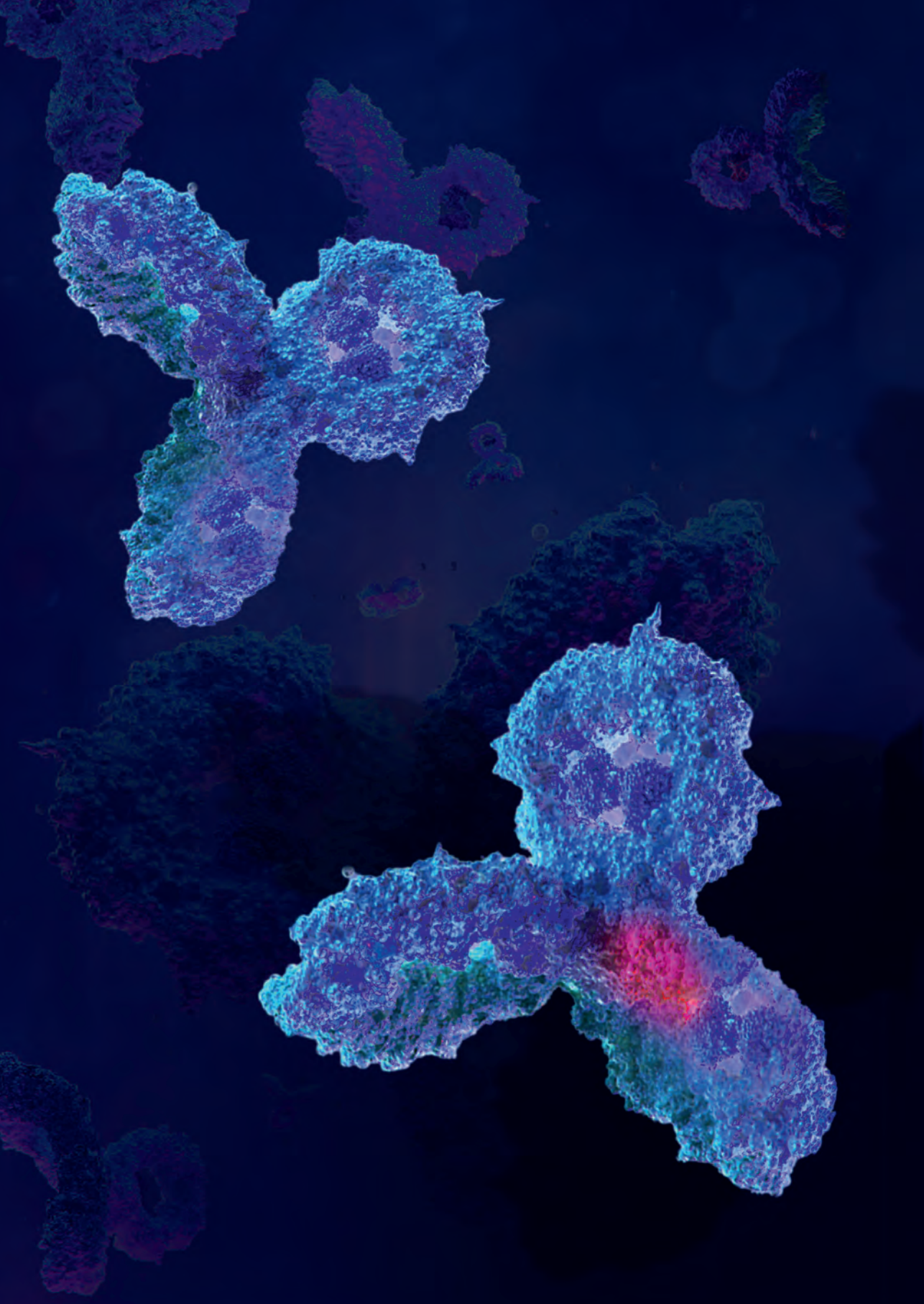


# Chapter 2

## Comparability of (critical) quality attributes



# Chapter 2.1

## Reporting of quality attributes in scientific publications presenting biosimilarity assessments of (intended) biosimilars: a systematic literature review

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## Abstract

Last years, more than 46 unique biosimilars were approved by EMA and/or US-FDA following patent expiration of reference products. Biosimilars are not identical like generics but highly similar versions, where demonstrating biosimilarity of quality attributes (QAs) to a reference product is the basis of development and regulatory approval. Information on QAs assessed to establish biosimilarity may not always be publicly available, although this information is imperative to understand better the science behind biosimilars approval. This study aims to identify QA types reported in publications presenting biosimilarity assessments of (intended) biosimilars over time. English full-text publications presenting biosimilarity assessments of QAs for (intended) biosimilars between 2000 and 2019 identified from PubMed and EMBASE. Publication characteristics and QAs classified into: structural (physicochemical properties, primary structure, higher-order structures (HOSs), post-translational modifications (PTMs), and purity and impurities) and functional (biological and immunochemical activities) were extracted from publications. Seventy-nine publications were identified (79% open-access, 75% industry-sponsored, 62% including unapproved biosimilars, and 66% involving antibodies). Reporting frequencies varied for QA types: biological activity (94%), physicochemical properties (81%), PTMs (79%), primary structure (77%) purity and impurities (73%), HOSs (58%), and immunochemical activity (41%). The number of publications increased from 6 (7%) during 2009–2011 to 62 (79%) during 2015–2019. Eighteen (28%) publications reported all QA types relevant to an active-biological-substance. Reporting of most QA types increased over time that most evidenced by immunochemical activity (from 0% to 47%) which occurred after EMA monoclonal antibody (mAbs) guideline in 2012 and more publications on mAbs later on when compared to earlier period. Biosimilarity assessments of QAs have been published in peer-reviewed publications for about 60% of approved biosimilars. Publishing biosimilarity assessments and reporting QAs over time appears to be affected by regulatory actions that occurred in 2012-2015, including regulatory approval and development of regulatory guidelines for biosimilars. Availability of a complete, publicly accessible and unbiased biosimilarity assessment of QAs, as part of a trusted and transparent regulatory process, will contribute to increase confidence and acceptance of biosimilars in clinical practice.

## Introduction

Recombinant DNA technology has enabled the production of therapeutic proteins as effective, mechanism-based treatments for a variety of diseases [1]. Since the first recombinant human insulin was granted regulatory approval in 1980 [2], multiple generations of recombinant DNA therapeutics ranging from single polypeptide chains such as hormones and cytokines to substantial and complex coagulation factors and antibodies have been developed and have received regulatory approval [3-8]. Biologicals offer important treatment options and accounted for 47% of all medicinal products containing novel molecular (chemical or biological) entities that were approved between 2014 and July 2018 in the US [8].

As patents of several biologicals have expired, the door opened for the development of subsequent versions: the so-called biosimilars or follow-on biologics. The first regulatory pathway for the approval of biosimilars was developed in 2005 by the European Medicines Agency (EMA) [9, 10]. Subsequently, after years of debate, the US Food and Drug Administration (US-FDA) launched an abbreviated regulatory pathway for biosimilars in 2015 [11, 12]. These biosimilar regulations intended to enable wider and earlier patient access for important medicines and to realize remarkable cost savings to reduce pressure on health care budgets [13, 14]. Up to this date, no product-specific safety and/or efficacy concerns were identified in clinical practice for licensed biosimilars in Europe supporting the robustness of the current regulatory framework [15-17].

In contrast to chemically synthesized generics that are identical copies, biosimilars are highly similar versions with respect to quality characteristics, biological and clinical activity, safety, and efficacy of the previously authorized reference products. The comparability assessment of quality attributes (QAs) between a biosimilar and the reference product is the basis for establishing biosimilarity during the development and regulatory approval of biosimilars. Quality attributes are measurable product characteristics that describe the physical, chemical, biological, and microbiological properties of a drug molecule [18]. In contrast to chemical drugs, biologicals are large and, often, complex molecules produced by living systems. This, and the complexity of the molecular structure and production process for biologicals results in a drug molecule with intrinsic variability (isoforms) and subsequent variability in QAs. The QAs of biologicals are heterogeneous and susceptible to changes in production processes that may, intentionally or not, for the same product result in gradual or sudden changes over time [19, 20]. Thus, variability in QAs of all biologicals is inevitable between batches derived from the same process; even isoforms in a single batch hardly remain constant over (storage) time [21-23].

Demonstrating biosimilarity requires a stepwise comparability assessment between a biosimilar and a reference product. The comparability of QAs is the mainstay for detecting potential differences and establishing biosimilarity. As a result of the advancement in science

and analytical technology, the comparative efficacy (phase III) trials became less important for certain product classes such as filgrastim, teriparatide and insulin biosimilars [24, 25].

Over the last decade, more than forty-six unique biosimilars (> eighty-seven brand names) have received regulatory approval from the EMA and/or US-FDA and this number is expected to further increase over the coming years. Although there is a robust and reliable regulatory framework for the approval of biosimilars, the uptake of and trust in biosimilars in the US and some European countries is still very low [26-28]. Clinicians focus on clinical trial data whereas the regulatory approval of biosimilars heavily relies on the comparability/biosimilarity assessment of QAs. The selection of QAs needed to establish the biosimilarity is not standardized yet and information on QAs accessible in the public domain is variable and for quite a few products limited. Compendial European and US pharmacopeia monographs cannot be considered as reference because these may not capture all QAs of the reference product, and have not yet been developed for clinical use [29]. The information on QAs of biosimilars are documented by the developers in confidential registration dossiers that are not publicly available, and reflected by the regulators in public assessment reports that are generally not well-known by stakeholders [30]. Sharing information on QAs through peer-reviewed scientific publications in a transparent manner is imperative to better understand the science behind the regulatory approval of biosimilars. As the clinical profile of biological drugs is influenced by their structural and functional QAs, information on QAs also results in better understanding on the role of QAs on clinical parameters. Previous systematic reviews show that there is a substantial discordance in the extent of published evidence to support establishing the biosimilarity between biological molecules across therapeutic areas [31-33]. However, no overview on the types of QAs studied in scientific publications to establish the biosimilarity is available in literature. Therefore, this study aims to identify the types of QA reported in scientific publications and provide an overview of the dynamics of scientific publications presenting biosimilarity assessments of QAs for (intended) biosimilars over time.

## Methods

### Systematic literature search

PubMed and EMBASE databases were used to collect scientific publications in peer-reviewed journals that presented biosimilarity assessments (i.e., analytical comparison) of QAs for (intended) biosimilars. The word "intended", hereafter, refers to any version of a recombinant therapeutic protein that was not approved by the EMA or US-FDA as a biosimilar at the date of publication. A search strategy was constructed to systematically identify relevant scientific publications between January 1, 2000 and December 31, 2019. Search strings were created to include indexed terms and controlled keywords

that were selected to define a search domain, determinant, and outcomes. The search formula (Domains AND (Determinants OR Outcomes)) was applied, which is provided in Supplementary Table S1a-b. Screening of titles and abstracts was performed to verify the search strings. The search filter title/abstract was used to retrieve publications pertinent to the study objective. The search strings were executed on May 21, 2018 and were refreshed on January 1, 2020 to capture recently indexed scientific publications up to December 31, 2019. This search was conducted according to PRISMA guidelines [34].

### Inclusion criteria

Duplicate publications identified by the search strategy were removed by the first author (AMA). The titles and abstracts of identified publications were screened by the same author to identify relevant and eligible full-text publications, which were further categorized into primary source "original publication" and secondary source "review publication". The list of references of each review publication was manually checked by AMA to retrieve relevant publications that were not captured in the electronic searches. If there was doubt about the eligibility of a publication for inclusion, a consensus decision was reached after discussion between AMA, TJG, and HG. Publications were considered eligible when: (I) the full-text article was in English; (II) (intended) biosimilars were assessed; (III) the active biological substance of the reference (comparator) was clearly defined; and (IV) a comparability/biosimilarity assessment (i.e., analytical comparison) of QAs between and (intended) biosimilar and the reference product was presented. Review publications were excluded unless original data were presented. Publications that assessed (intended) biosimilars containing non-recombinant proteins such as human albumin or heparin were excluded. Publications presenting comparability assessments with the primary aim to show assay suitability or manufacturing capability were excluded. Conference abstracts, preclinical animal studies, and all types of clinical trials were also excluded. European public assessment reports (EPARs) and chemistry review reports of approved biosimilars published by the EMA or US-FDA, respectively, were not considered in this study.

### Characteristics of included scientific publications

Baseline characteristics of each included publication were registered, namely the date of publication, the access-status of the publication, the source of funding, the regulatory-status of the (intended) biosimilar at the date of publication, and the type of active biological substance. The date of publication was the calendar month and year at the time of (first online) publication, which was divided into three periods. The three periods were selected based on the year of first publication and time frames where relevant regulatory guidelines were published and updated by the EMA (2012) and US-FDA (2014/2015), and defined as 2009–2011, 2012–2014, and 2015–2019. The publication access-status was defined as open or non-open access publications. The source of funding was categorized

into an industry or academic sponsorship. If the source of funding was not clearly stated, the institution of the corresponding author was considered as the source of funding. The regulatory-status of the (intended) biosimilar was defined as either approved or unapproved on the basis of the regulatory approval from at least one of the stringent regulatory authorities (SRAs) at the date of publication, which were identified from the official websites of the EMA [https://www.ema.europa.eu/en] and the US-FDA [https://www.fda.gov/]. The active biological substance of the (intended) biosimilars was classified into three types: antibodies, hormones, and others such as clotting factors and enzymes.

### Quality attributes

A classification scheme for the QA types was developed based on information about QAs included in biosimilarity assessments as outlined in the EMA and US-FDA biosimilar guidelines [9, 11]. The constructed classification scheme was discussed with regulators involved in the quality assessment of biosimilars at the Dutch Medicines Evaluation Board (MEB) [https://english.cbg-meb.nl/]. The QAs were first classified into structural or functional QAs, which included seven types of QAs in total. The structural QAs included five types: physicochemical properties, primary structure, higher-order structures (HOSs), post-translational modifications (PTMs), and purity and impurities. The PTMs were further divided into two subtypes: enzymatic PTMs including glycosylation and non-enzymatic PTMs. The purity and impurities were divided into two subtypes: size and charge variants. The functional QAs included two types: biological and immunochemical activities. All QA types included in the classification scheme are relevant to recombinant therapeutic protein with the exception of the enzymatic PTMs and the immunochemical activity that are only specific to glycoproteins and monoclonal antibodies and fusion proteins, respectively (Box 1).

### Data analysis

All reported QAs in the scientific publications were extracted, analyzed, and assessed using descriptive statistics. From each publication, the reported QAs were identified and sorted according to the developed classification scheme for the QA types (Box 1). The reporting frequencies were calculated for each QA type and subtype, which were stratified by the characteristics of the publication: publication date, funding source, regulatory-status, and active biological substance(s) type of the (intended) biosimilar(s). The median number for the reported QA types in publication(s) per year was calculated to present the dynamics of reporting QA types over time. For biosimilars that were approved by EMA or US-FDA, the pertinent scientific publications were identified for each unique biosimilar per the company development code or, if not applicable, per brand names. If a biosimilar granted approval from both agencies, the first regulatory approval date was considered to calculate the time difference (in calendar months) between the date of publication and regulatory approval. Follow-up ended on December 31, 2019. The statistical calculations were conducted using IBM SPSS 25 Statistical software (SPSS Inc. Chicago, Illinois, USA).

**Box 1:** A constructed classification scheme for the quality attribute types in biosimilarity assessment of biosimilars. *Enzymatic-PTMs and Immunochemical activity only applies to glycoproteins and antibodies, respectively.

**Structural quality attributes**

- **Physiochemical properties**
- **Primary structure**
- **Higher order structures-HOSs**
- **Post-translation modifications-PTMs**
  - Enzymatic- PTMs*
  - Non-enzymatic-PTMs
- **Purity and impurities**
  - Size variants
  - Charge variants

**Functional quality attributes**

- **Biological activity**
- **Immunochemical activity***

## Results

### Systematic literature search and characteristics of included publications

The search identified 1159 scientific publications that were potentially eligible for inclusion. After removing the duplicates, a total of 1012 publications were identified, of which 933 were excluded after title/abstract screening, most of which were conference abstracts or publications not related to biosimilarity assessments of QAs for (intended) biosimilars. This resulted in 79 full-text publications eligible for inclusion and further analysis (Figure 1).

The baseline characteristics of the 79 included publications [35-112] are described in Table 1. A large proportion of the included publications were open access (79%) and funded by the industry (75%). Thirty of the included publications (38%) studied biosimilars that had received regulatory approval at the date of publication. Most of the included publications presented biosimilarity assessments for (intended) biosimilars containing antibodies (66%).

### Reporting of quality attributes over time

Reporting of QAs varied between publications where the biological activity (94%) and physicochemical properties (81%) were the most frequently reported QA types. When comparing the reporting of QA types between publications, it was found that physicochemical properties (92% unapproved versus 63% approved), and primary structure (86% unapproved versus 63% approved) were often reported in publications of unapproved biosimilars, whereas immunochemical activity (50% approved versus 35% unapproved,

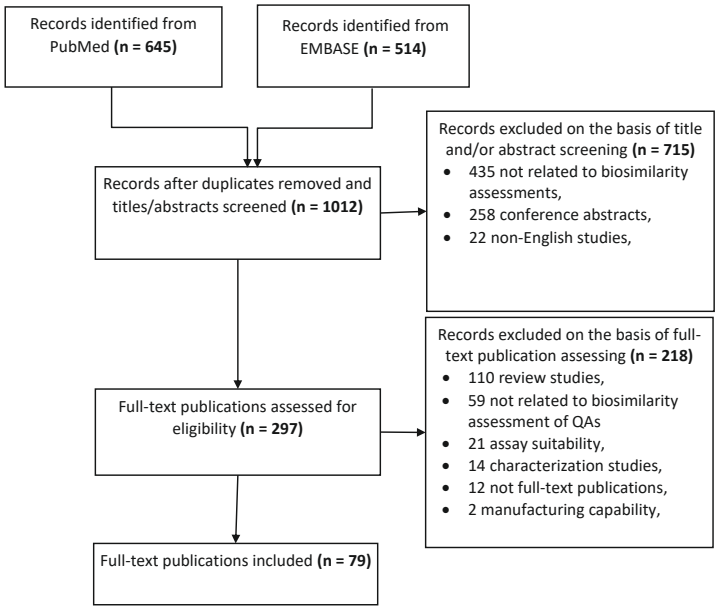


**Figure 1:** Flowchart depicting the inclusion criteria of eligible full-text scientific publications.

**Table 1:** Baseline characteristics of the included scientific publications.

| **Baseline characteristics** | **Publications n = 79 (100%)** |
|---|---|
| **Publication date** | |
| 2009–2011 | 6 (7%) |
| 2012–2014 | 11 (14%) |
| 2015–2019 | 62 (79%) |
| **Access-status of publications** | |
| Open-access | 62 (79%) |
| Non-open access | 17 (21%) |
| **Funding source** | |
| Academia/Public | 20 (25%) |
| Industry/Private | 59 (75%) |
| **Regulatory status of (intended) biosimilars at the date of publication** | |
| Approved | 30 (38%) |
| Unapproved | 49 (62%) |
| **Types of active biological substance of (intended) biosimilars** | |
| Antibodies | 52 (66%) |
| Hormones | 24 (30%) |
| Others (e.g., clotting factor and enzyme) | 3 (4%) |

and 25% academia versus 46% industry) were often reported in publications of approved biosimilars and publications sponsored by industry. The majority of publications sponsored by industry (48 out of 59; 81%) and publications of studied approved biosimilars (18 out of 30; 60%) included biosimilarity assessment of QAs for antibodies. Sixty-five of the included publications (82%) assessed (intended) biosimilars containing glycoproteins, for which enzymatic PTMs (e.g., glycosylation) are of relevance. In these 65 publications enzymatic PTMs were reported in 78%. The enzymatic PTMs were more often reported in publications of (intended) biosimilars containing antibodies, which accounted for 52 out of 65 (80%) of publications for (intended) biosimilars containing glycoproteins.

Most of the QAs were more frequently reported over time when comparing the periods 2009–2011 and 2015–2019—from 50% to 79% for primary structures, 67% to 82% for PTMs, 50% to 63% for HOSs, and 0% to 47% for immunochemical activity while reporting of some other QAs slightly decreased over time—from 100% to 71% for purity and impurities, 100% to 76% for general physicochemical properties, and 100% to 94% for biological activity—. Interestingly, reporting of immunochemical activity was first noted in the period 2012-2014 where reporting increased from 27% to 47%, which was in parallel with the increase of the number of publications and approvals for (intended) biosimilars of antibodies for which immunochemical activity is relevant (Table 2).
Of the included 79 publications, 24 (30%) reported all QA types that are relevant to the active biological substance of (intended) biosimilars being studied. The number of publications that reported all relevant QA types increased from 1 (17%) out of 6 publications during 2009-2011 (median= 4.0 QA types) to 21 (34%) out of 62 publications during 2015-2019 (median= 6.0 QA types) (Figure 2).

**Table 2:** Reporting of quality attribute types in scientific publications on biosimilarity assessments (n = 79), according to date of publication, source of funding, regulatory status of (intended) biosimilar on the date of publication, and type of therapeutic protein for the (intended) biosimilar.

| | Total n=79 (%) | Publication date | | | Funding sources | | Regulatory status | | Types of active biological substance | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2009-2011 n = 6 (%) | 2012-2014 n = 11 (%) | 2015-2019 n = 62 (%) | Academia n = 20 (%) | Industry n = 59 (%) | Ap-pro-ved n = 30 (%) | Unapproved n = 49 (%) | Antibodies n = 52 (%) | Hormones n = 24 (%) | Others n = 3 (%) |
| **Structural quality attributes** | | | | | | | | | | | |
| Physico-chemical properties | 81 | 100 | 100 | 76 | 85 | 80 | 63 | 92 | 77 | 88 | 100 |
| Primary structure | 77 | 50 | 82 | 79 | 80 | 76 | 63 | 86 | 79 | 75 | 67 |
| Higher-order structures-HOSs | 58 | 50 | 36 | 63 | 55 | 59 | 57 | 59 | 56 | 63 | 67 |
| Post translation modifi-cations-PTMs | 79 | 67 | 64 | 82 | 85 | 76 | 73 | 82 | 79 | 79 | 67 |
| Enzymatic PTMs* | 61 | 50 | 46 | 65 | 65 | 59 | 53 | 65 | 75 | 29 | 67 |
| Non-Enzymatic PTMs | 56 | 17 | 55 | 60 | 70 | 51 | 47 | 61 | 54 | 63 | 33 |
| Purity and impurities | 73 | 100 | 73 | 71 | 80 | 71 | 67 | 78 | 67 | 83 | 100 |
| Size variants | 68 | 100 | 73 | 65 | 65 | 70 | 63 | 71 | 62 | 83 | 67 |
| Charge variants | 57 | 50 | 36 | 61 | 50 | 59 | 57 | 57 | 62 | 46 | 67 |
| **Functional quality attributes** | | | | | | | | | | | |
| Biological activity | 94 | 100 | 91 | 94 | 95 | 93 | 93 | 94 | 94 | 92 | 100 |
| Immuno-chemical activity* | 41 | 0 | 27 | 47 | 25 | 46 | 50 | 35 | 62 | NA | NA |

*Enzymatic-PTMs and Immunochemical activity only applies to glycoprotein and antibodies, respectively

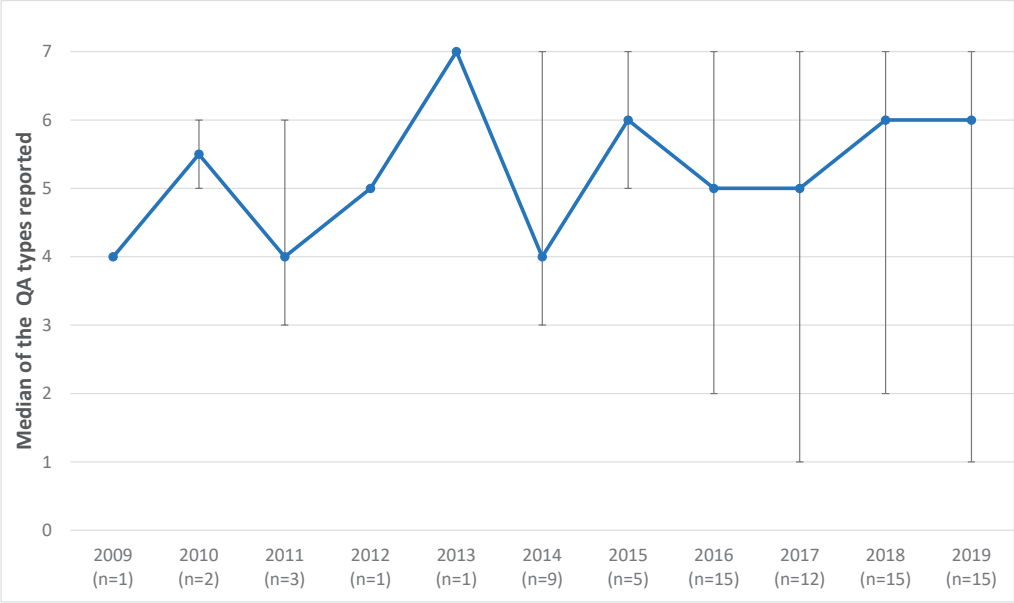


**Figure 2:** The median, minimum, and maximum values of the number of quality attribute types reported in the included publications over time.

## Dynamics of scientific publications of (intended) biosimilars over time

The first scientific publication that presented a biosimilarity assessment of QAs of (intended) biosimilars was published in 2009 while the first open-access publication was found in 2011. The number of scientific publications presenting biosimilarity assessments of QAs increased from 6 (7%) publications in the first period 2009–2011 to 11 (14%) publications in the second period 2012–2014 and 62 (79%) publications in the last period 2015–2019 (Figure 3).

The first period (2009–2011) included biosimilarity assessments for (intended) biosimilars containing hormones while more complex (intended) biosimilars containing antibodies became available in latter periods. These publications presented biosimilarity assessments of QAs for (intended) biosimilars against reference products for 19 distinctive active biological substance(s). The number of publications for monoclonal antibodies varied and ranged from 1 for *tocilizumab* to 14 for 18 (intended) biosimilars of *rituximab*. Most of the hormones were supported with a single publication, except for 23 *filgrastim, 14 epoetin* and 3 *follitropin alfa (intended) biosimilars, which* were supported with 9, 4, 3 publications, respectively (Supplementary Figure S1).

During the study period, the increase in the number of publications was in parallel with the increase of regulatory approval of biosimilars by the EMA and US-FDA (Figure 3). As of December 2019, the EMA and US-FDA have approved 46 unique biosimilars with

87 brand names as alternative to 15 reference products (Supplementary Table S2). More than half (56%) of the 46 unique biosimilars have at least one published biosimilarity assessment of QAs presented in a total of 36 publications where the majority (n= 33, 92%) was available as open-access publications. The remaining 43 publications studied (intended) biosimilars that were not yet approved by EMA and/or US-FDA as of December 2019. The overall average duration of time from the date of regulatory approval until publishing the first biosimilarity assessment of QAs for approved biosimilars was twelve months (SD= 27 months). Time from date of regulatory approval until scientific publication of the first biosimilarity assessment was the longest for biosimilars containing hormones (average mean= 33 months, SD= 22 months) after approval and shortest for biosimilars containing antibodies (average mean= 2.5 months, SD= 19 months) before approval (Supplementary Figure S2).

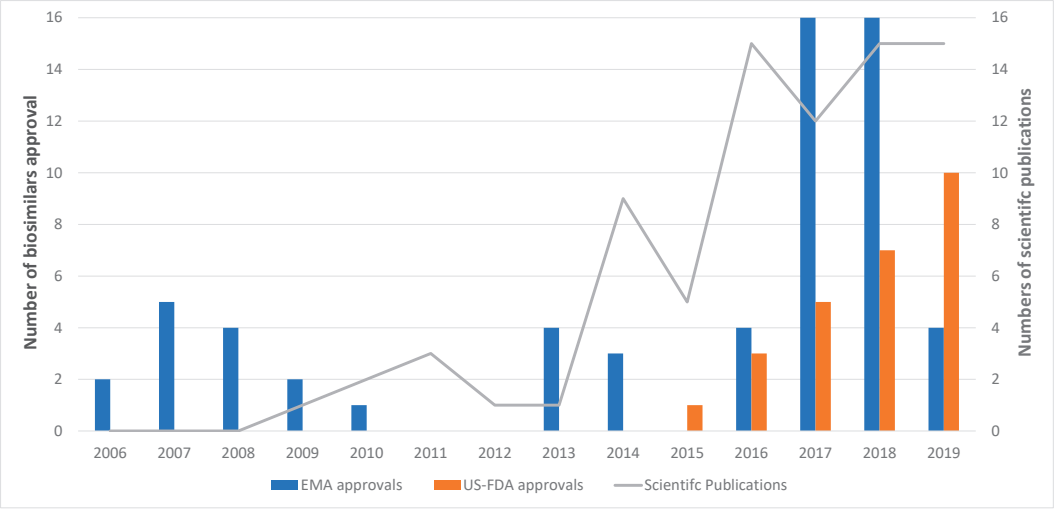


**Figure 3:** Dynamics of scientific publications presenting biosimilarity assessments of QAs in relation to the year of regulatory approval of biosimilars by EMA and/or US-FDA.

## Discussion

Our study found that reporting frequencies of the QA types in biosimilarity assessments of (intended) biosimilars varied among the included scientific publications. The most frequently reported QA types were the physicochemical properties and biological activity as these provide first and last insights, respectively, into the (dis)similarity between the (intended) biosimilar and the reference product at the molecular level. Reporting of most QA types increased over the study period, specifically the immunochemical activity

that was reported after the publication of the EMA guidance entitled "*guideline on similar biological medicinal products containing monoclonal antibodies* (mAbs)– *non-clinical and clinical issues"* in 2012 [113]. Although only 26 of the 46 unique biosimilars that have received regulatory approval, as of December 2019, from the EMA and/or US-FDA have a biosimilarity assessment of QAs in a scientific publication, the number of publications has increased over time; furthermore, only one-third of included publications reported all QA types that are relevant to the active biological substance of (intended) biosimilars being assessed.

A large variability in the completeness of reporting the QA types between publications was found, while demonstrating the biosimilarity would require assessing all QA types that are relevant to the active biological substance of an (intended) biosimilar [9, 11]. To illustrate this, the enzymatic PTMs (e.g., glycosylation) and immunochemical activity are specific to (intended) biosimilars containing glycoproteins and antibodies, respectively, but were not reported in all pertinent publications. However, the variability in reporting QAs is likely to be driven by the relevance of the QA type for the type of protein. For example, low reporting frequencies of enzymatic-PTMs in publications of hormones is likely due to the fact that hormones, in most cases, are non-glycoproteins where no existence of glycosylation precursors exist. The variability in reporting QA types might also be due to spreading out information on QAs in more than one publication where a few biosimilars have multiple biosimilarity assessments of QAs presented in different publications.

The foundation of establishing biosimilarity is the comparability assessment of QAs between a biosimilars and the reference product, followed by confirmation of biosimilarity by non-clinical studies, pharmacokinetics (PK), pharmacodynamics (PD) and comparative clinical efficacy and safety data where indicated [25]. The importance of the structural and functional relationship of QAs in establishing biosimilarity is continuously being better understood and characterized with the advancement in science and analytical technology. For example, the primary structure is essential in determining HOSs and might influence biological activity [114]. Thus, regulators strictly require identical amino acid sequences as a matter of principle because different sequence is from a regulatory perspective a different active substance. Alterations in "correct" folding of protein drugs may affect the receptor or antigen binding, and likely may hamper the biological and clinical activity and safety [115]. The PTMs and the purity and impurity QAs including size and charge variants often play a role in the biological activity, and such differences can substantially alter the PK/PD and/or immunogenicity via direct or indirect pathways [116]. Although differences in certain structural QAs can influence functional QAs, differences in functional QAs, including biological and immunochemical activity-for antibodies only- might have an impact on clinical parameters such as the serum half-life or the mode of action(s) [117-119]. The evaluation of functional QAs can

help to predict biosimilarity in the clinical performance and adds important knowledge for extrapolation across therapeutic indications [120]. Our data show that publications of unapproved biosimilars focused more on physiochemical properties and primary structure as these structural QA types only provide first insights into the biosimilarity between two molecules. On the other hand, the publications of approved biosimilars, which are often mAbs and fusion protein, focused more on biological and immunochemical activity as these types can link with the clinical activity and provide final insights into the biosimilarity at the molecular level. Moreover, the impact of (minor) differences in structural attributes could be assessed by testing functional attributes [119, 121, 122] Given the relationships between the QA types and their potential impact on clinical outcomes, it is important to pay equal attention to all relevant structural and functional attributes before concluding the biosimilarity at the quality level. Also, it is essential to report information on all relevant QAs that have or not met predefined biosimilarity criteria. Reporting all QA types relevant to the active biological substance was found in one-third of publications and seemed to increase over time, showing the willingness of publication-sponsors to share a comprehensive biosimilarity assessment of QAs.

The number of publications increased considerably during the study period, although the number is still a marginal fraction of all scientific publication on biosimilars. This positive trend indicates an improvement in knowledge sharing on biosimilarity assessments of QAs, which was not identified in previous systematic reviews [31-33]. This is perhaps because our search covered a longer time frame and a wider range of protein types, and was specifically designed to identify publications reporting biosimilarity assessment of QAs and assessed the QAs in more details. The increase in publications is likely a direct result of the increased development of biosimilars following patent expiration of reference biologicals by the industry and the growing interest in approval of biosimilars. The patent expiration of reference biologicals played an important role in the timing of arrival to markets where the first wave of approved biosimilars were hormones followed by monoclonal antibodies, the same shifted scope in the protein type of (intended) biosimilar was observed in scientific publications over time. The variabilities in the number of publications between the active biological substances of (intended) biosimilars (Supplementary Figure S1) is consistent with previous findings [32]. The majority of approved biosimilars (75%) were granted regulatory approval between 2015 and 2019, which is in line with the percentage of publications published during the study period (2015-2019). Our data also shows that scientific publications presenting biosimilarity assessments of QAs are available for approximately two-third of the approved biosimilars, revealing a knowledge gap for QAs of some biosimilars in peer reviewed scientific publications. Although the regulatory process has been shown to approve biosimilars which are as safe and efficacious as the reference product, biosimilars still face a sluggish and very low market penetration and uptake in the US and some European countries [26, 27].

Disseminating comprehensive data on biosimilarity assessment, including the QAs, in the public domain is necessary for gaining acceptance of biosimilars among prescribers, payers and patients, thereby achieving sustainable market uptake.

Our data shows some heterogeneity in publishing on QAs between publications that are derived from industry or academic institutions, which is likely explained by the fundamentally different motivations and expectations related to publishing. The motivation of the industry is to develop a biosimilar that meets the regulatory requirements, and as such they always perform a complete assessment of QAs, whereas the academia' immediate unit of success is the publications of what they think is relevant and interesting. The latter might not always include all QAs as what would be expected for an industry driven biosimilar assessment.

The majority of the included publications were funded by industry involved in biosimilar development. In addition, open-accessed publications has increased over time (Supplementary Figure S3), confirming the positive impact of scientific publications about similar drugs on industry rate of publication about its drug in the public domain [123]. This suggests that biosimilar developers are more willing to share the results of their biosimilarity assessment of QAs through open-access publications with the scientific and medical community. Transparency in publishing of comprehensive and unbiased biosimilarity assessment of QA data contributes to better understanding of the science behind regulatory approval and may increases confidence in biosimilars in medical practice. Academic institutions sponsored fewer publications in our review when compared to the industry. This might relate to their limited capacity and resources e.g., facilities and equipment, although three of the academia sponsored publications included in our review reported all relevant QA types to the active biological substance of (intended) biosimilars being assessed. Other factors that play a role in limiting the academic contribution are intellectual property rights and inaccessibility to batches of biosimilars that are not yet marketed [21].

Our data also shows that reporting the QA types over time is likely influenced by the development of regulatory guidelines of biosimilars that were published by the EMA and US-FDA during the study period. This regulatory guidance effect is most evident for reporting of immunochemical activity that was not reported in several included publications of (intended) biosimilars containing antibodies before the publication of EMA guidance on biosimilars containing monoclonal antibodies in 2012 [113]. The EMA updated guideline entitled *"Similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues",* and the US-FDA released a guideline entitled *"quality considerations in demonstrating biosimilarity of a therapeutic protein product to a reference product"* in 2014, and 2015, respectively [9, 11] Although both guidelines, especially compared to the first version of the EMA quality guideline,

placed more emphasis on all QA types included in the classification scheme, an increase in reporting was only found for primary structure, HOSs and PTMs.

Presenting biosimilarity assessment of QAs in scientific publications is one of several strategies to improve learning in biosimilar development, and to maintain communications with the scientific and medical community. The development of biosimilars together with continuous advancement in science and analytical technology facilitates the understanding about the active biological substance by the regulators and medical community. In the past, reference companies actively stated that producing biosimilars was almost an impossible task due to structural and manufacturing process complexity of biological drugs [124]. Several analytical analyses of different batches of reference products have shown that there is always some batch-to-batch variability in QAs as a result of, among others, changes in the production process [21-23]. The availability of a complete assessment of QAs could result in better understanding of the role of QAs in establishing biosimilarity and comparability not only for biosimilars at approval time but also for the reference biologicals as well as biosimilars when changes in the manufacturing process after the regulatory approval are introduced. However, among several QAs of biologicals, only a subset of these is potentially relevant to efficacy, safety, and dosing of a drug, which are also known as critical quality attributes (CQAs). As such, CQAs must be routinely monitored and controlled to keep them within an appropriate limit, range, or distribution to assure the quality of a biological drug [18]. A future challenge is to identify the CQAs and understand their relation to functional and clinical outcomes. This might result in a list of CQAs that matter most for establishing the biosimilarity, which could reshape the current regulatory requirements of biosimilars by reducing unnecessary comparative clinical trials currently required for licensing [25].

To our knowledge, this review is the first study that identifies the QA types reported in biosimilarity assessments presented in full-text scientific publications and describes the dynamics of publishing biosimilarity assessments and reporting of QA types over time. We constructed a classification scheme of QA types, based on regulatory guidelines and input from regulatory experts, to allow for a uniform assessment of the included publications. The study also sheds lights on how many biosimilars that were granted regulatory approval from the EMA and/or US-FDA have a published biosimilarity assessment of QAs to support the core evidence of biosimilarity in the literature.

Nevertheless, the present study has several limitations. First, a quality assessment for the included publications was not undertaken as there is no tool available to assess the strength/validity of the technical and analytical studies. Second, QAs might be missed due to heterogeneity in how these are defined between scientific publications, as no official classification system for QAs in biosimilarity assessments exists. However, we applied a classification scheme co-developed in collaboration with regulatory experts thus it is unlikely that QAs were missed. Third, it cannot be determined whether QAs not reported

were actually not tested or tested but not published by the author(s) because the present study only relied on published data. Finally, certain publications might be not included due to different languages or being unable to pick up in our search strings. However, we developed a search strategy in which the reference lists of review papers were manually checked to identify publications potentially missing in the electronic search, which resulted in an insignificant number of additional publications relevant to the study objective.

## Conclusion

We observed a clear increase in the number of scientific publications that present biosimilarity assessments of QAs for (intended) biosimilars over time, in line with an increased number of (intended) biosimilars for antibodies and hormones under development, with a large variability in the completeness of reporting QAs in these. Publishing of biosimilarity assessments and reporting of QA types over time appears to be affected by regulatory actions that occurred in 2012-2015, including the regulatory approval and the development of regulatory guidelines for biosimilars. Availability of a complete, publicly accessible (open access) and unbiased biosimilarity assessment of QAs, as part of a trusted and transparent regulatory process, will contribute to increased confidence and acceptance of biosimilars in clinical practice.

## Acknowledgments

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# Supplementary data

**Supplementary Table S1a:** Search terms and strategy for PubMed.

| Coherent | Search terms |
|---|---|
| **Domain** | "Biosimilar Pharmaceuticals"[Mesh] OR biosimilar*[Title/Abstract] OR similar biological medicinal*[Title/Abstract] OR subsequent entry biologic*[Title/Abstract] OR second entry biologic*[Title/Abstract] OR Off patent*[Title/Abstract] OR multisource product*[Title/Abstract] OR follow up biologic*[Title/Abstract] OR follow on biologic*[Title/Abstract] OR biogeneric*[Title/Abstract] OR intended cop*[Title/Abstract] OR similar biotherapeutic*[Title/Abstract] OR bio similar*[Title/Abstract] OR therapeutic protein product*[Title/Abstract] OR therapeutical proteins[Title/Abstract]) |
| **Determinant OR Outcome** | (biosimilarity[Title/Abstract] OR analytic*[Title/Abstract] OR quality assessment*[Title/Abstract] OR similarity assessment*[Title/Abstract] OR quality characteri*[Title/Abstract] OR quality attribute*[Title/Abstract] OR biocomparability[Title/Abstract] OR physicochemical* OR biological activit*[Title/Abstract] OR structural attribute*[Title/Abstract] OR functional attribute*[Title/Abstract] OR nonclinical assessment*[Title/Abstract] OR comparability* [Title/Abstract]) |

**Supplementary Table S1b:** Search terms and strategy for EMBASE.

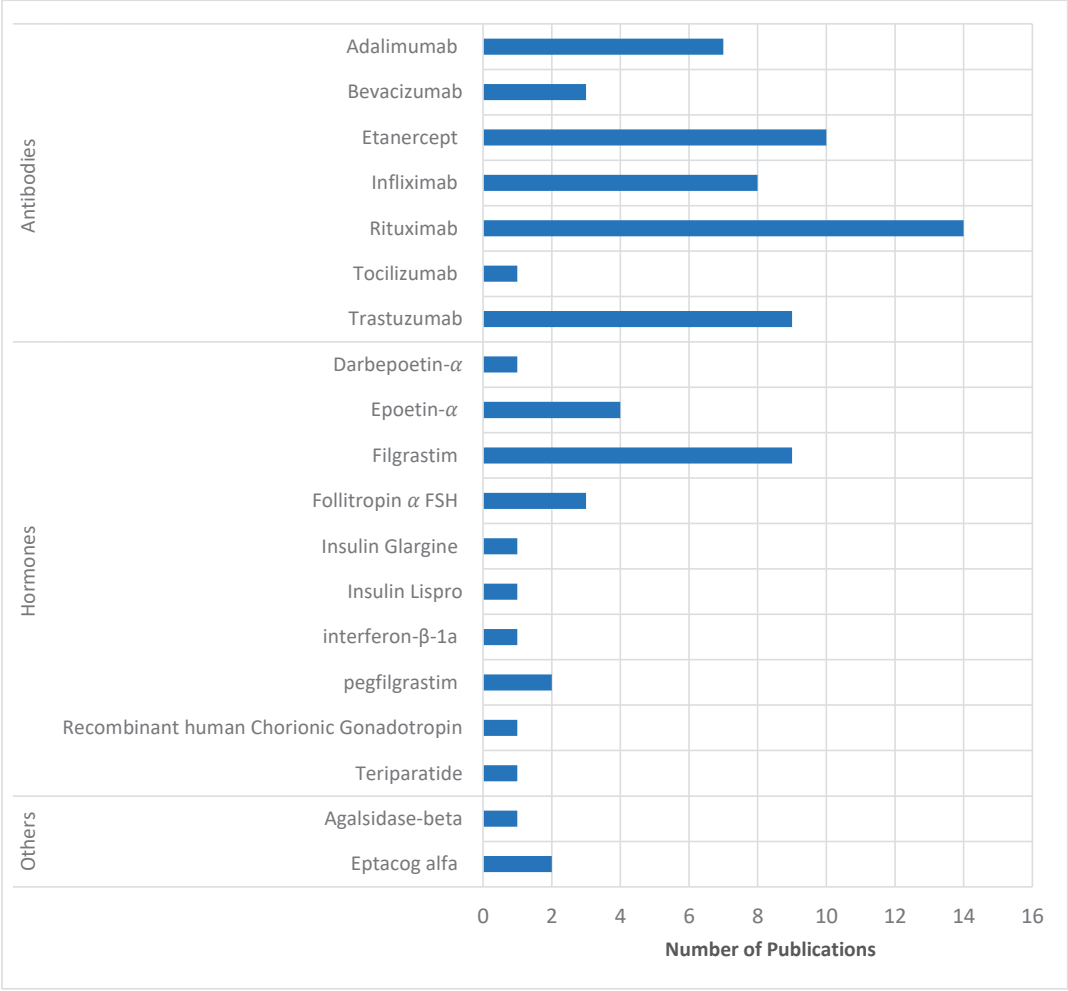
| Coherent | Search terms |
|---|---|
| **Domain** | ('biosimilar agent'/exp OR 'biosimilar agent' OR 'biosimilar drug*':ti,ab OR 'biosimilar*':ti,ab OR 'biosimilar agent':ti,ab OR 'biosimilar pharmaceutical':ti,ab OR 'similar biological medicinal*':ti,ab OR 'subsequent entry biologic*':ti,ab OR 'second entry biologic*':ti,ab OR 'off patent*':ti,ab OR 'multisource product*':ti,ab OR 'follow up biologic*':ti,ab OR 'follow on biologic*':ti,ab OR 'biogeneric*':ti,ab OR 'intended cop*':ti,ab OR 'similar biotherapeutic*':ti,ab OR 'bio similar*':ti,ab OR 'therapeutic protein product*':ti,ab OR 'therapeutical proteins':ti,ab) |
| **Determinant OR Outcome** | ('biosimilarity':ti,ab OR 'analytic*':ti,ab OR 'quality assessment*':ti,ab OR 'similarity assessment*':ti,ab OR 'quality characteri*':ti,ab OR 'quality attribute*':ti,ab OR 'biocomparability':ti,ab OR 'physicochemical*':ti,ab OR 'biological activit*':ti,ab OR 'structural attribute*':ti,ab OR 'functional attribute*':ti,ab OR 'nonclinical assessment*':ti,ab OR comparability*;ti,ab) |

**Supplementary Table S2:** the availability and accessibility of scientific publications presenting biosimilarity assessment of QAs for biosimilars (n=46) that were approved by EMA and US-FDA as of December 2019.

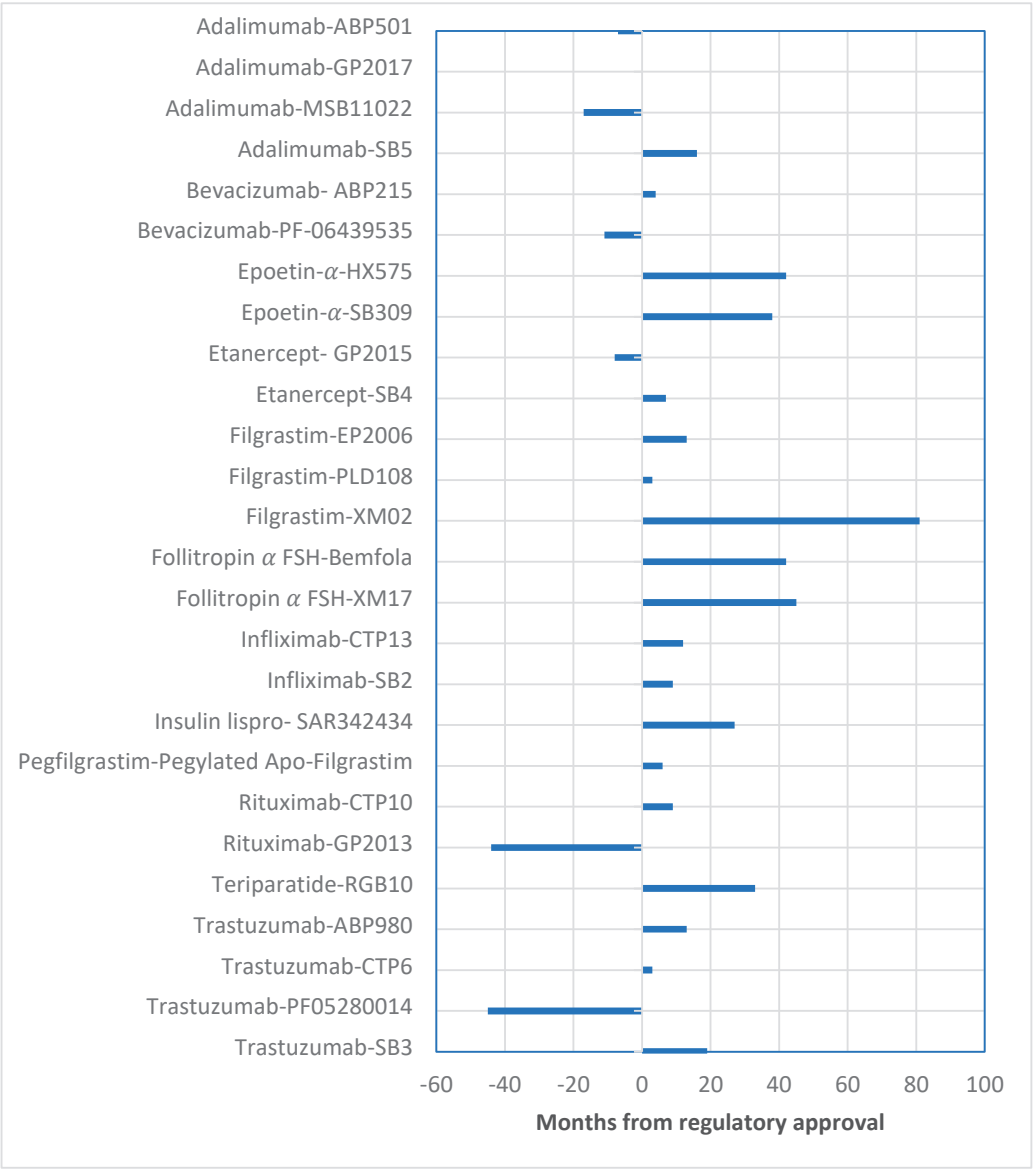
| Active substance | Development code | Brand names | First regulatory approval in EU/US (mm-yy) | Ref. | Access-status (yes/no) |
|---|---|---|---|---|---|
| Somatropin | EP2000 | Omnitrope | Apr-06 | - | - |
| Somatropin | Valtropin | Valtropin** | Apr-06 | - | - |
| Epoetin alfa | HX575 | Abseamed<br>Binocrit<br>Epoetin Alfa Hexal | Aug-07 | [1, 2] | Yes |
| Epoetin zeta | SB309 | Retacrit<br>Silapo | Dec-07 | [1, 2] | Yes |
| Filgrastim | XM02 | Ratiograstim<br>Biograstim**<br>Tevagrastim<br>Filgrastim<br>Ratiopharm** | Sep-08 | [3] | No |
| Filgrastim | EP2006 | Filgrastim Hexal<br>Zarzio<br>Zarxio | Feb-09 | [3-6] | Yes (2)<br>No (2) |
| Filgrastim | PLD108 | Nivestim<br>Nivestym | Jun-10 | [7] | No |
| Infliximab | CTP13 | Inflectra<br>Remsima | Sep-13 | [8-10] | Yes |
| Follitropin alfa | XM17 | Ovaleap | Sep-13 | [11] | Yes |
| Filgrastim | Apo-Filgrastim | Grastofil<br>Accofil | Oct-13 | - | - |
| Follitropin alfa | NA | Bemfola | Mar-14 | [12] | Yes |
| Insulin glargine | LY2963016 | Abasaglar (previously Abasria) | Sep-14 | - | - |
| Etanercept | SB4 | Benepali<br>Eticovo | Jan-16 | [13] | Yes |
| Infliximab | SB2 | Flixabi<br>Renflexis | May-16 | [8, 14] | Yes |
| Enoxaparin sodium | Inhixa | Inhixa | Sep-16 | - | - |
| Enoxaparin sodium | Thorinane | Thorinane | Sep-16 | - | - |
| Insulin glargine | MK-1293 | Lusduna** | Jan-17 | - | - |
| Teriparatide | RGB10 | Movymia<br>Terrosa | Jan-17 | [15] | yes |
| Rituximab | CTP10 | Truxima<br>Blitzima<br>Ritemvia<br>Rituzena<br>(previously Tuxella)* | Feb-17 | [16] | Yes |
| Adalimumab | ABP501 | Amgevita<br>Solymbic** | Mar-17 | [17, 18] | Yes |

**Supplementary Table S2 (Continued)**: the availability and accessibility of scientific publications presenting biosimilarity assessment of QAs for biosimilars (n=46) that were approved by EMA and US-FDA as of December 2019.

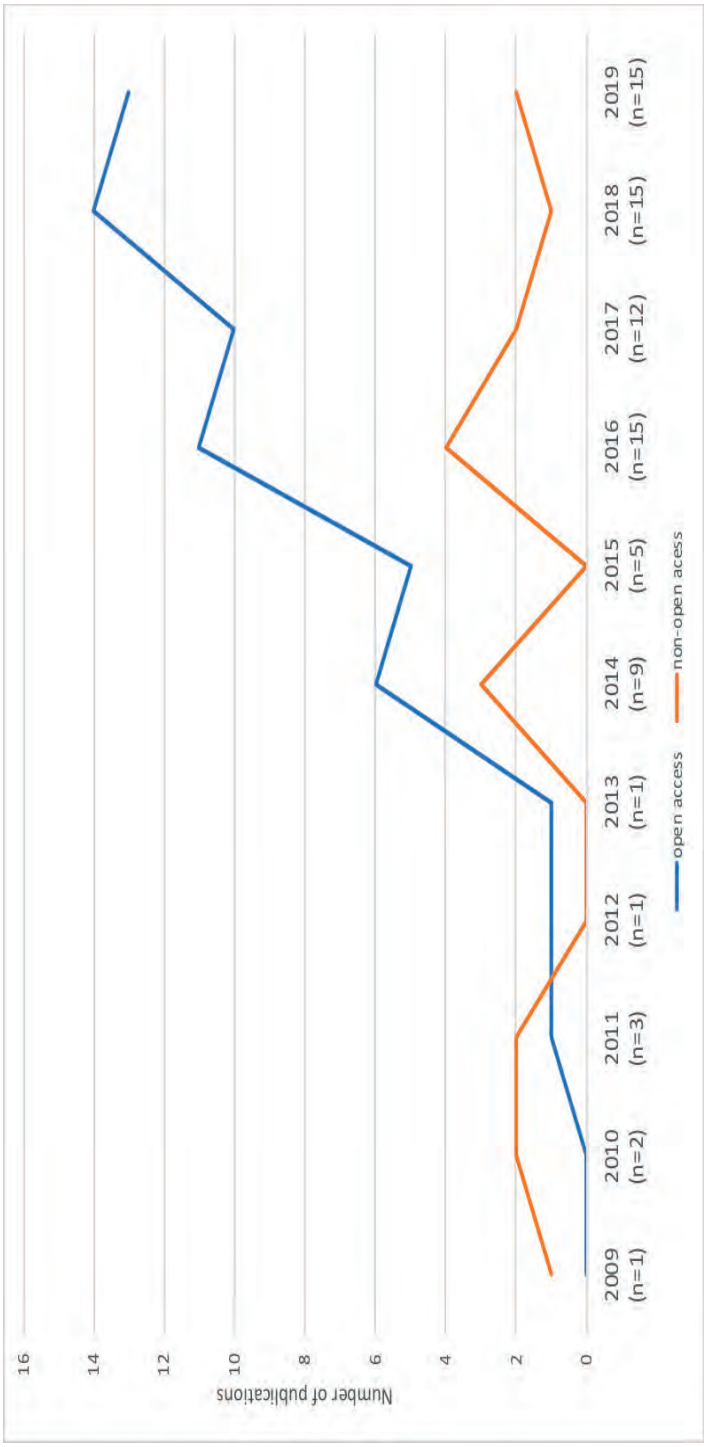
| Active substance | Development code | Brand names | First regulatory approval in EU/US (mm-yy) | Ref. | Access-status (yes/no) |
|---|---|---|---|---|---|
| Etanercept | GP2015 | Erelzi | Jun-17 | [19, 20] | Yes |
| Rituximab | GP2013 | Rixathon Riximyo | Jun-17 | [21, 22] | Yes |
| Insulin lispro | SAR342434 | Insulin lispro Sanofi | Jul-17 | [23] | Yes |
| Adalimumab | SB5 | Imraldi Hadlima | Aug-17 | [24, 25] | Yes |
| Adalimumab | BI695501 | Cyltezo** | Nov-17 | - | - |
| Trastuzumab | SB3 | Ontruzant | Nov-17 | [26, 27] | Yes |
| Bevacizumab | ABP215 | Mvasi | Jan-18 | [28] | Yes |
| Trastuzumab | CTP6 | Herzuma | Feb-18 | [29] | Yes |
| Insulin glargine | MYL1501D | Semglee | Mar-18 | - | - |
| Trastuzumab | ABP980 | Kanjinti | May-18 | [30, 31] | Yes |
| Infliximab | PF-06438179/ GP111 | Zessly Ixifi | May-18 | - | - |
| Trastuzumab | PF-05280014 | Trazimera | Jul-18 | [32] | Yes |
| Adalimumab | GP2017 | Hefiya Hyrimoz Halimatoz | Jul-18 | [33] | Yes |
| Adalimumab | FKB327 | Hulio | Sep-18 | - | - |
| Pegfilgrastim | Pegylated Apo-Filgrastim | Pelgraz | Sep-18 | [34] | Yes |
| Pegfilgrastim | CHS-1701 | Udenyca | Sep-18 | - | - |
| Pegfilgrastim | B12019 | Pelmeg | Nov-18 | - | - |
| Pegfilgrastim | MYL-1401H | Fulphila | Nov-18 | - | - |
| Pegfilgrastim | LA-EP2006 | Ziextenzo | Nov-18 | - | - |
| Trastuzumab | MYL-1401O | Ogivri | Dec-18 | - | - |
| Bevacizumab | PF-06439535 | Zirabev | Feb-19 | [35] | Yes |
| Adalimumab | MSB11022 | Kromeya Idacio | Apr-19 | [36] | Yes |
| Pegfilgrastim | USV | Grasutek | Apr-19 | - | - |
| Rituximab | PF-05280586 | Ruxience | Jul-19 | - | - |
| Infliximab | ABP710 | Avsola | Dec-19 | - | - |
| Adalimumab | PF 06410293 | Abrilada | Nov-19 | - | - |




**Supplementary Figure S1:** The number of included publications (n= 79) per the active biological substance(s) of (intended) biosimilars.




**Supplementary Figure S2:** The time difference between the date of publication and marketing authorization of biosimilars (n=26) that were approved by EMA or US-FDA and have scientific publication with biosimilarity assessment of QAs as of December 2019.




**Supplementary Figure S3:** The number of publications presenting biosimilarity assessment on quality attributes over time between open and non-open access publications.

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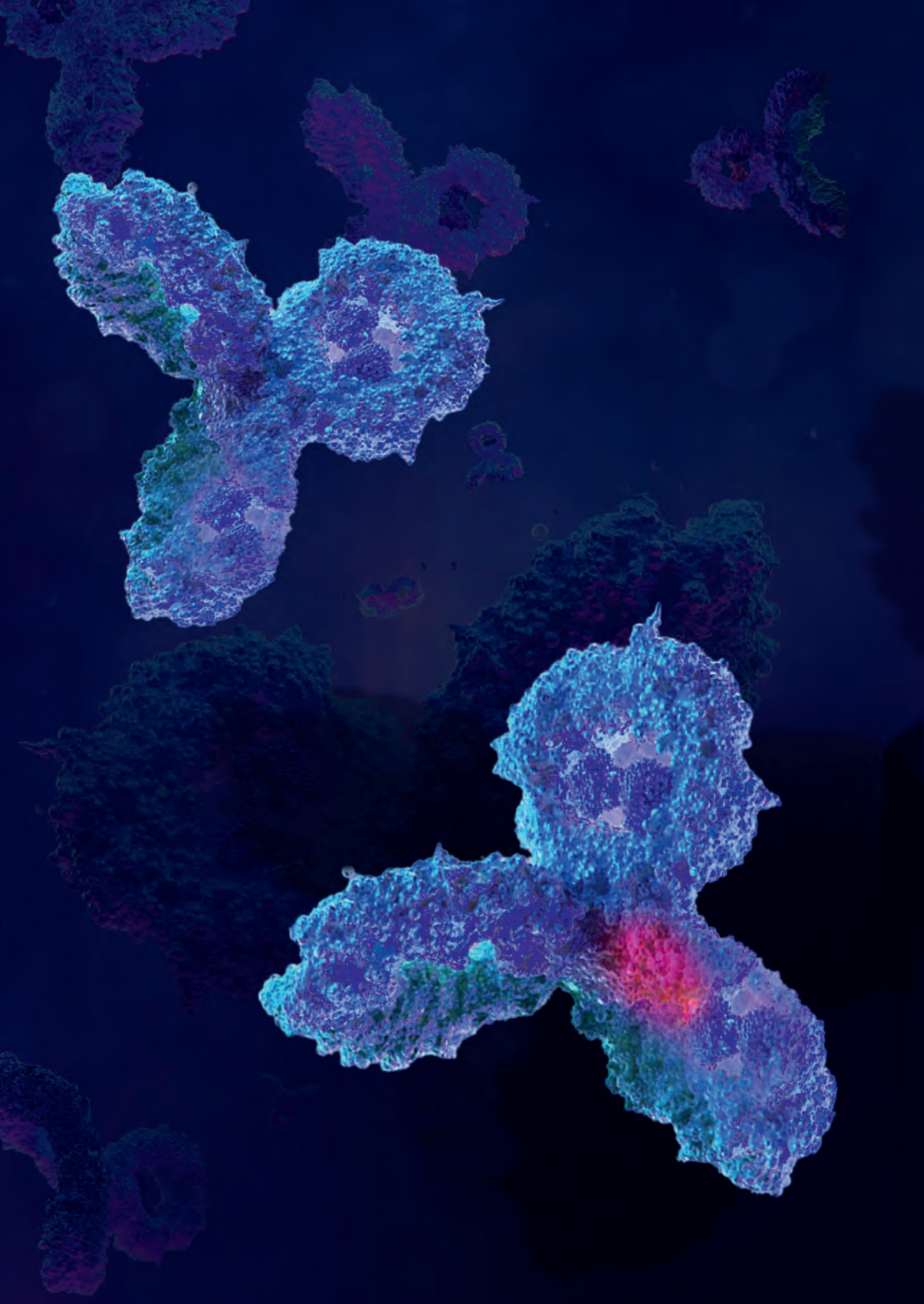

# Chapter 2.2

## Comparison of consistency and complementarity of reporting biosimilar quality attributes between regulatory and scientific communities: an adalimumab case study

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Author's contribution: AMA designed the study, performed data management, conducted data curation, analysis, and validation, prepared the first- draft of the manuscript, and implemented significant contribution from co-authors up to the final publication. Throughout the process, AMA asked for and implemented input and feedback from supervision team and co-authors, who performed critical review of the manuscript and provided significant contributions to the study.

## Abstract

Biosimilar approval relies on the comparability of quality attributes (QAs), for which information can be derived from regulatory or scientific communities. Limited information is known about whether these sources are consistent with or complementary to each other. The consistency and complementarity of QA reporting in biosimilarity assessments for adalimumab biosimilars approved by the European Medicines Agency in European public assessment reports (EPARs) and scientific publications was assessed. A classification of 77 different QAs (53 structural and 24 functional attributes) was used to assess the types of and information on QAs reported. Six adalimumab biosimilars were analyzed, for which the number of QAs reported in EPARs and publications varied (range = 47 [61%]–60 [78%]). The proportion of QAs consistently reported in both sources varied (range = 28%–75%) among biosimilars; functional QAs (mean = 21 QAs [88%]; range = 19–23) were more consistently reported than structural QAs (mean = 33 QAs [62%]; range = 27–34). The EPARs frequently reported biosimilarity interpretation without providing test results (9–57 QAs in EPARs versus 0–8 QAs in publications), whereas publications frequently reported both test results and interpretations (13–40 QAs in publications versus 0–3 QAs in EPARs). Both sources provided information on the biosimilarity of QAs in a complementary manner and the same biosimilarity interpretation of test results for reported QAs (mean = 90%; range = 78%–100%), with a small discrepancy in biosimilarity interpretations of a few clinically relevant QAs related to post-translation modifications and biological activity. Comprehensive reporting of QAs can contribute to an improved understanding of the role of structural and functional attributes in establishing biosimilarity and the mechanism of action of biological substances in general.

## Introduction



Since 2006, regulatory authorities have approved biosimilars, which are highly similar and clinically equivalent forms of off-patent reference biologicals. The increasing availability of biosimilars contributes to wider patient access to treatments for a variety of diseases due to the low prices of biosimilars. The regulatory assessment of biosimilars primarily relies on data regarding the comparability of quality attributes (QAs), which must remain within the range of variability established by analyzing multiple batches of the reference biological. Quality attributes are measurable structural or functional characteristics that describe specific physical, chemical, biological or microbial properties of a product [1]. Adalimumab (Humira®, AbbVie Inc.) is a fully humanized monoclonal antibody (mAb) that targets tumor necrosis factor-α [2] and has the largest number of approved mAb biosimilars and the broadest spectrum of therapeutic indications among TNF-α inhibitors, including infliximab and etanercept [3].

Stakeholders from the pharmaceutical industry, regulators, payers, healthcare professionals and patients can use different information sources to obtain comprehensive knowledge about the QAs of biosimilars. Two main publicly accessible information sources that report biosimilarity assessments are the regulatory community (e.g., European public assessment reports [EPARs]) and the scientific community (e.g., scientific publications) [4]. An EPAR is a regulatory document published by the European Medicine Agency (EMA) that outlines the regulatory procedures of a specific medicinal product and summarizes the evidence submitted by the applicant and the scientific assessment of the Committee for Medicinal Products for Human Use (CHMP) [5]. Scientific publications are published in peer-reviewed journals, by means of which the results from the biosimilarity assessment of QAs are communicated with the scientific community. For both sources, variation in the reporting of QAs has been acknowledged. A previous study from our group showed substantial variation in the reporting of QAs among the EPARs of various adalimumab biosimilars; the regulatory interpretation on biosimilarity was frequently provided for QAs, but the test results of the QAs were less detailed [6]. We have additionally shown that scientific publications on the biosimilarity assessment of QAs are available for only 60% of all biosimilars approved in the European Union (EU) and the United States, and the reporting of the QA types in these publications is highly variable and frequently incomplete [7].

The QA information available in the two publicly accessible sources is derived from biosimilarity or comparability assessments performed to support the development and marketing applications of biosimilars. The publication of information on QAs assessed to establish biosimilarity is likely influenced by the purpose of the information source. The EPARs represent the regulatory process of the registration dossier submitted by industry, whereas the scientific publications reflect the process of data generated and

interpreted by researchers affiliated with academia or industry. Only a limited number of studies have assessed whether and how information presented in these two publicly available information sources overlap. These studies focus on assessing the reporting of safety and efficacy data and have found substantial discord between regulatory reports and scientific publications [8-14]. To our knowledge, there are no studies that explore the reporting of QAs in the two sources and whether these QAs are consistent with or complementary to each other. Because the comparison of QAs is a fundamental step in the development and regulatory process of biosimilars and forms the basis for regulatory assessments of biosimilarity, a comprehensive and consistently reported set of QAs is needed to understand the science behind regulatory approval and increase confidence in biosimilars in clinical practice.

Therefore, the present study aimed to assess the consistency and complementarity of QA reporting in the biosimilarity assessment in EPARs from the regulatory community and in scientific publications from the scientific community using adalimumab biosimilars as a case example.

## Method

### Study cohort

Data were collected from the two information sources, EPARs and scientific publications, that reported on QAs in biosimilarity or comparability assessments of adalimumab biosimilars that were granted marketing authorization through a centralized procedure of the EMA until May 31, 2020. The EPARs included scientific discussions and technical summaries—after deletion of confidential data—submitted in the registration dossiers by the applicant. The EPARs were updated throughout the product life cycle after regulatory approval; however, only the initial EPARs published at the time of approval were considered for this study. EPARs were retrieved from the official website of the EMA (http://www.ema.europa.eu). Full-text scientific publications in peer-reviewed journals with biosimilarity assessments of adalimumab biosimilars were identified from the PubMed and EMBASE databases according to the search strategy presented in Supplementary Table-S1a–b (search date May 31, 2020). Both scientific publications published before and after biosimilar approval were included. Conference abstracts were not included, as these lack detailed data on QAs. Adalimumab biosimilars for which there were no scientific publications on the biosimilarity or comparability assessment of QAs were excluded.

### Data collection and extraction

Baseline characteristics for each adalimumab biosimilar were collected from each information source, including the company code(s), brand name(s), marketing authorization

holder, dates of publication of the initial EPAR and corresponding scientific publications and date of EU marketing authorization. A company code is a specific acronym including letters and numbers assigned by the developer and is used to define the active biological substance produced from the same development program. Certain adalimumab biosimilars are produced by the same manufacturer but marketed under different brand names, for example, Hefiya®, Halimatoz®, Hyrimoz®; however, the company code for these biosimilars is GP2017, for which the registration dossier and corresponding initial EPARs are identical. Thus, the company codes were considered identifiers to confirm that the scientific publications corresponded to the same adalimumab biosimilar described in the EPARs. If multiple brand names were associated with the same company code, only the EPAR of one brand name (e.g., Hefiya® for GP2017) was included in the study for subsequent analysis. The EPARs of brand names with the same company code were cross-checked to ensure that all EPARs presented identical information on biosimilarity assessment. The date of marketing authorization was defined as the calendar month and year when a marketing authorization was granted by the European Commission. The date of publication of the EPAR is generally the same date of the European Commission's decision. The date of publication of a scientific publication was defined as the calendar month and year when a publication first became accessible online.

**Outcomes**

The outcomes of this study were a) the types of QAs and b) information on the QAs reported in the EPARs or the scientific publications for the included biosimilars.

*Types of quality attributes*

The QAs reported in the initial EPARs and corresponding scientific publications were mapped according to the classification scheme developed in collaboration with regulators involved in quality assessments of biosimilars [7]. This scheme divided the QAs into structural and functional attributes, including a total of seven types with various subtypes, resulting in a list of 77 (53 structural and 24 functional) QAs identified from publicly available information relevant to a biological drug (Figure 1).

*Information on quality attributes*

Information on QAs reported in EPARs and scientific publications was investigated by assessing the extent of the information reported as well as the biosimilarity interpretation of the test result of QAs. The extent of information on QAs reported in each EPAR and corresponding scientific publication was classified into four categories (Table 1). The extent of information on QAs was defined based on the reporting of the test results and biosimilarity interpretation for reported QAs. The test results are presented in terms of the quantitative or qualitative acceptance criteria of a given QA, which included

numerical limits, range and distribution, as shown in the examples in Table 1, or other suitable visual assessment measures such as spectra for higher-order structures and chromatograms for purity and impurities. The biosimilarity interpretation was defined as the interpretation of the test result in terms of biosimilarity for a given QA provided by regulators in the EPARs and independent researchers in the scientific publications. The reporting of the biosimilarity interpretation of the test result of QAs was divided into two types: similar or different. The biosimilarity interpretation was defined as similar when the assessment included wording such as "identical", "same", "match", "(highly) similar", "comparable" and "consistent". The biosimilarity interpretation was defined as different when the assessment included wording such as "(minor) difference(s)" or "not similar".

### Data analysis

The reported QAs identified in EPARs and the corresponding scientific publications of adalimumab biosimilars were coded according to the classification scheme of QAs presented in Figure 1. The reporting of QAs (yes/no) was identified in each source; then the consistency and complementarity of the two sources in the QA reporting were assessed. A QA was considered consistently reported if it was reported at least once in both EPAR and scientific publications. A QA was considered complementarily reported if it was reported at least once in either EPAR or scientific publications. The same analysis was applied to assess the reporting of the extent of information on QAs for each biosimilar according to the above-mentioned four categories (Table 1). The proportion of consistently reported QAs and complementarily reported QAs was calculated. For adalimumab biosimilars where the biosimilarity interpretation (with or without the test results being presented) was reported in both EPAR and scientific publication, an assessment of whether both sources had the same interpretation was conducted. The same interpretation was considered if regulators in the EPAR and researchers in the scientific publication came up with the same biosimilarity interpretation of the test result for a given QA in both information sources (i.e., both reported "similar" or both reported "different").

## Results

### Characteristics of initial European public assessment reports and scientific publications

As of May 31, 2020, the EMA had approved 11 adalimumab biosimilars. These products were developed from seven unique biosimilars since several were marketed under different brand names. Although the marketing authorization holders had voluntarily withdrawn Solymbic®, Cyltezo® and Kromeya® from the EU market for commercial purposes, these were considered in the present study since the study aimed to assess the consistency and

| Structural quality attributes (n = 53, 70%) | | | | | | | Functional quality attributes (n = 24, 30%) | |
|---|---|---|---|---|---|---|---|---|
| **Physiochemical properties** | **Primary structures** | **Higher order structures** | **Post transaltions modifications (PTMs)** | | **Purity & Impurities** | | **Biological activity** | **Immunochemical activity** |
| | | | **Enzymatic PTMs** | **Non-Enzymatic PTMs** | **Size variants** | **Charge variants** | | |
| Molecular Mass | Amino acid sequence | Secondary structure | Glycosylation | Glycation | Aggregates | Main forms | Binding activity | Binding to C1q |
| Protein concentration | C-terminal variants | Tertiary strucutre | Glycosylation site | Oxidation | Sub-micron Particles | Acidic forms | Binding affinity | Binding to FcRn |
| Isoelectric point | N-terminal variants | Quaternary strucutre | Glycosylation site occupancy | Deamidation | Monomer | Basic forms | Binding specificity | Binding to Fcγ-RI |
| Visible Particles | Trisulfide variants | Thermodynamics properties | Glycoforms | Truncation | Dimer | | Binding to s-TNF-α | Binding to Fcγ-RIa |
| Subvisible Particles | Disulfide bridges | | Galactosylated glycans | Amidation | Isoforms | | Binding to tm-TNF-α | Binding to Fcγ-RIIa |
| Hydropphobcity | Thloether Bonds | | High mannose glycans | Isomerization | Fragments | | Neutralization of TNF-α | Binding to Fcγ-RIIIa |
| | Free-thiol SH | | Fucosylated glycans | Cysteinylation | Medium molecular weights | | Inhibition of apoptosis | Binding to Fcγ-RIIb |
| | | | Afucosylated glycans | Acetylation | Non-glycosylated heavy chain | | Induction of apoptosis | Binding to Fcγ-RIIIb |
| | | | Total afucosylated glycans | Formylation | | | Inhibition of proliferation | Binding to TNF-β |
| | | | Sialylated glycans | Methylation | | | Induction of regulatory macrophages | |
| | | | Neuraminic N-acetyl acid (NANA) | Hydroxylation | | | Inhibition of cytokine release | |
| | | | Neuraminic N-glycolyl acid (NGNA) | Phosphorylation | | | Inhibition of adhesion molecule expression | |
| | | | Galactose alpha-1,3-galactose | | | | ADCC activity | |
| | | | | | | | CDC activity | |
| | | | | | | | ADCP activity | |

**Figure 1:** Classification scheme for 77 common quality attributes (QAs) of biologicals. Definitions: ADCC: antibody-dependent cellular cytotoxicity, ADCP: antibody-dependent cellular phagocytosis, CDC: complement-dependent cytotoxicity, C1q: complement component 1q, TNF $\alpha$: tumor necrosis factor-alpha, s-TNF $\alpha$: surface tumor necrosis factor-alpha, tm-TNF $\alpha$: transmembrane tumor necrosis factor-alpha, Fc: fragment crystallizable, FcR: Fc receptor.

**Table 1:** Definitions of the four reporting categories for the quality attributes (QAs) assessed to establish biosimilarity and reported in the European public assessment reports (EPARs) and corresponding scientific publications.

| | | **Reporting of biosimilarity interpretation of the QAs** | |
|---|---|---|---|
| | | **No** | **Yes** |
| **Reporting of test results for the QAs** | **No** | QAs reported include no test results and no biosimilarity interpretation of the reported QAs, for example,<br>- The amino acid sequence and N-glycosylation site were compared.<br>- Protein concentration was determined.<br>- Binding to FcRn and Fcγ-RIIIa was studied, and a comparison of ADCC activity was performed.<br>- Neutralization of TNFα, binding to s-TNFα and binding to tm-TNFα were addressed. | QAs reported include the biosimilarity interpretation but not test results of the reported QAs, for example,<br>- The amino acid sequence and N-glycosylation site of the biosimilar were identical to those of the reference.<br>- The protein concentration was similar to that of the reference.<br>Minor differences with no clinical relevance were observed in glycation, galactosylated N-glycans, high mannose N-glycans, fucosylated N-glycans and sialylated glycans.<br>- The FcRn, C1q binding, CDC, ADCC and neutralization of TNFα were comparable with those of the reference. |
| | **Yes** | QAs reported include the test results but not the biosimilarity interpretation of reported QAs, for example,<br>- The levels of high mannose N-glycans (biosimilar: 1.9–2.5%; reference: 5.3–12.0%).<br>- The KD ranges for Fcγ-RIIIa binding (biosimilar: 6.2–10.1 nM; reference: 3.8–8.0 nM)<br>- The EC50 values for inhibition of cytokine release (204 pM, 294 pM and 200 pM for the three batches of biosimilars tested and 177 pM, 168 pM and 222 pM for the three batches of reference tested).<br>- The ADCC activity (biosimilar: 89–107%; reference: 84–115%) | QAs reported include the test results and biosimilarity interpretation of the reported QAs, for example,<br>- Minor differences with no clinical relevance were observed in the levels of high mannose N-glycans (biosimilar: 1.9–2.5%; reference: 5.3–12.0%).<br>- The ADCC activity (biosimilar: 89–107%; reference: 84–115%) was comparable/similar between the two products. |

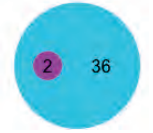
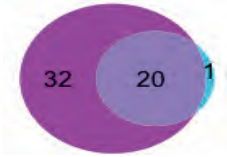
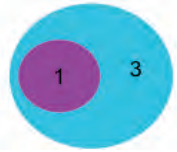
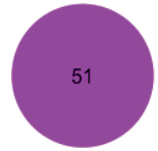
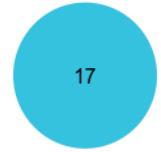
ADCC, antibody-dependent cellular cytotoxicity; CDC, complement dependent cytotoxicity; $EC_{50,}$ half-maximal effective concentration; TNFα, tumor necrosis factor-alpha; s-TNFα, surface tumor necrosis factor-alpha; tm-TNFα, transmembrane tumor necrosis factor-alpha; Fc, Fragment crystallizable; FcR, Fc receptor; $K_{D,}$ equilibrium dissociation constant; nM, nanomoles; pM, picomoles.

complementarity of information on QAs reported in biosimilarity assessments at the time of regulatory approval. For six of the seven unique biosimilars (85%), the biosimilarity assessment of QAs was reported in at least one corresponding scientific publication; one unique biosimilar—BI695501[15]—was excluded as it had no corresponding scientific publications. Thus, the following unique biosimilars were included for subsequent analysis: ABP501[16-19], SB5 [20-22], GP2017 [23-26], FKB327 [27, 28], MSB11022 [29-31] and PF-06410293 [32, 33]. The biosimilarity assessments of QAs were available through scientific publications before the publication of the initial EPAR for ABP502, GP2017, MSB11022 and PF-06410293. The relevant scientific publications were published, on average, one month (range = 1–29 months, standard deviation = 17 months) before the initial EPARs were available (Table 2).

**Table 2:** Characteristics of included European public assessment reports (EPARs) and scientific publications of adalimumab biosimilars

| Company code | Brand name | Marketing authorization holder | EU marketing authorization date (mm/yy) | Initial EPAR publication date mm/yy (ref.) | Scientific publication date mm/yy (ref.) |
|---|---|---|---|---|---|
| ABP501 | Amgevita® Solymbic®* | Amgen Europe B.V. | 03-2017 | 04-2017 [16,17] | 07-2016 [18,19] |
| SB5 | Imraldi® | Samsung Bioepis NL B.V. | 08-2017 | 08-2017 [20] | 10-2018 [21,22] |
| BI695501 | Cyltezo®* | Boehringer Ingelheim International GmbH | 11-2017 | 11-2017 [15] | None |
| GP2017 | Hefiya® Halimatoz® Hyrimoz® | Sandoz GmbH | 07-2018 | 08-2018 [23-25] | 07-2018 [26] |
| FKB327 | Hulio® | Mylan S.A.S. | 09-2018 | 09-2018 [27] | 05-2020 [28] |
| MSB11022 | Idacio® Kromeya®* | Fresenius Kabi Deutschland GmbH | 04-2019 | 04-2019 [29,30] | 11-2016 [31] |
| PF06410293 | Amsparity® | Pfizer Europe MA EEIG | 02-2020 | 02-2020 [23] | 01-2020 [33] |

*Solymbic®, Cyltezo® and Kromeya® were approved by the European Medicines Agency but voluntarily withdrawn by the applicant for commercial reasons.

| Biosimilar | Number of QAs reported | Reporting of test result −/ interpretation − | Reporting of test result +/ interpretation − | Reporting of test Result −/ interpretation + | Reporting of test result +/ interpretation + |
|---|---|---|---|---|---|
| **ABP501** (EPAR = 36, publication = 45, both = 53 QAs) |  53% Consistent (47%complementary) |  6% Consistent (94% complementary) |  0% Consistent (100% complementary) |  0% Consistent (100% complementary) |  5% Consistent (95% complementary) |
| **SB5** (EPAR = 49, Publication = 49, both = 56 QAs) |  75% Consistent (25% complementary) |  9% Consistent (91% complementary) | None |  9% Consistent (91% complementary) |  0% Consistent (100% complementary) |
| **GP2017** (EPAR = 52, Publication = 21, both =53 QAs) |  38% Consistent (62% complementary) |  25% Consistent (75% complementary) | None |  0% Consistent (100% complementary) |  0% Consistent (100% complementary) |

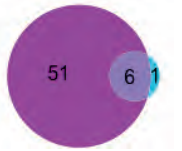
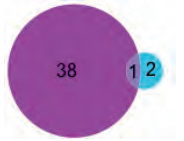
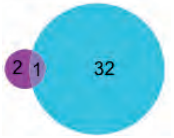
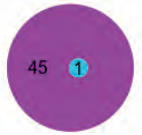
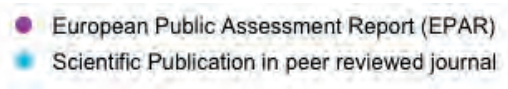
| Biosimilar | Number of QAs reported | Reporting of test result −/ interpretation − | Reporting of test result +/ interpretation − | Reporting of test Result −/ interpretation + | Reporting of test result +/ interpretation + |
|---|---|---|---|---|---|
| **FKB327** (EPAR = 57, Publication = 47, both = 60 QAs) |  75% Consistent (25% complementary) | None | None |  10% Consistent (90% complementary) |  0% Consistent (100% complementary) |
| **MSB11022** (EPAR = 42, Publication = 36, both = 53 QAs) |  47% Consistent (53% complementary) | None | None |  2% Consistent (98% complementary) |  3% Consistent (97% complementary) |
| **PF06410293** (EPAR = 46, Publication = 14, both = 47 QAs) |  28% Consistent (72% complementary) | None | None |  2% Consistent (98% complementary) |  0% Consistent (100% complementary) |

**Figure 2:** Proportional Venn diagrams presenting the number of quality attributes (QAs) described in the European public assessment reports (EPARs) and corresponding scientific publications for the adalimumab biosimilars approved in the EU.



### Types of reported quality attributes

The number of QAs reported in the EPARs and scientific publications varied among adalimumab biosimilars and ranged from 47 (61%) QAs for PF06410293 to 60 (78%) QAs for FKB327 (Table 3). Overall, the proportion of QAs consistently reported in both the EPARs and scientific publications further varied among biosimilars and ranged from 28% for PF06410293 to 75% for SB5 and FKB327 (Figure 2). More QAs were presented in the EPARs (range = 36–57 QAs) than in the scientific publications (range = 14–49 QAs). For all biosimilars, both sources provided complementary information on a greater number of QAs than the total QAs reported in each information source individually (e.g., for FKB327, EPAR = 57 QAs, publications = 47 QAs, both sources = 60 QAs; Figure 2). With respect to the type of QAs, functional QAs were reported more frequently (23/24; 96%) and consistently (mean = 21 QAs (88%); range = 19–23) than structural QAs (47/53; 89%; mean = 33 QAs [62%]; range = 27–34) in the EPARs and scientific publications (Table 3). For example, the binding to soluble-TNFα is a functional attribute directly related to the mode of action, which was reported in both information sources for all adalimumab biosimilars (data shown in the supplementary Figure S1). A list of QAs, the type and extent of information on each QA described in the EPARs and corresponding scientific publications for the same biosimilar are presented in Figure S1.

### Information on reported quality attributes

The reporting of biosimilarity interpretation without providing the test results was more frequent in EPARs (range = 9–57 QAs) than scientific publications (range = 0–8 QAs). Conversely, the reporting of test results and biosimilarity interpretations was more common in scientific publications (range = 13–40 QAs) than EPARs (range = 0–3 QAs). The consistency of reporting the extent of information on QAs (as defined in Table 1) between the EPARs and scientific publications of included biosimilars was low and ranged from 0%, which mainly applied to the reporting categories "test results without biosimilarity interpretation" and "test results with biosimilarity interpretation", to 10% for the category "no test results but with biosimilarity interpretation" for FKB327. The EPARs and scientific publications of three biosimilars (ABP501, SB5 and GP2017) lacked test result reporting and biosimilarity interpretation for several of the reported QAs (Figure 2).

The biosimilarity interpretation for reported QAs (with or without the test result of QAs being presented) was, in general, identical for a majority of reported QAs in the two information sources for included biosimilars. The QAs with same biosimilarity interpretation in both sources ranged from seven out of nine (78%) for ABP501 to 25 out of 25 (100%) for FKB327 and 13 out of 13 (100%) for PF06410293, whereas the QAs with different biosimilarity interpretations in both sources ranged from two out of 45 QAs (4%) for FKB327 to 6 out of 35 (17%) QAs for SB5. The proportion of QAs reported with the same biosimilarity interpretation in both sources was, on average, 90% (range = 78%–100%) for included biosimilars (Table 3). The types of QAs with the same biosim-

ilarity interpretation in both sources were frequently related to biological and immunochemical activity. Different biosimilarity interpretations of the test results between the two sources, where one source indicated similarity while the other indicated (minor) differences for the same QA, was observed for a few QAs among the included biosimilars. The types of QAs with different biosimilarity interpretations in both sources were frequently related to post-translation modifications and biological activity. For example, the biosimilarity interpretation of the test result of glycoforms was "minor differences" for a majority of EPAR and scientific publication pairs, except for the biosimilar SB5, where "minor differences" were reported in the EPAR and "similar" in the publication. Another example is the biosimilarity interpretation of the test result of antibody-dependent cellular cytotoxicity (ADCC activity), which was "similar" for a majority of EPAR and scientific publication pairs, except for the biosimilar GP2017, where "minor differences" were reported in the EPAR and "similar" in the publication. Although the test results of ADCC activity for biosimilars (ABP501, SB5, GP2017, and FKB327) was interpreted as "similar" in pertinent scientific publications, this same biosimilarity interpretation of ADCC activity for ABP501 (60–120%), SB5 (95–142%), GP2017 (85–183%) and FKB327 (69.5–130.9%) was based on different acceptance ranges presented in pertinent publications.

The test results and their biosimilarity interpretations were reported in the EPAR as well as the scientific publications for only two QAs of ABP501 (protein concentration and FcγRIIIa binding) and one QA for MSB11022 (FcγRIIIa binding). For both biosimilars, the same biosimilarity interpretation of the test result of reported QAs ("similar") was reported in both sources, although the numerical value of the test result differed between the two sources with the use of a strict range of acceptance criteria in the scientific publications (Table S2).

**Table 3:** Reporting of types of quality attributes stratified by the company code of adalimumab biosimilars in the European public assessment reports (EPARs) and scientific publications.

| | **All QAs (n = 77, %)** | **Types of QAs** | | **QAs with biosimilarity interpretation in both sources (n= QAs with same interpretation, %)** |
|---|---|---|---|---|
| | | **Structural (n =53, %)** | **Functional (n =24, %)** | |
| All biosimilars | 70 (91%) | 47 (89%) | 23 (96%) | * |
| ABP501 | 53 (69%) | 32(60%) | 21 (88%) | 9 (7, 78%) |
| SB5 | 56 (73%) | 33 (62%) | 23 (96%) | 35 (29, 83%) |
| GP2017 | 53 (69%) | 34 (64%) | 19 (79%) | 16 (13, 81%) |
| FKB327 | 60 (78%) | 40 (75%) | 20 (83%) | 45 (43, 96%) |
| MSB11022 | 53 (69%) | 31 (58%) | 22 (92%) | 25 (25, 100%) |
| PF06410293 | 47 (61%) | 27 (51%) | 20 (83%) | 13 (13, 100%) |

*No single QA was reported with interpretation in both information sources for all included biosimilars

## Discussion

The present study assessed the consistency and complementarity of the types of and information on QAs reported by regulators in the EPARs and researchers in the scientific publications of adalimumab biosimilars. Overall, the proportion of QAs consistently reported in both sources ranged from 28% for PF06410293 to 75% for SB5 and FKB327. Combining the information on QAs presented in both sources provided a more complete reporting of the biosimilarity assessment. Functional QAs were more frequently and consistently reported than structural QAs, which might be explained by their direct relation to clinical relevance. With respect to the extent of information on QAs, the EPARs more frequently reported biosimilarity interpretation without providing the test results, while the reporting of both test results and biosimilarity interpretation was more common in scientific publications. In general, both sources frequently reported the same biosimilarity interpretation of the test result for reported QAs, while a small discrepancy in reporting the biosimilarity interpretation or the acceptance criteria was detected for a few clinically relevant numbers of QAs (e.g., glycoforms and ADCC activity).

Along with the surge of biosimilars introduced to the European market over the last decade, the need for comprehensive and reliable information among decision makers (e.g., clinicians, pharmacists, payers and regulators) about the justification of biosimilarity has become pertinent. Data supporting the claim of biosimilarity, particularly those related to QAs, is reported by the EMA in EPARs and has increasingly been reported by industry in scientific publications [7]. The present study identified information for 70 (91%) of the 77 pre-defined QAs in the EPARs and scientific publications of adalimumab biosimilars. As expected, reporting on QAs varied between the two sources among the included biosimilars. This variation was in part due to the different aims of the two sources and was consistent with previous findings of substantial differences in reporting safety and efficacy information in regulatory reports and scientific publications [8-14]. Therefore, both sources should be systematically consulted to obtain comprehensive information on QAs for an improved understanding of how biosimilarity was established at the molecular level.

Functional QAs were more frequently described in EPARs and scientific publications than structural attributes (88% versus 62%). For adalimumab, the binding to and neutralizing of both the soluble and membrane-bound TNF-α were functional QAs relevant to the mechanism(s) of action (MoA), which was consistently reported in both sources for all included biosimilars. By the binding to Fc gamma receptors (FcγRs), and component 1q (C1q), adalimumab can additionally mediate effector functions such as ADCC and CDC activity [34], which were additionally described in both sources for at least five adalimumab biosimilars (Figure S1). The relevance of ADCC or CDC activity to the efficacy of adalimumab is not well established but may be important, particularly in inflammatory bowel disease [3]. The underlying reason for functional attributes to be

more comprehensively and consistently reported could relate to the fact that they reflect the clinically relevant MoA and provide useful information in predicting the outcomes of clinical studies [35-37]. Moreover, functional attributes provide not only the final insight into (dis)similarity at the quality level but also the basis for supporting the extrapolation of biosimilars across all indications authorized for the reference product [38-41]



Although we were not able to study the clinical relevance of our findings, it is known that (minor) differences in QAs (e.g., post-translational modifications and size and charge variants) may directly or indirectly impact functional attributes and clinical profiles [42-44]. The clinical profiles of biologicals, including biosimilars, are influenced by structural and functional attributes. Subsets of these attributes are likely related to clinical profiles and are frequently referred to as critical quality attributes (CQAs). Although there is no consensus on which attributes are CQAs, these need to be identified and controlled to ensure that clinical effects and product safety are not impacted by (minor) differences. In practice, (minor) differences in QAs between a biosimilar and reference biological are expected due to different production processes. This further applies to batch-to-batch variability during the life cycle of the reference biological due to introducing changes to enhance the production process [45]. The biosimilar only has to show biosimilarity to the reference product as part of the initial approval. After approval, the biosimilar is considered a stand-alone product and can undergo changes to the production process without the need to show biosimilarity to the reference biological. Examples of the potential clinical impact of structural differences in biologicals include increased immunogenicity due to increased aggregates; a decrease in antibody specificity and affinity due to increased deamidation in the complementarity-region (CDR) and a decrease in neonatal Fc receptor (FcRn) binding leading to an increase in drug clearance due to increased oxidation. It is additionally known that differences in glycoforms can have a significant impact on functional attributes. An increase in afucosylated glycans can positively impact FcγRIIIa binding, leading to increased ADCC activity, while an increase in sialylated glycans negatively impacts FcγRIIIa binding, hence decreasing ADCC activity. Furthermore, an increase in galactosylated glycans leads to increased C1q binding and hence increased CDC, while an increase in high mannose glycans can lead to increased drug clearance.

For the majority of adalimumab biosimilars included in the present study, the reporting of the extent of information on QAs in the two sources was inconsistent but reasonably complementary. For example, biosimilarity interpretation without providing the test results of QAs was frequently reported in the EPARs (range = 9–57 QAs in EPARs versus 0–8 QAs in publications), whereas a combination of the test results and biosimilarity interpretation was frequently present in the scientific publications (range = 13–40 QAs in publications versus 0–3 QAs in EPARs). Although the scientific publications were available before the EPARs for most included biosimilars, both sources provided the same biosimilarity interpretation for a majority of reported QAs. This alignment in biosimilarity

interpretation between the two sources is reassuring for the biosimilar system. There was only a small discrepancy in reporting biosimilarity interpretation for the glycoforms and ADCC activity of SB5 and GP2017, respectively. For both examples, the test results were interpreted as having "(minor) differences" in EPARs and being "similar" in publications. The EPARs stated that these (minor) differences were appropriately justified in the dossier and considered clinically meaningless. Nonetheless, the scientific justifications underlying these (minor) differences and the test results were frequently not presented in the EPARs, which did not allow for further insight into the extent of (minor) differences.

This means that for an improved understanding of the science behind the regulatory approval of biosimilars, there is a need to know both the test results and the interpretations. It may be not as important to report the test results for all QAs but important to place more emphasis on CQAs. The discrepancy in reporting the biosimilarity interpretation of the test results in terms of biosimilarity between the two sources could be explained by the following. (1) The wording chosen to describe the biosimilarity interpretation may differ between the EPAR and publication and be subjective; for example (minor) difference might mean the same as (highly) similar. (2) The test result of QAs presented in publications may differ from those submitted in the dossier for regulatory decision. (3) The acceptance criteria for defining the biosimilarity limit or range of a given QA may differ between the two sources as well as across publications. The acceptance criteria might be influenced by the number and age of batches of the reference product at the time of analysis [46]. Based on our analysis, a more strict biosimilarity range of reported QAs was present in publications when compared to EPARs (Table S2). The publications additionally used different acceptance criteria for biosimilarity, for example, the ranges of ADCC activity for ABP501 (60–120%), SB5 (95–142%), GP2017 (85–183%) and FKB327 (69.5–130.9%), although all the included biosimilars were compared to the same reference product. These differences in the ranges of ADCC activity between publications could be related to the variability between batches of the reference product, which may additionally raise questions on what the range considered by regulators to be acceptable for biosimilarity is.

The differences in QA reporting between the EPARs and scientific publications reflect the different purposes of the two sources (i.e., information affecting regulatory decisions versus information focusing on study and data). Regulators, who have access to a complete quality, nonclinical and clinical data of biosimilars during the regulatory process, may be more concerned with the consistency and accountability of decisions. Researchers, frequently affiliated with biosimilar companies, might be more focused on presenting positive news, that is QAs with favorable results in terms of biosimilarity, such as highly similar attributes. The present study could not detect any signatures of bias, although selective reporting on QAs in both sources could not be excluded and would need further study. For instance, the biosimilarity assessment for functional attributes of GP2017 was only reported in a single scientific publication [26]. The dissemination of a comprehensive

biosimilarity assessment of all relevant and critical QAs in the public domain contributes to an enhanced understanding of the relationship between structural and functional attributes and provides insight into MoA and clinically relevant attributes. For example, drifts in FcγRIIIa binding and ADCC activity due to changes in the level of afucosylated glycans, which occurred transiently for multiple batches with different expiry dates of the reference trastuzumab product [47], were associated with a reduced event-free survival (EFS) rate [48]. These drifts would likely not have been discovered without the analysis of multiple batches of the reference biological by the biosimilar company.

The present study was not without caveats. Only adalimumab biosimilars were examined in this study, raising a concern about generalizability. The data extraction of QAs may have been affected by the various terminologies used to describe the same QAs, particularly in scientific publications, because no consensus classification was available. We attempted to minimize this drawback by using a classification of QAs of a biological drug, which may not have reflected all QAs required by regulators to establish biosimilarity. As the study only relied on published QA data in the selected information sources, it was difficult to determine whether or not the unreported QAs were tested by the authors or assessed by the regulators.

Reporting the types of QAs and information on QAs may differ between scientific publications and EPARs as well as across biosimilars, but both sources provide information on the biosimilarity assessment of QAs in a complementary fashion. Functional attributes are consistently reported in comparison to structural attributes in the two sources, suggesting that MoA and clinically relevant QAs are reported in both sources, whereas less clinically relevant QAs are reported in one of the two sources. The EPARs are comprehensive regarding reporting the regulatory interpretation of QA biosimilarity, whereas scientific publications are focused on presenting both the test results and biosimilarity interpretation of QAs. There were no essential differences between the two sources' biosimilarity interpretations of the QA test results, which is reassuring the robustness of biosimilar regulation system as it has evolved in Europe over the last decade. Greater transparency and consistency in reporting QAs could lead to an improved understanding of the science behind biosimilar approval, which heavily relies on a comprehensive assessment of structural and functional attributes. The comprehensive reporting of QAs can contribute to improving the understanding of the role of QAs in establishing biosimilarity and the MoA of biological substances in general, which is essential for not only marketing authorization decisions but also informed decision making once a product is approved.

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# Supplementary data

**Supplementary Table S1a:** Search terms and strategy for PubMed.

| Coherent | Search terms |
|---|---|
| **Domain** | "Biosimilar Pharmaceuticals"[Mesh] OR biosimilar*[Title/Abstract] OR similar biological medicinal*[Title/Abstract] OR subsequent entry biologic*[Title/Abstract] OR second entry biologic*[Title/Abstract] OR Off patent*[Title/Abstract] OR multisource product*[Title/Abstract] OR follow up biologic*[Title/Abstract] OR follow on biologic*[Title/Abstract] OR biogeneric*[Title/Abstract] OR intended cop*[Title/Abstract] OR similar biotherapeutic*[Title/Abstract] OR bio similar*[Title/Abstract] OR therapeutic protein product*[Title/Abstract] OR therapeutical proteins[Title/Abstract]) |
| **Determinant OR Outcome** | (biosimilarity[Title/Abstract] OR analytic*[Title/Abstract] OR quality assessment*[Title/Abstract] OR similarity assessment*[Title/Abstract] OR quality characteri*[Title/Abstract] OR quality attribute*[Title/Abstract] OR biocomparability[Title/Abstract] OR physicochemical* OR biological activit*[Title/Abstract] OR structural attribute*[Title/Abstract] OR functional attribute*[Title/Abstract] OR nonclinical assessment*[Title/Abstract] OR comparability* [Title/Abstract]) |

**Supplementary Table S1b:** Search terms and strategy for EMBASE.

| Coherent | Search terms |
|---|---|
| **Domain** | ('biosimilar agent'/exp OR 'biosimilar agent' OR 'biosimilar drug*':ti,ab OR 'biosimilar*':ti,ab OR 'biosimilar agent':ti,ab OR 'biosimilar pharmaceutical':ti,ab OR 'similar biological medicinal*':ti,ab OR 'subsequent entry biologic*':ti,ab OR 'second entry biologic*':ti,ab OR 'off patent*':ti,ab OR 'multisource product*':ti,ab OR 'follow up biologic*':ti,ab OR 'follow on biologic*':ti,ab OR 'biogeneric*':ti,ab OR 'intended cop*':ti,ab OR 'similar biotherapeutic*':ti,ab OR 'bio similar*':ti,ab OR 'therapeutic protein product*':ti,ab OR 'therapeutical proteins':ti,ab) |
| **Determinant OR Outcome** | ('biosimilarity':ti,ab OR 'analytic*':ti,ab OR 'quality assessment*':ti,ab OR 'similarity assessment*':ti,ab OR 'quality characteri*':ti,ab OR 'quality attribute*':ti,ab OR 'biocomparability':ti,ab OR 'physicochemical*':ti,ab OR 'biological activit*':ti,ab OR 'structural attribute*':ti,ab OR 'functional attribute*':ti,ab OR 'nonclinical assessment*':ti,ab OR comparability*;ti,ab) |

| Category | | Quality attributes | ABP501 | | SB5 | | GP2017 | | FKB327 | | MSB11022 | | PF06410293 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | EPAR | Pubs. | EPAR | Pubs. | EPAR | Pubs. | EPAR | Pubs. | EPAR | Pubs. | EPAR | Pubs. |
| Physiochemical properties | QA1 | Molecular weight | ○ | ● | ◕ | ● | ◕ | | ◕ | ● | | ◕ | ◕ | ● |
| | QA2 | Protein concentration | ● | ● | ◕ | ◕ | ◕ | | ◕ | ● | ◕ | | ◕ | |
| | QA3 | Isoelectric point | | ● | | | ◕ | | ◕ | ● | | ● | | ● |
| | QA4 | Visible Particles | ◕ | | | | ◕ | | ◕ | ● | | | | |
| | QA5 | Subvisible Particles | ◕ | ● | ◕ | | ◕ | | ◕ | ● | | | | |
| | QA6 | Hydrophobicity | | | | | ◕ | | ◕ | | | | | |
| Primary structure | QA7 | Amino acid sequence | ◕ | ● | ○ | ● | ◕ | ○ | ◕ | ◕ | ◕ | ● | ◕ | ◕ |
| | QA8 | C-terminal variants | ◕ | | ◕ | ● | ◕ | | ◕ | ● | ◕ | ● | | |
| | QA9 | N-terminal variants | | | ○ | ◕ | ◕ | | ◕ | ◕ | ◕ | ● | | |
| | QA10 | Trisulfide variants | | | | | | | ◕ | ◕ | | | | |
| | QA11 | Disulfide bridges | ○ | ● | ○ | ● | ◕ | | ◕ | ◕ | ◕ | ● | ◕ | |
| | QA12 | Thioether Bonds | | | | | ◕ | | ◕ | | | | | |
| | QA13 | Free-thiol SH | | | ◕ | ● | ◕ | | ◕ | | ◕ | | ◕ | |
| Higher order structures | QA14 | Secondary structure | ○ | ● | ◕ | ● | ◕ | ○ | ◕ | ● | | ● | ◕ | |
| | QA15 | Tertiary structure | ○ | ● | ◕ | ● | ◕ | ○ | ◕ | ● | | ● | ◕ | |
| | QA16 | Quaternary structure | | | | | ◕ | | | | | | | |
| | QA17 | Thermodynamics properties | ○ | ● | | ● | ◕ | | ◕ | ● | | ● | ◕ | |
| Post-translation modifications-PTMs | QA18 | Glycosylation | ◕ | ● | ◕ | ● | ◕ | | ◕ | ● | ◕ | | ◕ | |
| | QA19 | Glycosylation site | ○ | ● | ◕ | ● | | | ◕ | ● | | | ◕ | |
| | QA20 | Glycosylation site occupancy | | ● | ○ | | ◕ | | ◕ | ● | | ◕ | ◕ | |
| Enzymatic- PTMs | QA21 | Glycoforms | ◕ | ● | ◕ | ● | ◕ | | ◕ | ● | ◕ | | ◕ | |
| | QA22 | Galactosylated glycans | ◕ | ● | ○ | ○ | ◕ | | ◕ | ● | ◕ | | ◕ | |
| | QA23 | High mannose glycans | ◕ | ● | ○ | ● | ◕ | | ◕ | ● | ● | | ◕ | |
| | QA24 | Fucosylated glycans | | | ◕ | | | | ◕ | ● | | | | |
| | QA25 | Afucosylated glycans | | ● | ◕ | ● | ◕ | | | ● | | | ◕ | |
| | QA26 | Total afucosylated glycans | | ● | | | | | | | ◕ | | ◕ | |
| | QA27 | Sialylated glycans | | ● | ◕ | ● | | | ◕ | ● | ◕ | | ◕ | |
| | QA28 | Neuraminic N-acetyl acid (NANA) | | | | ◕ | | | | | | | ◕ | |
| | QA29 | Neuraminic N-glycolyl acid (NGNA) | | | | ◕ | | | | | | | | |
| | QA30 | Galactose alpha-1,3-galactose | | | | | | | | | | | | |
| Non-Enzymatic- PTMs | QA31 | Glycation | | | | | ◕ | | ◕ | | ◕ | | | |
| | QA32 | Oxidation | | ◕ | ◕ | ◕ | ◕ | | ◕ | ● | ◕ | ● | | |
| | QA33 | Deamidation | | ◕ | ○ | ◕ | ◕ | | ◕ | ● | ◕ | ◕ | | |
| | QA34 | Truncation | ○ | | ○ | | ◕ | | ◕ | | | ● | | |
| | QA35 | Amidation | | | | ○ | ◕ | | ◕ | | | ● | | |
| | QA36 | Isomerization | | | | | ◕ | | ◕ | ◕ | | | | |
| | QA37 | Cysteinylation | | | | | | | ◕ | | | | | |
| | QA38 | Acetylation | | | | | | | | | | | | |
| | QA39 | Formylation | | | | | | | | | | | | |
| | QA40 | Methylation | | | | | | | | | | | | |
| | QA41 | Hydroxylation | | | | | | | | | | | | |
| | QA42 | Phosphorylation | | | | | | | | | | | | |

QAs reported in the EPAR or scientific publication DO include test results and DO include interpretation on biosimilarity
QAs reported in the EPAR or scientific publication DO NOT include test results and DO include interpretation on biosimilarity
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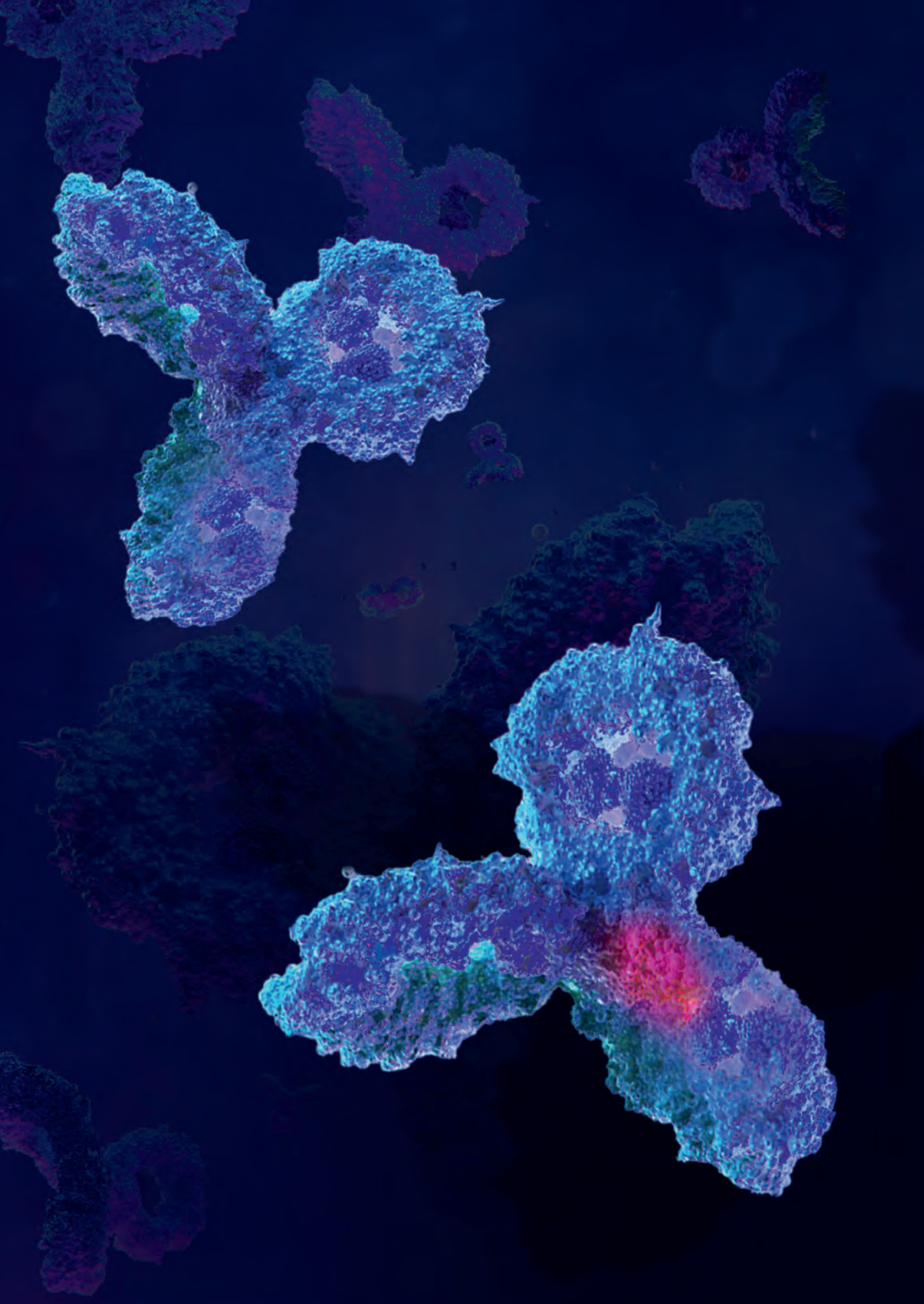
**Supplementary Figure S1:** The extent of information on reported quality attributes in biosimilarity assessment between EPARs and scientific publications for adalimumab biosimilars available at the EU market.

**Supplementary Table S2:** Comparison of quality attributes where test results and interpretation were reported for ABP501 and MSB11022 biosimilar.

| Biosimilar | Quality attributes | EPAR | | Publication | |
|---|---|---|---|---|---|
| | | **Test result** | **Interpretation** | **Test result** | **Interpretation** |
| ABP501 | Protein concentration (mg/ml) | ABP501 [range (n)]: 50.2 – 52.6 (4) | Similar | ABP501 [range (n)]: 47.9 – 52.6 (10) | Similar |
| | | US Reference [range (n)]: 51.1– 53.1 (3) | | US Reference [range (n)]: 48.1 – 52.3 (23) | |
| | | EU Reference [range (n)]: 50.6 – 51.6 (3) | | EU Reference [range (n)]: 49.6 – 53.7 (18) | |
| | Binding to FcγRIIIa (%) | ABP501 [mean (SD)]: 108 (12.3) | Similar | ABP501 [range (n)]: 67 – 113 (3) | Similar |
| | | US Reference [mean (SD)]: 101 (13.6) | | US Reference [range (n)]: 76 – 114 | |
| | | EU Reference [mean (SD)]: 113 (7.6) | | EU Reference [range (n)]: 86 – 104 | |
| MSB11022 | Binding to FcγRIIIa (nM) | MSB11022 [range (n)]: 6.2 – 10.1 (NR) | Similar | MSB11022 [range (n)]: 7.5 – 9.1 (3) | Highly similar |
| | | EU Reference [range (n)]: 3.8 – 8.0 (NR) | | EU and US Reference [range (n)]: 5.8 – 7.9 (23) | |

EU, European Union; US, United States; NR, not reported; FcγRIIIa, Fragment crystallizable gamma receptor; mg/ml; milligram/ milliter; nM, nanomole



# Chapter 2.3

## Type and extent of information on (potentially critical) quality attributes described in European public assessment reports for adalimumab biosimilars

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Author's contribution: AMA designed the study, performed data management, conducted data curation, analysis, and validation, prepared the first- draft of the manuscript, and implemented significant contribution from co-authors up to the final publication. Throughout the process, AMA asked for and implemented input and feedback from supervision team and co-authors, who performed critical review of the manuscript and provided significant contributions to the study.

## Abstract

Regulatory approval of biosimilars predominantly relies on biosimilarity assessments of quality attributes (QAs), particularly the potentially critical QAs (pCQAs) that may affect the clinical profile. However, a limited understanding exists concerning how EU regulators reflect the biosimilarity assessments of (pC)QAs in European public assessment reports (EPARs) by different stakeholders. The type and extent of information on QAs and pCQAs in EPARs were evaluated for seven adalimumab biosimilars. Seventy-seven QAs, including 31 pCQAs, were classified and assessed for type (structural and functional attributes) and extent (biosimilarity interpretation and/or test results) of information in EPARs. Reporting on the QAs (35–75%) varied between EPARs, where the most emphasis was placed on pCQAs (65–87%). Functional attributes (54% QAs and 92% pCQAs) were reported more frequently than structural attributes (8% QAs and 22% pCQAs). About 50% (4 structural and 12 functional attributes) of pCQAs were consistently reported in all EPARs. Regulators often provided biosimilarity interpretation (QAs: 83% structural and 80% functional; pCQAs: 81% structural and 78% functional) but rarely include test results (QAs: 1% structural and 9% functional and pCQAs: 3% structural and 9% functional). Minor differences in structural attributes, commonly in glycoforms and charge variants, were often observed in adalimumab biosimilars but did not affect the functions and clinical profile. Despite the variability in reporting QAs in EPARs, the minor observed differences were largely quantitative and not essentially meaningful for the overall conclusion of biosimilarity of the seven adalimumab biosimilars.

## Introduction

Biological drugs have become important treatment options for numerous diseases, including cancer and inflammatory diseases [1]. After patent expiration of the reference biologicals, biosimilars contribute to improved patient access to treatment due to competition, resulting in lower prices. Unlike small molecule drugs, biological drugs, including biosimilars, are large and complicated molecules produced through a complex process using living microorganisms. Variability within and between batches is an inherent feature of the production of biologicals [2,3]. Therefore, biosimilars are, generally, not exact replications of the reference biological but are highly similar [4].



The leading regulatory and health authorities in highly regulated markets, such as the European Medicines Agency (EMA), the United States Food and Drug Administration (US FDA), and the World Health Organization (WHO), have established frameworks and guidelines for the development, assessment, and approval of biosimilars [5–8]. Biosimilar development and regulatory approval predominantly rely on demonstrating the biosimilarity to the reference biological, which involves a stepwise comparability assessment. The comparability assessment of quality attributes (QAs) is a fundamental step, and it forms the basis for establishing biosimilarity and determining the scope and range of the in-vitro and clinical studies needed for biosimilar approval [9–12]. Minor differences in QAs between the biosimilar and reference biological may exist but should not be clinically relevant to obtaining regulatory approval.

Quality attributes are measurable molecular characteristics that describe the physical, chemical, biological, and microbiological properties of a drug molecule [13]. Some QAs are classified as potentially critical QAs (pCQAs) because they may affect the biological activity (potency) and the clinical drug profile, which includes pharmacokinetics (PK), pharmacodynamics (PD), safety, immunogenicity, and efficacy [14]. This criticality can be illustrated by a recent example where a biosimilar company discovered a drift in antibody-dependent cell-mediated cytotoxicity (ADCC) activity due to shifts in afucosylated glycans of the reference biological trastuzumab [15], which was associated with a reduced event-free survival rate [16]. Several studies have provided valuable insight into various risk assessment tools for identifying pCQAs [17–22]. Some pCQAs apply to all biologicals, but some pCQAs are specific to a biological and information about these may (d)evolve over time as more knowledge of the product and manufacturing process becomes available. The pharmaceutical industry generally defines which QAs are considered pCQAs based on the available information and the manufacturer risk assessment [23–32]. For biosimilars, the test results of all QAs must remain within the range of variability set by analyzing different batches of the reference biological. Scientific justification is needed if any deviation occurs in the QAs, especially in pCQAs.

This rigorous assessment should also be followed when changes are introduced to the manufacturing processes of approved biologicals, including biosimilars [33–36].

Since the regulatory approval of the first biosimilar in Europe in 2006, 49 unique biosimilars marketed under 69 brand names for 15 reference biologicals have received a positive opinion from the EMA's Committee for Medicinal Products for Human Use (CHMP) as of November 2020 [37]. Currently, the reference biological adalimumab, sold under the brand name Humira® by AbbVie Corporation, USA, has the largest number of biosimilars approved in the EU market. Adalimumab is an anti-tumor necrosis factor-α (TNF-α) monoclonal antibody that prevents the interaction of TNF-α with its receptors and is indicated for the treatment of various immune-mediated inflammatory diseases [38–40].

Despite the established and stringent regulatory pathway of biosimilars in Europe, the adoption of biosimilars in clinical practice is challenged by a lack of knowledge and understanding of the scientific rationale behind their approval [41–43]. In Europe, regulators have taken actions to increase transparency for the biosimilar approval process to improve stakeholder understanding of biosimilars through various communication media. The European public assessment report (EPAR) is an unbiased source through which the EMA publishes and broadcasts information to stakeholders about regulatory assessments for all medicinal products approved by the European Commission (EC) [37]. Previous studies have provided an in-depth overview of the clinical evidence reported in EPARs that supports approval of biosimilars in general [44,45] and approval of adalimumab biosimilars in particular [46]. These studies have shown that variations exist in reporting clinical data that confirm the biosimilarity of biosimilars to a reference biological, but they have not explored the reporting of the QAs that are the basis of biosimilar approval. The biosimilarity assessment of QAs is increasingly reported in scientific publications of biosimilars [47], which needed to be systematically consulted with the corresponding EPARs to obtain comprehensive information on biosimilarity at the quality level [48]. However, a limited understanding exists concerning how EU regulators reflect the biosimilarity assessment of (pC)QAs in EPARs by different stakeholders.

Therefore, this study aims to evaluate the QAs and pCQAs reported in EPARs using adalimumab biosimilars as a case study in terms of (1) consistency of QA and pCQA reporting between biosimilars of the same reference biological (i.e., adalimumab), (2) Type of the reported QAs and pCQAs (i.e., structural or functional attributes), and (3) how biosimilarity interpretation and test results were described for the reported (pC)QAs. We hypothesized that EU regulators are more focused in the reporting of pCQAs and the biosimilar interpretation because these are more likely to be of clinical relevance.

## Methods

### Study cohort

In this study, the initial EPARs of all adalimumab biosimilars approved by the EMA before 30 November 2020 were included. The initial EPARs of adalimumab biosimilars were retrieved from the official EMA website (http://www.ema.europa.eu (accessed on 1 June 2020) [37]. The EPAR contains a summary of the submitted registration dossier and the scientific assessment undertaken by the CHMP, a body that advises the EC on marketing authorization of medicines for human use. Only the initial EPAR of each adalimumab biosimilar released following the final EC decision was included in this study because biosimilarity assessments of QAs and pCQAs between biosimilar and reference biological are presented only in the initial EPARs.



The initial EPARs were used to extract baseline characteristics for each adalimumab biosimilar, including the company code(s), brand name(s), date of the initial EPAR publication, and member states of the rapporteurs responsible for the assessment. Some adalimumab biosimilars are produced by the same manufacturer but marketed under different brand names (e.g., the company code for Hefiya®, Halimatoz®, and Hyrimoz® is GP2017) for which the registration dossier and initial EPARs are identical. In such cases, only the EPAR of one brand name (e.g., Hefiya® for GP2017) was included in the study for subsequent analysis. The date of the initial EPAR publication was defined as the month and year when the EPAR was published by the EMA, which is generally the same date as the EC decision on marketing authorization. The member state was defined as the rapporteurs' European country of origin. The rapporteurs are the two CHMP members who led the regulatory assessment of a marketing authorization application.

### Information on (potentially critical) quality attributes in EPARs

The study outcome was the determination of how EU regulators report information on the biosimilarity assessment of QAs and pCQAs in the EPARs. Two aspects were studied: the type and extent of information on the reported QAs and pCQAs.

*Types of reported (potentially critical) quality attributes*

The types of QAs and pCQAs reported in the biosimilarity assessment were identified from the quality, non-clinical, and clinical sections of the initial EPARs. A general classification scheme of QAs was used to extract information from the EPARs. Information about the development of the classification scheme has been described elsewhere [47]. In short, the first draft was developed by the authors based on information from the EMA and US FDA biosimilar guidelines [5–7] and publicly available information relevant to the molecular characterization of a biological drug. The classification scheme was validated by regulators involved in the quality assessment of biosimilars at the Dutch Med-

icines Evaluation Board (MEB) to ensure that no critical and relevant QAs were missed. The classification scheme divides the QAs into seven types with additional subtypes of structural (physiochemical properties, primary structure, higher-order structure, PTMs and purity and impurities) and functional attributes (biological and immunochemical activity), resulting in the classification of 77 (53 structural and 24 functional) QAs of biologicals considered in the biosimilarity assessment (Figure 1) [47,48].

Subsequently, a list of pCQAs was defined in a two-step process. First, the pCQAs of adalimumab were identified from scientific publications presenting comparability or biosimilarity studies of adalimumab products, including the reference biological (Humira®) and corresponding biosimilars [23–32]. The publications were selected from an updated search of our previous systematic review [47]. From this search, an initial list of 29 pCQAs of adalimumab was constructed based on the pCQAs proposed by the authors. Second, the initial list was compared with the pCQAs identified for monoclonal antibodies, in general, in the previous literature [17–22] to verify and broaden the initial selection of pCQAs. If a new pCQA was identified in this second step, the authors (A.M.A., T.J.G., and H.G.) discussed its relevancy to adalimumab and reached a consensus on the inclusion of the attribute. In this way, two pCQAs were added to the initial list, resulting in a final list of 31 (18 structural and 13 functional) pCQAs considered relevant to adalimumab products. These pCQAs were classified according to the previously described scheme (Figure 1).

*Extent of Reported Information on (Potentially Critical) Quality Attributes*

The extent of the information on QAs and pCQAs provided in the EPARs was categorized by whether a biosimilarity interpretation was reported (yes/no) and whether test results were reported (yes/no) for a given QA or pCQA. The four possible combinations of answers resulted in four categories for each reported QA and pCQA (Table 1) [48].

Biosimilarity interpretation was defined as reported (yes) if the EPAR contained keywords demarcating the regulatory interpretation of the biosimilarity of a QA and pCQA as identical, similar, or having minor differences. The interpretation of similar included wording such as “same,” “match,” “(highly) similar,” “comparable,” and “consistent”.

Test results were defined as reported (yes) if the EPAR included the quantitative or qualitative acceptance criteria of a given QA and pCQA, which included the numerical limits, range, and distribution, as shown in the examples in Table 1, or other suitable visual assessment measures, such as the spectra for higher-order structures and chromatograms for purity and impurities.

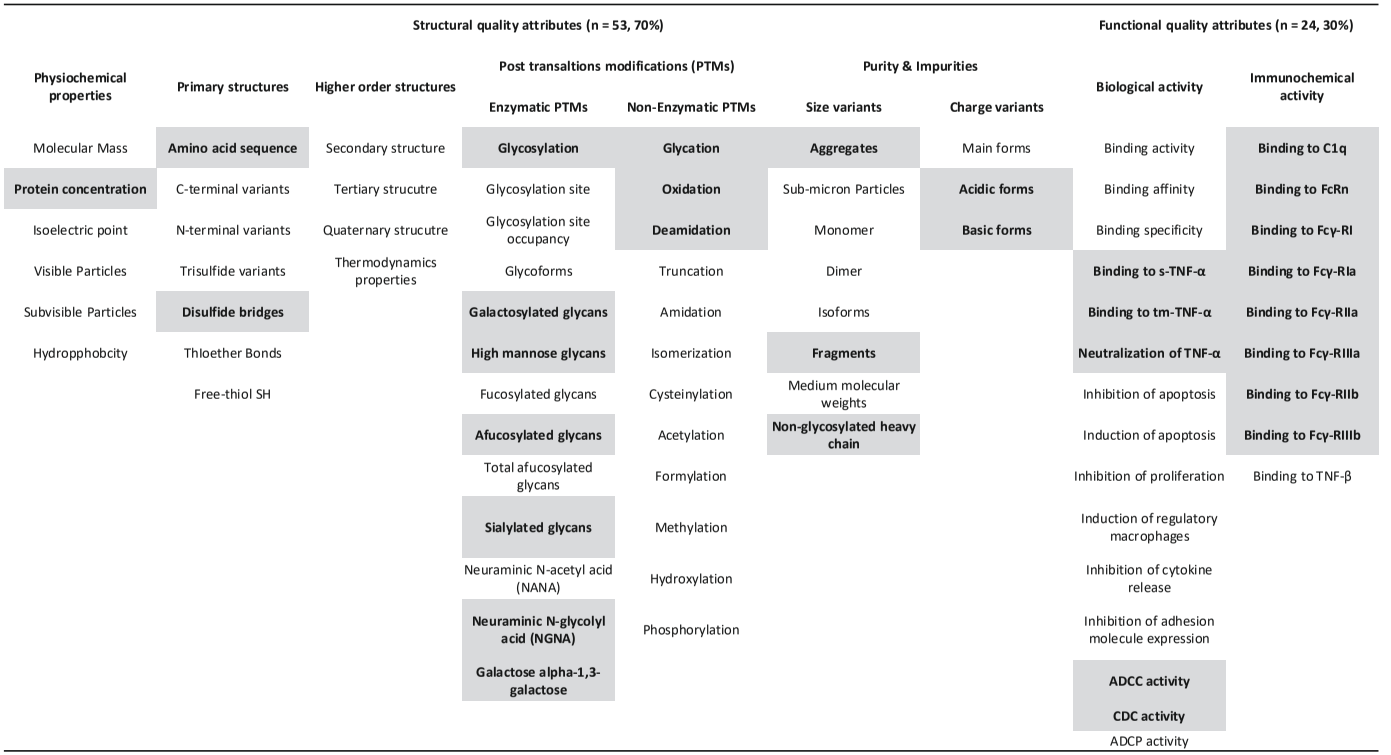


**Figure 1:** Classification scheme for 77 common quality attributes (QAs) of biologicals including 31 potentially critical quality attributes (pCQAs) relevant to adalimumab. The pCQAs are presented in gray boxes. Definitions: ADCC: antibody-dependent cellular cytotoxicity, ADCP: antibody-dependent cellular phagocytosis, CDC: complement-dependent cytotoxicity, C1q: complement component 1q, TNFα: tumor necrosis factor-alpha, s-TNFα: surface tumor necrosis factor-alpha, tm-TNFα: transmembrane tumor necrosis factor-alpha, Fc: fragment crystallizable, FcR: Fc receptor.

**Table 1:** Definitions of the four reporting categories for the quality attributes (QAs) and potentially critical quality attributes (pCQAs) reported in biosimilarity assessments in the initial European public assessment reports (EPARs) [48].

| Reporting catagories | Biosimilarity Interpretation | | |
|---|---|---|---|
| | **No** | | **Yes** |
| **Test results** | **No** | Reported QAs and pCQAs include no biosimilarity interpretation and no test results, for example:<br>- The amino acid sequence and N-glycosylation site were compared.<br>- The protein concentration was determined.<br>- Binding to FcRn and Fcγ-RIIIa was studied, and a comparison of ADCC activity was performed.<br>- Neutralization of TNFα, binding to s-TNFα, and binding to tm-TNFα were addressed. | Reported QAs and pCQAs include the biosimilarity interpretation but not test results, for example:<br>- The amino acid sequence and N-glycosylation site of the biosimilar were identical to those of the reference.<br>- The protein concentration was similar to that of the reference.<br>- Minor differences with no clinical relevance were observed in glycation, galactosylated N-glycans, high mannose N-glycans, fucosylated N-glycans, and sialylated glycans.<br>- The FcRn, C1q binding, CDC, ADCC, and neutralization of TNFα were comparable with those of the reference. |
| | **Yes** | Reported QAs and pCQAs include the test results but not the biosimilarity interpretation, for example:<br>- The levels of high mannose N-glycans (biosimilar: 1.9–2.5%; reference: 5.3–12.0%).<br>- The $K_D$ ranges for Fcγ-RIIIa binding (biosimilar: 6.2–10.1 nM; reference: 3.8–8.0 nM)<br>- The $EC_{50}$ values for inhibiting cytokine release (204 pM, 294 pM and 200 pM for the three batches of tested biosimilars and 177 pM, 168 pM and 222 pM for the three batches of tested reference biological).<br>- ADCC activity (biosimilar: 89–107%; reference: 84–115%) | Reported QAs and pCQAs include the biosimilarity interpretation and test results, for example,<br>- Minor differences with no clinical relevance were observed in the levels of high mannose N-glycans (biosimilar: 1.9–2.5%; reference: 5.3–12.0%).<br>- ADCC activity (biosimilar: 89–107%; reference: 84–115%) was comparable/ similar between the two products. |

ADCC: antibody-dependent cellular cytotoxicity, CDC: complement-dependent cytotoxicity, $EC_{50}$: half-maximal effective concentration, TNFα: tumor necrosis factor-alpha, s-TNFα: surface tumor necrosis factor-alpha, tm-TNFα: transmembrane tumor necrosis factor-alpha, Fc: fragment crystallizable, FcR: Fc receptor, $K_D$: equilibrium dissociation constant, nM: nanomoles, pM: picomoles.

### Data analysis

The frequency of the reported QAs and pCQAs stratified by structural and functional attributes was used to express the consistency in reporting the QAs and pCQAs of adalimumab biosimilars by EU regulators in EPARs. A QA and pCQA was considered to be consistently reported if EU regulators describe it in all included EPARs. The proportion of reported QAs and pCQAs for the four reporting categories (see Table 3) was calculated and stratified by structural and functional attributes to compare the extent of information on reported QAs and pCQAs in EPARs. If the regulatory interpretation of the biosimilarity or test results were presented for a given QA or pCQA in the EPARs, the type of interpretation (identical, similar, or minor differences) and the acceptance biosimilarity criteria were identified.



## Results

### Characteristics of the included European public assessment reports of adalimumab biosimilars

As of 30 November 2020, seven unique adalimumab biosimilars (11 brand names) had received marketing authorization from the EC. Three of the seven biosimilars (i.e., ABP501, GP2017, and MSB11022) were marketed under more than one brand name. Rapporteurs from 11 member states prepared the initial EPARs of the seven adalimumab biosimilars. Rapporteurs from two (Finland and Austria) of the 11 member states were involved in more than one EPAR of adalimumab biosimilars (Table 2).

### Types of reported (potentially critical) quality attributes

In general, the frequency of reported QAs (range: 27 (35%)–58 (75%)) varied between EPARs of adalimumab biosimilars, with most emphasis placed on the reporting of the pCQAs (range: 20 (65%)–27 (87%)). The proportion of reported pCQAs was comparable for all biosimilars. Overall, 16 (21%) of all QAs were reported in all EPARs of adalimumab biosimilars. Of the 31 pCQAs, 29 (94%) were reported at least in one EPAR, and 16 (52%) were consistently reported in all included EPARs (Table 3). Two (6%) pCQAs related to structural attributes were not reported in any included EPAR: post-translation modifications (PTMs) including neuraminic N-glycolyl acid and galactose alpha-1,3-galactose (Figure S1).

Overall, functional attributes (54% QAs and 92% pCQAs) were more often consistently reported than structural attributes (8% QAs and 22% pCQAs) in EPARs of adalimumab biosimilars (Table 2). Consistent reporting of functional pCQAs was high, with 12 (92%) out of 13 pCQAs reported in all EPARs, including binding to soluble- and transmembrane-TNFα (s-TNFα and tm-TNFα), (ADCC), and complement-dependent cytotoxicity

(CDC) activity and binding to complement component 1q (C1q), neonatal Fc receptor (FcRn), and six Fcγ-receptors. Of the 18 structural pCQAs, only four (22%) were consistently reported in all EPARs, including amino acid sequence and disulfide bridges, glycosylation, and aggregates (Figure S1).

**Table 2:** Characteristics of the included initial European public assessment reports (EPARs) of adalimumab biosimilars [49–59].

| Company Code | Date of Initial EPAR Publication (mm/yyyy) | Brand Names | EU Member State of Rapporteurs (Rapporteur and Co-rapporteur) |
|---|---|---|---|
| ABP501 | 04-2017 | Amgevita® Solymbic®* | Sweden and Italy |
| SB5 | 08-2017 | Imraldi® | Finland and Austria |
| BI695501 | 11-2017 | Cyltezo®* | Austria and Germany |
| GP2017 | 08-2018 | Hefiya® Halimatoz® Hyrimoz® | Austria and Ireland |
| FKB327 | 09-2018 | Hulio® | Belgium and United Kingdom |
| MSB11022 | 04-2019 | Idacio® Kromeya®* | Netherlands and Lithuania |
| PF06410293 | 02-2020 | Amsparity® | Finland and Romania |

* Solymbic®, Cyltezo® and Kromeya® were approved by the European Medicines Agency (EMA) but voluntarily withdrawn by the applicant for commercial reasons.

**Table 3:** Reporting of the quality attributes (QAs) and potentially critical quality attributes (pCQAs) stratified by structural and functional attributes and the company code of adalimumab biosimilars in the included European public assessment reports (EPARs).

| Company Code | All QAs (n = 77, 100%) | Type of QAs | | All pCQAs (n = 31, 100%) | Type of pCQAs | |
|---|---|---|---|---|---|---|
| | | Structural (n = 53, %) | Functional (n = 24, %) | | Structural (n = 18, %) | Functional (n = 13, %) |
| ABP501 | 36 (47%) | 18 (34%) | 18 (75%) | 20 (65%) | 7 (39%) | 13 (100%) |
| SB5 | 49 (64%) | 27 (51%) | 22 (92%) | 27 (87%) | 14 (78%) | 13 (100%) |
| BI695501 | 27 (35%) | 12 (23%) | 15 (63%) | 20 (65%) | 7 (39%) | 13 (100%) |
| GP2017 | 52 (68%) | 34 (64%) | 18 (75%) | 27 (87%) | 14 (78%) | 13 (100%) |
| FKB327 | 58 (75%) | 39 (74%) | 19 (79%) | 27 (87%) | 14 (78%) | 13 (100%) |
| MSB11022 | 42 (55%) | 20 (38%) | 22 (92%) | 25 (81%) | 12 (67%) | 13 (100%) |
| PF06410293 | 46 (60%) | 27 (51%) | 19 (79%) | 24 (77%) | 12 (67%) | 12 (92%) |
| Consistent for all biosimilars | 16 (21%) | 4 (8%) | 12 (54%) | 16 (52%) | 4 (22%) | 12 (92%) |

### Extent of information on reported (potentially critical) quality attributes

In general, no differences were observed in the extent of the reported information between the QAs and pCQAs in all EPARs of adalimumab biosimilars. Regulators frequently provided biosimilarity interpretations of the reported QAs (83% structural and 80% functional) and pCQAs (81% structural and 78% functional) but rarely included test results with or without biosimilarity interpretations of the reported QAs (1% structural and 9% functional) and pCQAs (3% structural and 9% functional) (Figure 2).

The total number of reported QAs included with a biosimilarity interpretation in EPARs was 69 QAs and the number varied (range: 10–58 QAs) for adalimumab biosimilars. The interpretation of the biosimilarity of the reported QAs was most frequently reported as being similar (range: 7–44 QAs) than having minor differences (range: 1–18 QAs) (Table S1). Thirty-one QAs, including fifteen pCQAs, were observed with minor differences in at least one adalimumab biosimilar. The most common structural pCQAs with minor differences were the four glycoforms (galactosylated glycans, high mannose glycans, afucosylated glycans, and sialylated glycans) and the charge variants (acidic and basic variants). While functional pCQAs were more often similar between the biosimilar and reference biological, minor differences were observed for the functional pCQAs tm-TNFα binding, ADCC activity, and C1q binding in two adalimumab biosimilars: GP2017 and PF-06410293 (Figure S1).

Regulators provided both biosimilarity interpretations and test results in EPARs for only five pCQAs, including the protein concentration and binding to FcγRIIIa for ABP501 and the high mannose glycans, ADCC activity, and binding to FcγRIIIa for MSB11022 (Table S2). Of those five pCQAs, only the test results of high mannose glycans, which were slightly lower in the MSB11022 biosimilar (range = 1.9–2.5%) compared to the reference biological (range = 5.3–12.0%), were interpreted by the regulators as minor difference. Figure S1 shows reporting of the type and extent of information on QAs and pCQAs described in the EPARs of adalimumab biosimilars included.

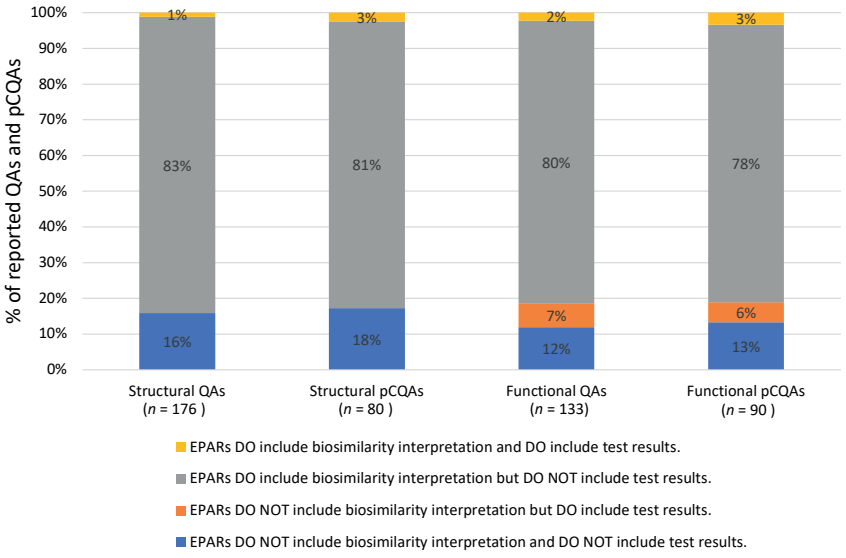


**Figure 2:** Comparison of the extent of reported information on quality attributes (QAs) and potentially critical quality attributes (pCQAs) stratified by the types of QAs and pCQAs (structural and functional) reported in all EPARs of adalimumab biosimilars included.

## Discussion

The present study evaluated the type and extent of information on QAs and pCQAs reported in EPARs by EU regulators for seven adalimumab biosimilars approved in Europe as of November 2020. In general, reporting of QAs (ranging from 27 (35%) to 58 (75%)) varied between EPARs of adalimumab biosimilars, where the most emphasis was on reporting pCQAs (ranging from 20 (65%) to 27 (87%)). About 50% (4 structural and 12 functional attributes) of pCQAs were consistently reported in all EPARs. Functional attributes (54% QAs and 92% pCQAs) were more frequently and consistently reported than structural attributes (8% QAs and 22% pCQAs). Minor differences between adalimumab biosimilars and the reference biological in certain structural attributes, most commonly in glycoforms and charge variants, were often observed by regulators. Regulators reported on the biosimilarity interpretation but rarely presented the test results underlying their interpretation in EPARs. However, QA and pCQA data not reported in the EPARs do not necessarily indicate that they were neither submitted by companies nor assessed by regulators during the stringent regulatory process.

This study highlights some variations in reporting biosimilarity assessments at the quality level in EPARs. Despite this variability in QA reporting, pCQAs were most frequently and consistently reported by EU regulators in EPARs. The variation in QA report-

ing between EPARs is consistent with the variability in reporting clinical data, which was explained by the flexibility in regulatory requirements (i.e., a case-by-case basis) [44,45]. However, such flexibility cannot explain the variability in reporting of QAs and pCQAs for biosimilars, particularly those containing the same active substance and compared to the same reference biological (e.g., Humira® in the case of adalimumab), that were assessed based on the same regulatory standards for establishing biosimilarity. The variability in QA reporting may be explained by the fact that the EPARs are prepared by various rapporteurs (i.e., regulators) from different member states. Nevertheless, regulators diligently reported the pCQAs, which are all considered to be of relevance because these may potentially affect functions (biological and immunochemical activity) and the clinical profile, including the pharmacokinetics, pharmacodynamics, safety, immunogenicity and efficacy of the drug. It is, however, important to note that learning on pCQAs is an ongoing process, which will likely result in changes to the current list over time.



The direct or indirect relationship between structural and functional QAs and the clinical profile influences the determination of pCQAs [19]. This relationship can be illustrated by the four structural pCQAs, including the amino acid sequence, disulfide bridges, aggregates, and glycosylation, which were consistently reported in EPARs. A mismatch in amino acid sequence and disulfide bridges can change the structural conformation affecting the biological activity and clinical performance, which were identical to the reference biological for all adalimumab biosimilars. Aggregates can elicit immunogenic responses by inducing neutralizing antibodies, hypersensitivity reactions, and infusion-related reactions in vivo. The propensity of aggregation may increase with some structural attributes (e.g., disulfide bridges, oxidation, and deamidation) if these are inadequately controlled. For all adalimumab biosimilars, aggregate levels were similar to the reference biological. Glycosylation is a PTM that occurs through an enzymatic process at specific sites in a protein drug and can influence the biological activity (potency and efficacy), serum half-life clearance (pharmacokinetics), and immunogenicity (safety). Minor differences in glycosylation were observed in adalimumab biosimilars, which are the most frequent notable differences in biosimilars and reference biologicals in general [9–12].

In practice, minor differences in QAs and pCQAs are expected for biosimilars due to the use of various manufacturing processes, cell lines, and materials [35]. These minor differences have also been observed between batches of a reference biological, primarily when a company introduces manufacturing changes [2,3,39]. The galactosylated glycans, high mannose glycans, afucosylated glycans, and sialylated glycans are types of glycoforms where minor differences have most commonly been reported (Figure S1). Galactosylated glycans may influence C1q binding and CDC activity, whereas high mannose glycans may influence pharmacokinetics parameters. However, structure-activity relationship studies and pivotal pharmacokinetics trials indicate that these are not

affected by minor differences in galactosylated and high mannose glycans [49,50,52–58]. The same applies to afucosylated and sialylated glycans, which may influence Fcγ-receptors and ADCC activity [52–59]. These examples demonstrate the importance of structure-activity relationship studies and pharmacokinetics and pharmacodynamics trials in assessing the potential effect of minor differences in pCQAs in biosimilarity assessments. Minor differences in acidic and basic variants in several adalimumab biosimilars were attributed to changes in c-terminal lysin [49,50,52–55,59], which is generally cleaved in human serum with no effect on clinical profiles, and were thus considered noncritical QAs. Minor differences for certain functional pCQAs were attributed to minor differences in certain structural QAs and pCQAs, which were observed and reported by EU regulators in EPARs for GP2017 and PF06410293. For both biosimilars, the minor differences in ADCC activity disappeared when using an in-vitro assay with more physiological conditions in peripheral blood mononuclear cells. For GP2017, the aggregate levels were slightly higher using size-exclusion chromatography and slightly lower using analytical ultracentrifugation than the reference biological, which was considered a minor and clinically irrelevant difference by regulators. This ADCC and aggregate example indicates the importance of using orthogonal methods to assess the (dis)similarity of QAs. Based on these observations, minor differences in these pCQAs seem to be quantitative (i.e., numerical values) but do not preclude the overall conclusion for biosimilarity and are considered clinically irrelevant.

The underlying reason functional pCQAs are more frequently and consistently reported in EPARs could relate to their direct relationship with the mechanisms of action (MoAs). The primary MoA of adalimumab involves binding to, and neutralizing TNF-α. Adalimumab also mediates effector functions, such as ADCC and CDC activity, by binding to tm-TNF-α, C1q (for CDC), and Fcγ-receptors. The relevance of ADCC or CDC activity to the primary MoA and efficacy of adalimumab is not well established but may be important, particularly in inflammatory bowel disease [46]. Binding to tm-TNFα can trigger potential biological functions known as "referred signaling," which may play a role in some therapeutic indications (e.g., inflammatory bowel disease). For GP2017, regulators reported minor differences in the binding to tm-TNFα, for which the scientific justifications provided by the company were not available in the EPAR for GP2017. However, the developer company of GP2017 reported functional and pharmacological characterizations demonstrating indistinguishable binding profiles and subsequent induction of reverse signaling to support the rationale for extrapolation across indications [28]. Therefore, functional pCQAs provide the final insight into the (dis)similarity at the quality level and useful information in predicting the outcomes of clinical studies [9–11], forming the basis for supporting the extrapolation of biosimilars across all indications authorized for the reference biological [60–63].

Regulators frequently describe the biosimilarity interpretation of reported QAs and pCQAs but rarely present the test result data, impeding the interpretation by EPAR users. For example, in EPARs, minor differences are frequently expressed subjectively as "slightly lower" or "slightly higher," but the exact extent to which the difference is minor remains unclear for most reported QAs and pCQAs. A more appropriate method would be in line with what was reported in the EPAR of MSB11022, in which the ranges of high mannose glycans (ranging from 1.9% to 2.5%) and the reference adalimumab (ranging from 5.3% to 12.0%) were reported. Such information on the test results allows for a better understanding of the regulatory interpretation and scientific justification behind the regulatory approval of biosimilars.



The present study used a classification scheme to investigate in a standardized manner how EU regulators present information on the biosimilarity of QAs and pCQAs in EPARs. The focus on the pCQAs to be considered in biosimilarity assessment, which may affect the clinical profiles of adalimumab products, was a strength of this investigation. The selection of adalimumab pCQAs was based on the literature review, providing an overview concerning which QAs are considered pCQAs with the current knowledge. This study stresses the importance of EPARs as a source of information that provides insight into the scientific evidence underpinning the regulatory approval of biosimilars.

Our study does have some limitations, which are noted as follows. First, these study findings are restricted to adalimumab biosimilars, which may hamper the generalizability to biosimilars of other biological molecules. Nevertheless, even if a biosimilarity assessment of another molecule is conducted with a different set of QAs and pCQAs, the findings, especially the focus on reporting the pCQAs, are expected to be comparable to other types of biosimilars because all EPARs are published by the same regulatory agency (i.e., EMA). Second, the generalizability of our findings to the regulatory reports from various jurisdictions, such as in the US FDA review reports, is unknown and beyond the scope of this study. Third, the QA classification scheme may not have captured all pCQAs of adalimumab because no consensus list is currently available. However, a literature search for publications on comparability and biosimilarity studies of adalimumab products was performed, and no pCQAs were identified that were not included in our classification.

Our observations reveal that minor differences in certain QAs between biosimilars and reference biological can occur at the same level of variability between pre- and post-manufacturing change batches of the reference biological [35,39,64], which reassures the biosimilar regulation system. Although EU regulators have focused on describing pCQAs, these critical attributes were not explicitly defined in EPARs. Because biosimilar companies have conducted extensive analyses to define pCQAs based on their risk assessments, it would be preferable if regulators clearly define which QAs are identified as pCQAs by the companies. A clear definition of pCQAs in EPARs would enable stake-

holders to better understand the links between QAs and the clinical profile and the meaning of the QAs concerning patient safety and product efficacy. The pCQAs may also (d)evolve over the drug life cycle based on the knowledge gained regarding the product and process. Standardized reporting of pCQAs in EPARs would benefit regulatory learning by allowing future researches to track pCQAs over time. Learning of pCQAs over time might result in reducing the need for comparative clinical trials and streamlining biosimilar approvals [9–12].

Although the EMA quality guidance of biosimilars provides high-level information on QAs, the guidance was last updated in 2014 and may not reflect the current state of knowledge and regulatory experience regarding QAs for biosimilars [5]. The lack of information on pCQAs in the guidance is understandable because these were not entirely known in the early years of biosimilar regulation. Nevertheless, the accumulated and long experience of EU biosimilar regulation as reflected in EPARs would fuel regulatory guidance with product-specific pCQAs, making the regulatory standard more visible and predictable.

As EPARs are considered an unbiased information source, there is great value in providing insight into the biosimilarity assessment of QAs for various stakeholders involved in biosimilar development, adoption, and regulation. The pharmaceutical industry can use EPARs to learn from past successes and failures and predict the regulatory process, and EPARs as such may contribute to reducing the time and cost of biosimilar development [65]. Healthcare professionals (HCPs) can use EPARs to understand the QA assessment's crucial role during the regulatory approval of biosimilars [66,67]. Reporting more extensive information about pCQAs in EPARs could help HCPs understand the predominant role of QAs and the reduced weight of evidence from comparative clinical trials in biosimilar approval. Among HCPs, pharmacists are uniquely positioned to take a leading role in informing other HCPs and patients about the scientific evidence underpinning biosimilar approval. Such efforts could increase confidence in and acceptance of using biosimilars in medical practice to fully capture the societal and patients benefits offered by biosimilars. Non-European regulatory authorities can use EPARs to support their own decision-making process, relying on the regulatory assessment undertaken by competent authorities in the world [68–73]. Therefore, EPARs could contribute to accelerating the regulatory review process and patients access to biosimilars in non-European jurisdictions.

For a comprehensive understanding of biosimilarity concepts and the predominant role of QAs in the approval of biosimilars, continued improvement in presenting biosimi-

larity assessments of QAs in EPARs is recommended. One method could include applying a structured uniform approach to QA reporting in EPARs. Such an approach may enhance the completeness and consistency of QA data and avoid missing crucial regulatory reflection on clinically relevant pCQAs. Greater consistency in QA reporting could make the EPAR a valuable and reliable tool for stakeholders to support evidence-based education to address the lack of knowledge and understanding of the scientific rationale behind biosimilar approval. Biosimilarity assessments of QAs in EPARs could be summarized in a standardized format that includes the type of evaluated QAs, explicit definition of the pCQAs, the test methods used and their results, the biosimilarity interpretation and scientific justification of the differences, if applicable. This summary could be achieved through adopting the International Pharmaceutical Regulators Program's regulatory review templates to optimize the current content with respect to biosimilarity assessment of QAs in EPARs [74]. Alternatively, initiating a project similar to the collaborative study between the EMA and European network for health technology assessment [75], which has resulted in a template to improve the contribution of EPARs in health technology assessments of relative drug effectiveness.



## Conclusion

In conclusion, we found variations in the frequency of reported QAs between EPARs of adalimumab biosimilars. The minor differences in the identified QAs did not affect functions and clinical performance and seem to be largely quantitative differences and not essentially meaningful for the overall conclusion of biosimilarity.

In line with our hypothesis, the pCQAs, specifically functional pCQAs, were reported most frequently and consistently in EPARs, as these reflect the MoA and can potentially affect the clinical profile. Greater consistency could be applied in reporting of QAs with more emphasis on pCQAs in EPARs, which could improve the understanding of the relationship between QAs and the clinical profile, which may positively contribute to adopting biosimilars in clinical practice.

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## Supplementary data

**Supplementary Table S1:** Types of biosimilarity interpretation of reported quality attributes (QAs) stratified by the company code of adalimumab biosimilars in the European public assessment reports (EPARs).

| Company code | All QAs (n=77; 100%) | QAs reported with biosimilarity interpretation (n) | Type of biosimilarity interpretation | | | |
|---|---|---|---|---|---|---|
| | | | Similar | | Minor differences | |
| | | | Structural QAs (n) | Functional QAs (n) | Structural QAs (n) | Functional QAs (n) |
| ABP501 | 36 (47%) | 11 | 5 | 2 | 4 | 0 |
| SB5 | 49 (64%) | 39 | 6 | 20 | 13 | 0 |
| BI695501 | 27 (35%) | 10 | 0 | 9 | 1 | 0 |
| GP2017 | 52 (68%) | 51 | 19 | 14 | 15 | 3 |
| FKB327 | 58 (75%) | 58 | 25 | 19 | 14 | 0 |
| MSB11022 | 42 (55%) | 42 | 16 | 22 | 4 | 0 |
| PF06410293 | 46 (60%) | 46 | 21 | 19 | 5 | 1 |

| Category | | Quality Attributes (QAs) | ABP501 | SB5 | BI655501 | GP2017 | FKB327 | MSB11022 | PF06410293 |
|---|---|---|---|---|---|---|---|---|---|
| Physicochemical properties | QA1 | Molecular Mass | ○ | ◐≠ | ○ | ◐= | ◐= | | ◐= |
| | **QA2** | **Protein concentration*** | ●= | ◐= | | ◐= | ◐= | ◐= | ◐= |
| | QA3 | Isoelectric point | | | | ◐= | ◐= | | |
| | QA4 | Visible Particles | ◐= | | ◐≠ | ◐= | ◐= | | |
| | QA5 | Subvisible Particles | ◐= | ◐= | | ◐= | ◐= | | |
| | QA6 | Hydrophobicity | | | | ◐≠ | ◐≠ | | |
| Primary structure | **QA7** | **Amino acid sequence*** | ◐= | ○ | ○ | ◐= | ◐= | ◐= | ◐= |
| | QA8 | C-terminal variants | ◐≠ | ◐≠ | | ◐≠ | ◐≠ | ◐= | |
| | QA9 | N-terminal variants | | ○ | | ◐≠ | ◐= | ◐= | |
| | QA10 | Trisulfide variants | | | | | ◐≠ | | |
| | **QA11** | **Disulfide bridges*** | ○ | ○ | ○ | ◐= | ◐= | ◐= | ◐= |
| | QA12 | Thioether Bonds | | | | ◐≠ | ◐= | | |
| | QA13 | Free-thiol SH | | ◐≠ | | ◐= | ◐= | ◐= | ◐= |
| Higher order structures | QA14 | Secondary structure | ○ | ◐= | ○ | ◐= | ◐= | | ◐= |
| | QA15 | Tertiary structure | ○ | ◐= | | ◐= | ◐= | | ◐= |
| | QA16 | Quaternary structure | | | | ◐= | | | |
| | QA17 | Thermodynamics properties | ○ | | | ◐= | ◐= | | ◐= |
| Post-Translation Modifications-PTMs: Enzymatic- PTMs | **QA18** | **Glycosylation*** | ◐= | ◐≠ | ○ | ◐≠ | ◐= | ◐= | ◐= |
| | QA19 | Glycosylation site | ○ | ◐= | ○ | | ◐= | | ◐= |
| | QA20 | Glycosylation site occupancy | | ○ | | ◐≠ | ◐= | | ◐= |
| | QA21 | Glycoforms | ◐≠ | ◐≠ | | ◐≠ | ◐≠ | ◐≠ | ◐≠ |
| | **QA22** | **Galactosylated glycans*** | ◐≠ | ○ | | ◐≠ | ◐≠ | ◐= | ◐≠ |
| | **QA23** | **High mannose glycans*** | ◐≠ | ○ | ○ | ◐≠ | ◐≠ | ●≠ | ◐≠ |
| | QA24 | Fucosylated glycans | | ◐≠ | | | ◐≠ | | |
| | **QA25** | **Afucosylated glycans*** | | ◐≠ | | ◐≠ | ◐≠ | | ◐≠ |
| | QA26 | Total afucosylated glycans | | | | | | ◐≠ | ◐≠ |
| | **QA27** | **Sialylated glycans*** | | ◐≠ | | | ◐≠ | ◐≠ | ◐= |
| | QA28 | Neuraminic N-acetyl acid (NANA) | | | | | | | ◐= |
| | **QA29** | **Neuraminic N-glycolyl acid (NGNA)*** | | | | | | | |
| | **QA30** | **Galactose alpha-1,3-galactose*** | | | | | | | |
| Post-Translation Modifications-PTMs: Non-Enzymatic- PTMs | **QA31** | **Glycation*** | | | | ◐≠ | ◐= | ◐= | |
| | **QA32** | **Oxidation*** | | ◐≠ | ○ | ◐= | ◐= | ◐= | |
| | **QA33** | **Deamidation*** | | ○ | ○ | ◐= | ◐= | ◐= | |
| | QA34 | Truncation | ○ | ○ | ○ | ◐= | ◐≠ | | |
| | QA35 | Amidation | | | | ◐= | ◐≠ | | |
| | QA36 | Isomerization | | | | ◐= | ◐= | | |
| | QA37 | Cysteinylation | | | | | ◐= | | |
| | QA38 | Acetylation | | | | | | | |
| | QA39 | Formylation | | | | | | | |
| | QA40 | Methylation | | | | | | | |
| | QA41 | Hydroxylation | | | | | | | |
| | QA42 | Phosphorylation | | | | | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Purity and impurities | Size variants | QA44 | Sub-micron Particles | ○ | | | | | | |
| | | QA45 | Monomer | | ◐≠ | | ◐= | ◐= | ◐= | ◐= |
| | | QA46 | Dimer | | | | ◐≠ | | ◐= | ◐= |
| | | QA47 | Isoforms | | | | | | ◐= | ◐= |
| | | **QA48** | **Fragments*** | | | ○ | ◐= | ◐= | ◐= | ◐= |
| | | QA49 | MMWs | | | | | ◐= | | |
| | | **QA50** | **Non-glycosylated heavy chain (NGHC)*** | | ◐≠ | | | | | |
| | Charge variants | QA51 | Main forms | | | | | ◐≠ | | ◐= |
| | | **QA52** | **Acidic forms*** | | ◐≠ | | ◐≠ | ◐≠ | | ◐= |
| | | **QA53** | **Basic forms*** | | ◐≠ | | ◐≠ | ◐≠ | | ◐= |
| Biological activity | | QA54 | Binding activity | | ○ | | ◐= | ◐= | ◐= | ◐= |
| | | QA55 | Binding affinity | | ◐= | | ◐= | ◐= | ◐= | ◐= |
| | | QA56 | Binding specificity | ○ | ○ | | ◐= | ◐= | ◐= | ◐= |
| | | **QA57** | **Binding to s-TNFα*** | ◑ | ◐= | ○ | ◐= | ◐= | ◐= | ◐= |
| | | **QA58** | **Binding to tm-TNFα*** | ◑ | ◐= | ○ | ◐≠ | ◐= | ◐= | ◐= |
| | | **QA59** | **Neutralization of TNFα*** | ◑ | ◐= | ○ | ◐= | ◐= | ◐= | |
| | | QA60 | Inhibition of apoptosis | ◑ | ◐= | | ◐= | ◐= | ◐= | ◐= |
| | | QA61 | Induction of apoptosis | ◑ | ◐= | ◐= | | | ◐= | |
| | | QA62 | Inhibition of proliferation | ◑ | | | | ◐= | ◐= | ◐= |
| | | QA63 | Induction of regulatory macrophages | | ◐= | | | ◐= | ◐= | ◐= |
| | | QA64 | Inhibition of cytokine release | ◑ | ◐= | | ○ | | ◐= | |
| | | QA65 | Inhibition of adhesion molecule expression | | ◐= | | | | ◐= | ◐= |
| | | **QA66** | **ADCC activity*** | ○ | ◐= | ○ | ◐≠ | ◐= | ●= | ◐≠ |
| | | **QA67** | **CDC activity*** | ○ | ◐= | ◐= | ◐= | ◐= | ◐= | ◐= |
| | | QA68 | ADCP activity | | | ◐= | | | | |
| Immunochemical activity | | **QA69** | **Binding to C1q*** | ○ | ◐= | ○ | ◐≠ | ◐= | ◐= | ◐= |
| | | **QA70** | **Binding to FcRn*** | ◐= | ◐= | ○ | ◐= | ◐= | ◐= | ◐= |
| | | **QA71** | **Binding to Fcγ-RI*** | ○ | ◐= | ◐= | ◐= | ◐= | ◐= | ◐= |
| | | **QA72** | **Binding to Fcγ-Ria*** | ◑ | ◐= | ◐= | ◐= | ◐= | ◐= | ◐= |
| | | **QA73** | **Binding to Fcγ-RIIa*** | ◑ | ◐= | ◐= | ◐= | ◐= | ◐= | ◐= |
| | | **QA74** | **Binding to Fcγ-RIIIa*** | ●= | ◐= | ◐= | ◐= | ◐= | ●= | ◐= |
| | | **QA75** | **Binding to Fcγ-RIIb*** | ○ | ◐= | ◐= | ◐= | ◐= | ◐= | ◐= |
| | | **QA76** | **Binding to Fcγ-RIIIb*** | ○ | ◐= | ◐= | ◐= | ◐= | ◐= | ◐= |
| | | QA77 | Binding to TNF-β | | ◐= | | | | | ◐= |

**Supplementary Figure S1:** The types of and extent of information on quality attributes (QAs) and potentially critical QAs (pCQAs, in bold and gray boxes) as part of biosimilarity assessment reported by regulators in the initial European public assessment reports (EPARs) of seven adalimumab biosimilars.

○ QAs reported in the EPAR DO NOT include biosmilarity interpretation and DO NOT include test results

◑ QAs reported in the EPAR DO NOT include biosmilarity interpretation and DO include test results

◐ QAs reported in the EPAR DO include biosmilarity interpretation and DO NOT include test results

● QAs reported in the EPAR DO include biosmilarity interpretation and DO include test results and

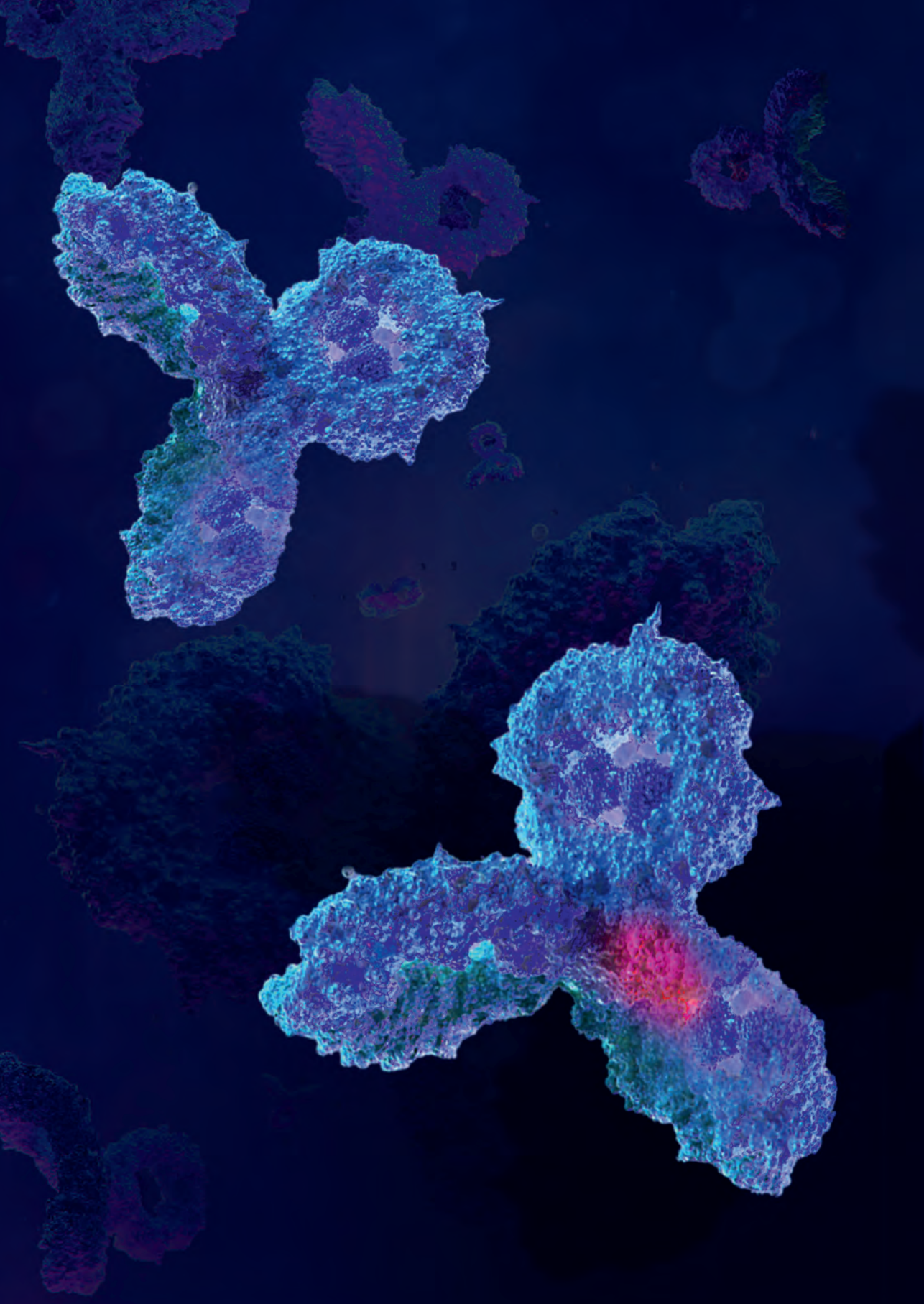
= Biosmilarity interpretation of the test result of QA was similar between biosimilar and reference biological

≠ biosmilarity interpretation of the test result of QA was different between biosimilar and reference biological

**Supplementary Table S2:** Comparison of potentially critical quality attributes (pCQAs) where test results and interpretation were reported for ABP501 and MSB11022 biosimilar.

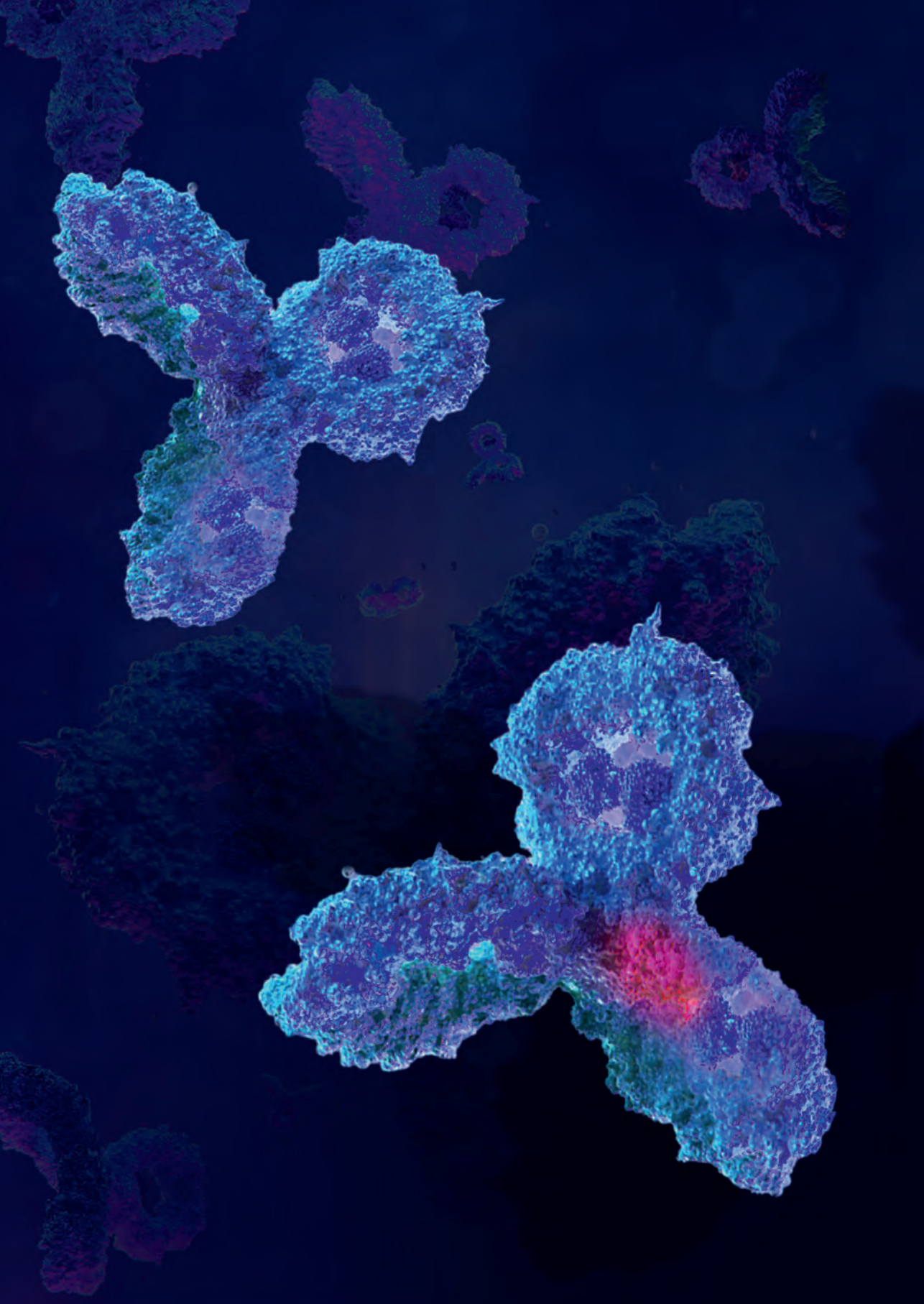
| Biosimilar | Quality attributes | Test result | Biosimilarity interpretation |
|---|---|---|---|
| ABP501 | Protein concentration (mg/ml) | ABP501 [range (n)]:<br>50.2 – 52.6 (4)<br><br>US Reference [range (n)]:<br>51.1– 53.1 (3)<br><br>EU Reference [range (n)]:<br>50.6 – 51.6 (3) | Similar |
| | Binding to FcgRIIIa (%) | ABP501 [mean (SD)]:<br>108 (12.3)<br><br>US Reference [mean (SD)]:<br>101 (13.6)<br><br>EU Reference [mean (SD)]:<br>113 (7.6) | Similar |
| MSB11022 | High mannose glycans (%) | MSB11022 [range (n)]:<br>1.9-2.5 (NR)<br><br>EU Reference [range (n)]:<br>5.3-12.0 (NR) | Minor differences |
| | Binding to FcgRIIIa (nM) | MSB11022 [range (n)]:<br>6.2 – 10.1 (NR)<br><br>EU Reference [range (n)]:<br>3.8 – 8.0 (NR) | Similar |
| | ADCC activity (%) | MSB11022 [$E_{max}$ range(n)]:<br>84-92 at F/F genotype<br>88-99 at V/F genotype<br><br>MSB11022 [$EC_{50}$ range(n)]:<br>41-56 at F/F genotype<br>26-37 at V/F genotype<br><br>EU Reference [$E_{max}$ range(n)]:<br>95-104 at F/F genotype<br>94-104 at V/F genotype<br><br>EU Reference [$E_{max}$ range(n)]:<br>79-162 at F/F genotype<br>70-174 at V/F genotype | Similar |

ADCC, Antibody Dependent Cellular Cytotoxicity, $EC_{50,}$ half-maximal effective concentration, $E_{max,}$ maximal effect at high drug concentrations, EU, European Union, US, United States. NR, not reported; FcgRIIIa, Fragment crystallizable gamma receptor; mg/ml; milligram/ milliter; nM, nanomole



# Chapter 3

## Post-approval quality surveillance



# Chapter 3.1

## Nature and timing of post-approval manufacturing changes of tumour necrosis factor α inhibitor products: a 20-year follow-up study of originators and biosimilars

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Author's contribution: AMA designed the study, performed data management, conducted data curation, analysis, and validation, prepared the first- draft of the manuscript, and implemented significant contribution from co-authors up to the final publication. Throughout the process, AMA asked for and implemented input and feedback from supervision team and co-authors, who performed critical review of the manuscript and provided significant contributions to the study.

## Abstract

The manufacturing of biopharmaceuticals is complex, and minor changes in the process may affect quality attributes (QAs) that may, in turn, impact clinical outcomes. Regulatory documents from the European Medicines Agency were used to characterize two aspects, nature and timing, of post-approval MCs for originators and biosimilars TNF-α inhibitors that were on the European market up to May 2021. The nature of MCs was evaluated in two ways: 1) the type of MCs related to the drug substance (DS) or drug product (DP), classified as manufacturing, quality control, composition, packaging, or stability with various subtypes; and 2) the risk level according to the potential impact of the MCs on QAs, classified as low, medium, or high. Timing was defined as the date of the regulatory decision on the MC in relation to the approval date. We identified 801 post-approval MCs implemented to originators (mean: 137, range: 112–175) and biosimilars (mean: 30, range: 0–133). Most of implemented MCs for originators and biosimilars were classified as low and medium risk (88.1%), and a small fraction were considered high-risk (11.9%). The average incidence rates were comparable for both originators and biosimilars (7.0/year for MCs, 0.8/year for high-risk MCs). In 20% of MCs introduced to biosimilars, the DP manufacturing site was involved (9% for originators). In contrast, 16% of MCs introduced to originators were related to the DS manufacturing processes (only 7% for biosimilars). In conclusion, while the overall MC incidence rate and the risk level of MCs was not substantially different between TNF-α inhibitor products, we observed some differences in a few types of MCs related to DS manufacturing process and DP manufacturing site between originators and biosimilars. As far as our data shows there is no reasons to assume that post-approval MCs will lead to differences between TNF-α-i originators and biosimilars in clinical practice.

## Introduction

The manufacturing of biopharmaceuticals is a complex process, and every step may influence the quality attributes (QAs) of the drug substance (DS) and/or drug product (DP). Furthermore, an inherent degree of structural heterogeneity occurs in biopharmaceuticals; hence, batch-to-batch variability within certain limits or ranges is acceptable from a regulatory standpoint. Manufacturing changes (MCs) may be implemented after marketing authorization (MA) of a biopharmaceutical, i.e., post-approval MCs. Among others, the reasons for implementing MCs include compliance with regulatory commitments and standards, maintaining product quality and consistency between batches, and increasing manufacturing scale, robustness, efficiency, and reliability [1-3].

Even minor changes in the manufacturing process can potentially impact clinically relevant QAs (i.e., critical quality attributes), which may, in turn, influence the clinical outcomes of biopharmaceuticals [4, 5]. Regulators require therefore the provision of adequate evidence from a comparability exercise to ensure that the quality, safety, and efficacy of DPs are unaffected following post-approval MCs. According to the International Conference on Harmonization (ICH Q5E), the QAs of pre- and post-MC batches must be comparable to minimise the risk that MCs adversely impact clinical performance [6]. The cornerstone of assessing comparability is the comparison of QAs based on a risk evaluation of the intended MCs. Sometimes the outcome may warrant (non-)clinical comparative studies. Additional clinical data are, however, rarely required for MC approval and is limited to a very few examples, including Aranesp® (darbepoetin alfa), following a process change to a serum-free bioreactor to reduce the risk of contamination, and Humira® (adalimumab), following a change in formulation and concentration to improve patient convenience [7].

The same scientific and technical principles of comparability apply to the development and regulatory approval of biosimilars. A biosimilar is a biological medicine that is similar to a reference product (i.e., 'originator') with no clinically meaningful differences in terms of quality. A successful demonstration of comparability to the originator at the QA level is the basis of biosimilar approval, but this cannot be achieved without well-designed and quality-driven reverse engineering of the originator production process [8]. Upon approval, biosimilars are considered standalone products with no need for comparison to the originator if post-approval MCs are introduced.

Previous studies found that a substantial number of post-approval MCs were implemented for originator biopharmaceuticals approved in the European Union (EU) and the USA [9, 10]. Most authorized MCs were classified as low (72%) or medium risk (23%), and only a small fraction (5%) as high risk with a potential impact on product quality and clinical outcomes [10]. This finding indicates that regulators have extensive experience in assessing post-approval MCs for originator biopharmaceuticals.

TNF-α inhibitor (TNF-α-i) products, including mAbs (infliximab, adalimumab, certolizumab pegol, and golimumab) and a fusion protein (etanercept), provide effective treatment options for several inflammatory diseases [11-14]. More than half of the 31 mAbs biosimilars (34 trade names) approved by the European Medicine Agency (EMA) as of 2021 are biosimilars of TNF-α-i products.

Previous studies have reflected on the number and risk level of post-approval MCs of originator mAbs from the MA date up to 2014 [9, 10]. Information on the nature and timing of post-approval MCs of biosimilars is scarce. In this study we aim to complement current evidence with a description and characterization of post-approval MCs of both originators and biosimilars of TNF-α-i -products in Europe (most recent observation date, May 2021).

## Method

### Setting and study design

A retrospective descriptive analysis was conducted for TNF-α-i products (originators and corresponding biosimilars) with data sourced from publicly available regulatory documents retrieved from the EMA's official website (www.ema.europa.eu; access date 31 May 2021). The study included the mAbs infliximab and adalimumab and the fusion protein etanercept, which were centrally authorized in the EU between January 1999 and May 2020. TNF-α-i products for which only the originators have been approved (i.e., certolizumab pegol, and golimumab) were excluded. Baseline characteristics of TNF-α-i products were obtained from the initial European Public Assessment Reports (EPARs) and included the trade name(s), company code of the development programme, and MA date in the EU. The company code of the development programme only applies to biosimilars because these are marketed under different trade names that originated from the same development programme. The biosimilars were ordered according to the MA date (i.e., Remsima® and Inflectra® were considered as the first biosimilars of infliximab [BS1] and Flixabi® the second [BS2], etc.).

### Post-approval manufacturing changes

The scope and dates for the regulatory decisions on post-approval MCs for the included TNF-α-i products were obtained from the EPARs, which contain information regarding the procedural steps and scientific information after authorization. This information is posted in the section "assessment history" on the EMA website (www.ema.europa.eu; access date 31 May 2021) and includes a detailed description of the nature of post-approval MCs. Since one assessment procedure may include more than one post-approval MC, every MC was considered and included as an independent MC. Each post-approval

MC was evaluated on two aspects: 1) the nature of the MC, including type and risk level; and 2) the timing of regulatory approval of MCs in relation to the MA date.

The classification of MC types was developed based on types of post-approval changes for MA of human medicines available in the European Commission regulation (No. 1234/2008) [15]. This classification includes four types of MCs related to the DS and six related to the DP, with various subtypes (Box 1). Post-approval MCs not related to quality or manufacturing (i.e., changes made to update the regulatory dossier and related to regional administrative information, safety, and efficacy of the products) were not considered in this study, and those made to update documentation of the quality dossier (e.g., changes to approved management protocol; submission of a new, updated, or deleted certificate of suitability to the European Pharmacopoeia) were outside the scope of this study.

The risk level of each post-approval MC was classified as low, medium, or high, based on definitions proposed by Lee et al. [1] and applied by Vezér et al. [10]. Lee et al. used the risk-level definitions as per the ICH Q5E [6].



**Box 1:** Classification of the type of manufacturing changes (MCs) according to European Commission regulation 1234/2008.

| Drug substance (DS) | Drug product (DP) |
|---|---|
| DS manufacturing | DP Composition |
| Manufacturing site | Strength |
| Manufacturing process | Formulation |
| Batch size | DP manufacturing |
| In-process test or limits | Manufacturing site |
| DS quality control | Manufacturing process |
| Specification parameters or limits | Batch size |
| Analytical test procedures | In-process test or limits |
| DS packaging system | Excipient quality control |
| Primary (immediate) packaging | Specification parameters or limits |
| DS stability | Analytical test procedures |
| Shelf life | DP quality control |
| Storage conditions | Specification parameters or limits |
| Stability protocol | Analytical test procedures |
| | DP packaging system |
| | Primary (immediate) packaging |
| | Secondary packaging |
| | DP stability |
| | Shelf life |
| | Storage conditions |
| | Stability protocol |

MCs that are not expected to adversely impact the QAs of the DS and DP and for which additional (non-)clinical data are not required for regulatory approval were classified as low risk (e.g., changes in the DP stability protocol). MCs that may result in minor differences in clinically not-relevant QAs and do not require additional (non-)clinical data for regulatory approval were classified as moderate risk (e.g., changes to in-process tests or limits applied during DS manufacture). MCs that may result in differences in clinically relevant QAs that potentially warrant additional (non-)clinical data for regulatory approval were classified as high risk (e.g., changes in DS purification or DP formulation).

Each post-approval MC was assessed and allocated to a specific risk level. The first assessment was performed by the authors (AMA) and thereafter validated by an expert in the quality and manufacturing of biopharmaceuticals (ED). In the event of discrepancies regarding the risk-level allocation, a decision was made by team consensus. The overall inter-rater reliability was 93.5% (kappa = 0.867).

The dates of regulatory approval of MCs were used to assess the timing of the implementation of post-approval MCs relative to the date of MA.

### Data analysis

Descriptive statistics were performed to evaluate the nature, including type and risk level, and timing of the post-approval MCs. Timing was assessed from the date of MA until the regulatory approval of MCs or until 31 May 2021 (end of follow-up), allowing for at least one year of follow-up for each TNF-α-i product. The absolute number and incidence rate of post-approval MCs were stratified by type, risk level, calendar year and by TNF-α-i products (active substance, originator, biosimilar).

Cumulative curves were plotted, using R software (version 4.1.2) to explore patterns in the timing of implementation of post-approval MCs in general and high-risk MCs for both originators and biosimilars over the study period. All descriptive analyses were performed using the statistical software package SPSS version 27 (SPSS Inc, Chicago, Illinois).

## Results

### Characteristics of tumour necrosis factor monoclonal antibodies

Sixteen TNF-α-i products approved between August 1999 and May 2020, namely, three originators (Remicade® [infliximab], Enbrel® [etanercept], and Humira® [adalimumab]) and 13 corresponding biosimilars, were included in the analysis. Up to 31 May 2021, in total 801 post-approval MCs were introduced to these products. The number of MCs varied substantially between products: originators (mean = 137; range = 112–175) and biosimilars (mean = 33; range = 0–133) (Table 1).

**Table 1:** Characteristics of originators and corresponding biosimilars of TNF-α-i products approved in the EU up to May 2020.

| Active substance | Trade name | Company code (BS order) | EU MA date (mm-yyyy) | Number of post-approval MCs (n) | Follow-up to May 2021 (years) | Average incidence rate of MCs/year (high-risk/year) |
|---|---|---|---|---|---|---|
| Infliximab | Remicade® | Originator (RP) | 08-1999 | 112 | 21.8 | 5.2 (0.8) |
| | Remsima® Inflectra® | CTP13(BS1)[b] | 09-2013 | 133 | 7.7 | 17.3 (2.7) |
| | Flixabi® | SB2 (BS2) | 05-2016 | 57 | 5.0 | 11.4 (1.4) |
| | Zessly® | GP111 (BS3) | 05-2018 | 9 | 3.0 | 3.0 (0.0) |
| Etanercept | Enbrel® | Originator (RP) | 02-2000 | 175 | 21.3 | 8.2 (0.9) |
| | Benepali® | SB4 (BS1) | 01-2016 | 43 | 5.3 | 8.1 (0.5) |
| | Erelzi® | GP2015 (BS2) | 06-2017 | 30 | 3.9 | 7.7 (0.0) |
| | Nepexto® | YLB113 (BS3) | 05-2020 | 8 | 1.0 | 8.0 (0.0) |
| Adalimumab | Humira | Originator (RP) | 09-2003 | 124 | 17.7 | 7.1 (1.0) |
| | Amgevita® Solymbic®[a] | ABP501 (BS1)[b] | 03-2017 | 11 | 4.2 | 2.6 (0.2) |
| | Imraldi® | SB5 (BS2) | 08-2017 | 45 | 3.8 | 12.0 (0.5) |
| | Cyltezo®[a] | BI695501 (BS3) | 11-2017 | 1 | 1.3[c] | 0.8 (0.0) |
| | Hefiya® Halimatoz® Hyrimoz® | GP2017 (BS4)[b] | 07-2018 | 28 | 2.8 | 9.9 (0.7) |
| | Hulio® | FKB327 (BS5) | 09-2018 | 18 | 2.7 | 6.8 (0.7) |
| | Idacio® Kromeya® | MSB11022 (BS6)[b] | 04-2019 | 7 | 2.1 | 3.4 (0.0) |
| | Amsparity® | PF06410293 (BS7) | 02-2020 | 0 | 1.3 | - |

[a]Trade names of biosimilars that received MA from the European Commission but were voluntarily withdrawn by the applicant for commercial reasons. [b]Biosimilars produced from the same development programmes and available on the EU market under several trade names. [C]The end of follow-up was the date of withdrawal of MA by EU. RP, reference product; BS, biosimilar; MA, marketing authorization; MCs, manufacturing changes; EU, European Union..

### Types of post-approval manufacturing changes

More than half of the MCs were related to manufacturing at the DS (26.8% for originators and 22% for biosimilars) and DP (27.7% for originators and 31.8% for biosimilars) levels; these changes were mainly related to the 'manufacturing site' and 'manufacturing process'. Approximately 25% of the total MCs were related to quality control at the DS (11.2% for originators and 14.1% for biosimilars) and DP (8.3% for originators and 5.1% for biosimilars) levels and were mainly related to 'specification parameters and limits'. Subtle differences were noted in absolute frequency between originators and biosimilars in few subtypes, namely, 'manufacturing process of the DS' (16% for originators versus 7% for biosimilars) and 'manufacturing site of the DP' (9% for originators versus 20% for biosimilars). The type of MCs implemented for biosimilars were not related to the type of MCs already implemented for originators. (Table 2).

### Risk level of post-approval manufacturing changes

The majority of the 801 implemented MCs for originators and biosimilars were classified as low (62.5%) or medium (25.6%) risk, while a small fraction were considered high-risk MCs (11.9%) (Table 2). The high-risk MCs involved both originators (15%) and biosimilars (10%). At least one high-risk MC was implemented with all originators and seven of included biosimilars during the study period. The high-risk MCs were relatively more often related to DS quality control, mainly concerning 'specification parameters and limits' (35.1% for originators and 23.7% for biosimilars), DP composition (15.8% for originators and 18.5% for biosimilars), and DS manufacturing, predominantly the 'manufacturing process' (17.5% for originators and 18.4% for biosimilars). In a limited number of cases, some high-risk MCs that were never implemented for originators were implemented for a few biosimilars, for example, high-risk MCs related to 'in-process test or limits of the DP', 'batch size of the DS', 'formulation of the DP', and 'primary (immediate) packaging of the DP'. Detailed information on the type and nature of high-risk MCs implemented for originators and biosimilars is available in supplementary Table S1.

### Timing of post-approval manufacturing changes

The follow-up time was, on average, 20 years for originators and 3 years for biosimilars. The implementation of MCs for originators and biosimilars follow a similar pattern, which is increasing overtime (Figure S1). Although there was a large variation between products in the absolute number of MCs (Table 1, Figure 1), no substantial variation in incidence rate i.e., taking follow-up time into account, was observed between originators and biosimilars. The overall average incidence rate of MCs per year was 7 for originators (range: 5.1–8.2) and 7.6 for biosimilars (range: 0.8–17.3) (Table 1). Similar patterns were observed when limiting to the high-risk MCs, where incidence rate was on average 0.9 MC for originators (range: 0.8 – 1.0) and 0.6 MC for biosimilars (range: 0.0 – 2.7) (Table 1, Figure 2). The type of MCs related to the stability, among other types, was introduced sooner after the regulatory approval for both originators and biosimilars.

**Table 2:** Type and risk level of manufacturing changes for TNF-α-i originators (1999–2020) and biosimilars (2013–2020).

| Types of MCs | All MCs (n = 801) | | Low-risk MCs (n = 501) | | Medium-risk MCs (n = 205) | | High-risk MCs (n = 95) | |
|---|---|---|---|---|---|---|---|---|
| | Originators 411 (100%) | Biosimilars 390 (100%) | Originators 255 (100%) | Biosimilars 246 (100%) | Originators 99 (100%) | Biosimilars 106 (100%) | Originators 57 (100%) | Biosimilars 38 (100%) |
| Drug substance (DS) | 146 (39.9%) | 162 (41.5%) | 28 (11.2%) | 34 (13.9%) | 98 (99%) | 106 (100%) | 38 (66.6%) | 22 (57.9%) |
| DS manufacturing | 110 (26.8%) | 86 (22%) | 5 (2%) | 6 (2.4%) | 87 (87.9%) | 67 (63.2%) | 18 (31.5%) | 12 (34.2%) |
| Manufacturing site | 18 (4.4%) | 31 (7.9%) | 0 (0%) | 0 (0%) | 14 (14.1%) | 30 (28.3%) | 4 (7%) | 1 (2.6%) |
| Manufacturing process | 67 (16.3%) | 28 (7.2%) | 0 (0%) | 0 (0%) | 57 (57.6%) | 21 (19.8%) | 10 (17.5%) | 7 (18.4%) |
| Batch size | 2 (0.5%) | 2 (0.5%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 2 (3.5%) | 2 (5.3%) |
| In-process test or limits | 23 (5.6%) | 25 (6.4%) | 5 (2%) | 6 (2.4%) | 16 (16.2%) | 16 (15.1%) | 2 (3.5%) | 3 (7.9%) |
| DS quality control | 47 (11.2%) | 55 (14.1%) | 23 (9%) | 25 (10.2%) | 4 (4%) | 21 (19.8%) | 20 (35.1%) | 9 (23.7%) |
| Specification parameters or limits | 24 (5.8%) | 30 (7.7%) | 0 (0%) | 0 (0%) | 4 (4%) | 21 (19.8%) | 20 (35.1%) | 9 (23.7%) |
| Analytical test procedures | 23 (5.6%) | 25 (6.4%) | 23 (9%) | 25 (10.2%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| DS packaging system | 1 (0.2%) | 3 (0.8%) | 0 (0%) | 0 (0%) | 1 (1%) | 3 (2.8%) | 0 (0%) | 0 (0%) |
| Primary (immediate) packaging | 1 (0.2%) | 3 (0.8%) | 0 (0%) | 0 (0%) | 1 (1%) | 3 (2.8%) | 0 (0%) | 0 (0%) |
| DS stability | 6 (1.4%) | 18 (4.7%) | 0 (0%) | 3 (1.2%) | 6 (6.1%) | 18 (14.1%) | 0 (0%) | 0 (0%) |
| Shelf life | 5 (1.2%) | 12 (3.1%) | 0 (0%) | 0 (0%) | 5 (5.1%) | 12 (11.3%) | 0 (0%) | 0 (0%) |
| Storage conditions | 1 (0.2%) | 3 (0.8%) | 0 (0%) | 0 (0%) | 1(1%) | 3 (2.8%) | 0 (0%) | 0 (0%) |
| Stability protocol | 0 (0%) | 3 (0.8%) | 0 (0%) | 3 (1.2%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |

DP, drug substance; MCs, manufacturing changes.

**Table 2 (continued)**: Type and risk level of manufacturing changes for TNF-α-i originators (1999–2020) and biosimilars (2013–2020).

| Types of MCs | All MCs (n = 801) | | Low-risk MCs (n = 501) | | Medium-risk MCs (n = 205) | | High-risk MCs (n = 95) | |
|---|---|---|---|---|---|---|---|---|
| | Originators 411 (100%) | Biosimilars 390 (100%) | Originators 255 (100%) | Biosimilars 246 (100%) | Originators 99 (100%) | Biosimilars 106 (100%) | Originators 57 (100%) | Biosimilars 38 (100%) |
| Drug product (DP) | 265 (60.1%) | 228 (58.5%) | 222 (88.1%) | 211 (86.1%) | 1 (1%) | 0 (0%) | 24 (33.4%) | 17 (42.2%) |
| DP Composition | 16 (3.8%) | 7 (1.8%) | 7 (2.8%) | 0 (0%) | 0 (0%) | 0 (0%) | 9 (15.8%) | 7 (18.5%) |
| Strength | 8 (1.9%) | 5 (1.3%) | 2 (0.8%) | 0 (0%) | 0 (0%) | 0 (0%) | 6 (10.5%) | 5 (13.2%) |
| Formulation | 8 (1.9%) | 2 (0.5%) | 5 (2%) | 0 (0%) | 0 (0%) | 0 (0%) | 3 (5.3%) | 2 (5.3%) |
| DP manufacturing | 114 (27.7%) | 124 (31.8%) | 100 (40%) | 119 (48.9%) | 1 (1%) | 0 (0%) | 8 (14%) | 3 (7.9%) |
| Manufacturing site | 39 (9.5%) | 80 (20.5%) | 39 (15.3%) | 80 (32.5%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| Manufacturing process | 45 (10.9%) | 18 (4.6%) | 36 (14.1%) | 17 (6.9%) | 1 (1%) | 0 (0%) | 8 (14%) | 1 (2.6%) |
| Batch size | 6 (1.5%) | 7 (1.8%) | 6 (2.4%) | 7 (2.8%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| In-process test or limits | 24 (5.8%) | 19 (4.9%) | 24 (9.4%) | 17 (6.9%) | 0 (0%) | 0 (0%) | 0 (0%) | 2 (5.3%) |
| Excipient quality control | 1 (0.2%) | 5 (1.3%) | 1 (0.4%) | 5 (2%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| Specification parameters or limits | 1 (0.2%) | 4 (1%) | 1 (0.4%) | 4 (1.6%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| Analytical test procedures | 0 (0%) | 1 (0.3%) | 0 (0%) | 1 (0.4%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| DP quality control | 34 (8.3%) | 20 (5.1%) | 33 (12.9%) | 15 (6.1%) | 0 (0%) | 0 (0%) | 1 (1.8%) | 5 (13.2%) |
| Specification parameters or limits | 9 (2.2%) | 9 (2.3%) | 8 (3.1%) | 4 (1.6%) | 0 (0%) | 0 (0%) | 1 (1.8%) | 5 (13.2%) |
| Analytical test procedures | 25 (6.1%) | 11 (2.8%) | 25 (9.8%) | 11 (4.5%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| DP packaging system | 56 (13%) | 45 (12%) | 55 (21.6%) | 44 (17.9%) | 0 (0%) | 0 (0%) | 1 (1.8%) | 1 (2.6%) |
| Primary (immediate) packaging | 18 (4.4%) | 10 (2.6%) | 17 (6.7%) | 9 (3.7%) | 0 (0%) | 0 (0%) | 1 (1.8%) | 1 (2.6%) |
| Secondary packaging | 38 (9.2%) | 35 (9%) | 38 (14.9%) | 35 (14.2%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| DP stability | 26 (6.3%) | 27 (7%) | 26 (10.3%) | 27 (10.9%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| Shelf life | 15 (3.6%) | 18 (4.6%) | 15 (5.9%) | 18 (7.3%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| Storage conditions | 4 (1%) | 4 (1%) | 4 (1.6%) | 4 (1.6%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| Stability protocol | 7 (1.7%) | 5 (1.3%) | 7 (2.7%) | 5 (2%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |

DP, drug product; MCs, manufacturing changes.

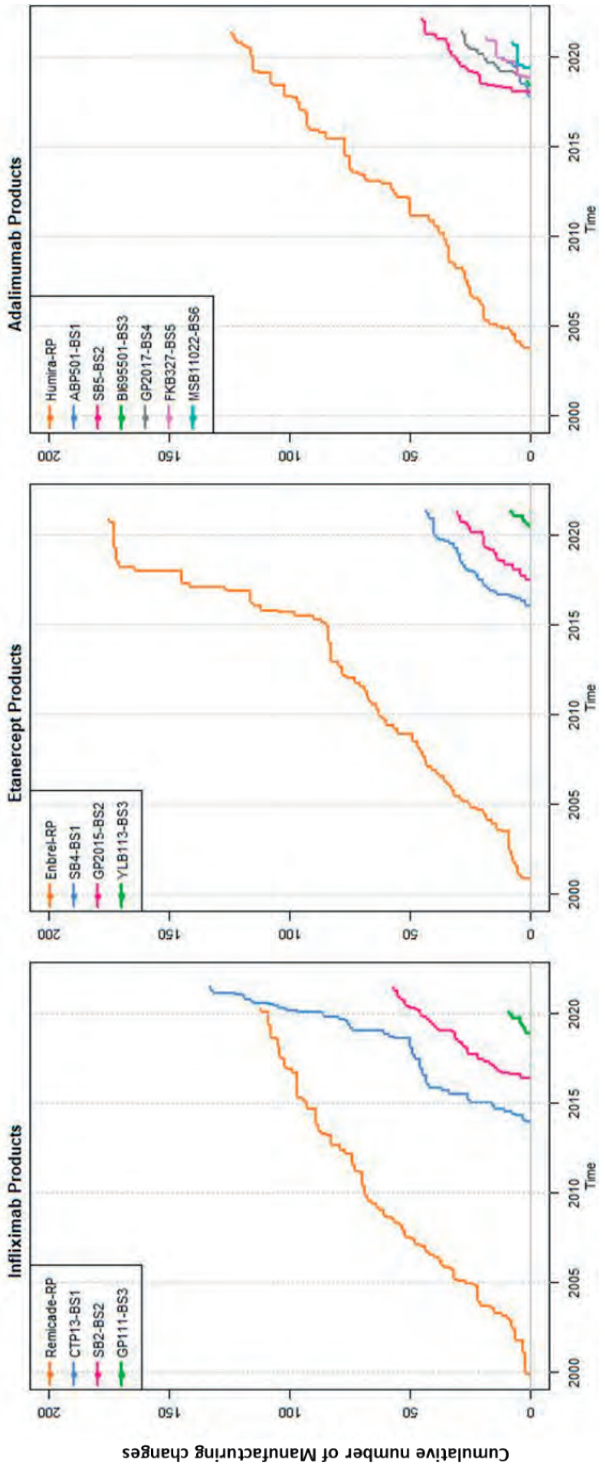


**Figure 1:** Cumulative number of post-approval MCs since the date of marketing authorization.

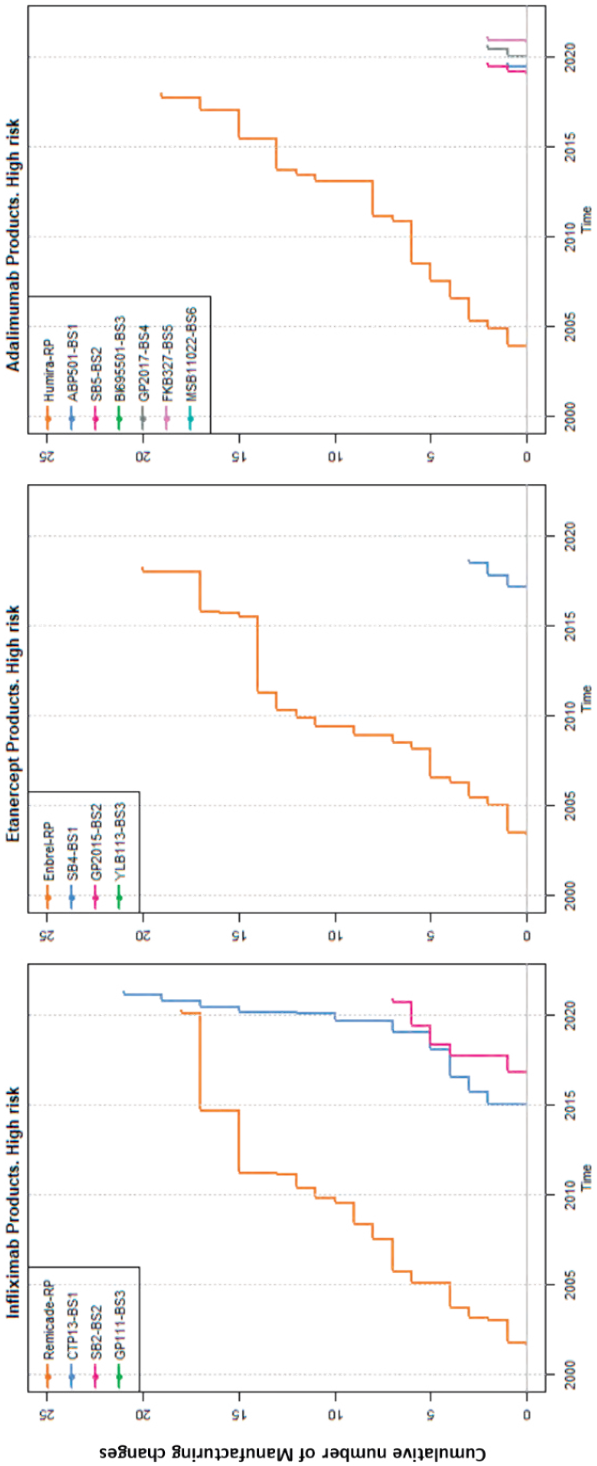


**Figure 2:** Cumulative number of post-approval high-risk MCs since the date of marketing authorization.

## Discussion

Approximately 800 post-approval MCs to the three originator TNF-α-I products and the 13 corresponding biosimilars were implemented during an average period of 20 years for originators and 3 years for the biosimilars corresponding to on average 7 MCs and 0.8 high risk MCs per year. Key differences between originators and biosimilars with regards to type of MC were only found for MCs related to the DS manufacturing process, which were twice as frequent for originators when compared with biosimilars, and the DP manufacturing site, which occurred more frequently for biosimilars. Approximately 10% of the post-approval MCs were classified as high risk and these were relatively more frequently related to DS quality control and DS manufacturing and to DP composition for both originator and biosimilars.



Our results are consistent with Vezér et al.'s [10] that showed that MCs are implemented frequently and even long after approval and that the vast majority were low or medium risk. We found on average an annual incidence rate of 7 MCs (both for originators and biosimilars) which is considerably higher than the annual incidence of 1.8 MCs reported by Vezér et al. This discrepancy could be explained by the fact that most post-approval MCs identified for originators in our study were implemented after the period studied by Vezér et al. The continuous modernization of manufacturing processes and optimization of the quality of biopharmaceuticals (both originators and biosimilars) likely contributes to this finding [9, 10]. Further, the relatively quick introduction of MCs related to stability for both originator and biosimilars after approval could relate to obligatory post-approval regulatory commitments or to support the extension of the shelf life based on a longer data period. We also found that the type of MCs for biosimilars were not related to the type of MCs already implemented for originator, which reflects that biosimilars and originators are standalone products after approval.

Our analysis found that MCs related to the DS manufacturing process were more frequently implemented for originators which includes advancements in knowledge and technical innovations introduced in recent decades to scale manufacturing and optimize the purification and characterization of biopharmaceuticals [16-21]. The higher frequency of implementing MCs related to the DP manufacturing site for biosimilars could be attributed to biosimilar companies scaling up or building of new production sites, enabling them to provide sufficient stock to meet market demand. It is important to note that these MCs mainly involved non-critical activities, such as the addition of sites for batch release, quality control tests, and secondary packaging. We argue that these subtle differences in post-approval MCs between originators and biosimilars most likely do not lead to differences in clinical practice. To the best of our knowledge, no safety and efficacy concerns have been reported from post-marketing pharmacovigilance systems following implementation of post-approval MCs for the studied TNF-α-i products.

At the time of approval, biosimilars are required to demonstrate biosimilarity against the originator based on comparability exercises [22, 23]. Regulators may allow differences in certain aspects between biosimilars and originator, such as the formulation (e.g., excipients), presentation (e.g., powder to be reconstituted versus solution ready for injection), and administration device (e.g., type of delivery pen), if these do not affect the biosimilarity on biological and pharmacological functions and clinical outcomes. After approval, the originator and biosimilars are considered standalone products and redemonstration of biosimilarity is not required following post-approval MCs. However, bringing innovative solutions for patient care may trigger companies to implement certain MCs after approval. This is illustrated by two examples developing novel formulation and new route of administration for biopharmaceuticals. The marketing authorization holder of adalimumab originator (Humira®) developed a new citrate-free formulation to reduce pain associated injection site reaction providing comfort for patients and improve adherence [7]. The marketing authorization holder of the first infliximab biosimilar (Remsima®/Inflectra®) developed the first infliximab for subcutaneous use, which allows self-administration and reduces time associated with the intravenous infusions to improve patient compliance and adherence [24, 25]. These examples show that both originators and biosimilars can bring novel solutions by optimizing and improving the quality of the product.

It is never clear in advance whether post-approval MCs might lead to changes in clinically relevant QAs and clinical outcomes. As an example, the mAb towards HER2, trastuzumab (Herceptin®), for which the company producing a trastuzumab biosimilar (Ontruzant®; SB3) discovered differences in the glycosylation and potency (antibody-dependent cell-mediated cytotoxicity) in the originator in batches with different expiry dates, which might potentially impact clinical outcomes [26]. These alterations were linked to multiple changes in the manufacturing site and process and resulted in seemingly reduced efficacy in patients who received the affected batches of Herceptin®, based on the 3-year follow-up of the Phase III trial [27, 28]. However, the 3-year follow-up result was not confirmed in the 5-year follow-up, which further confirmed the similarity in clinical outcomes in term of the response rate and long-term survival between the originator (Herceptin®) and biosimilar (Ontruzant®; SB3) [29]. Although this case demonstrates that clinical outcome of Herceptin® is unaffected by the drift in glycosylation and potency, it shows how important it is to understand the clinical meaning of small differences in clinically relevant QAs, known as critical QAs. Nevertheless, this trastuzumab case raised questions about the variability range that should be used for drifted or shifted QAs to support the comparability evidence for biosimilar approval. What can be learnt from the case of trastuzumab is that biosimilar companies need to consider post-approval MCs implemented to originators when establishing the variability range for biosimilar development and approval.

Companies are required to send a notification or request a regulatory approval before implementing (major) changes to the manufacturing process, and regulators

may demand comparability exercise of QAs to ensure batch-to-batch consistency and minimize the risk of potential divergence between batches from the same manufacturer (i.e., pre-, and post-change batches) [30-32]. Consistency in clinically relevant QAs is a key quality issue to ensure that therapeutic biological function and clinical outcomes are not affected by post-approval MCs. Several biopharmaceutical companies have reported results for a selection of QAs of multiple batches produced over extended periods to demonstrate consistency in manufacturing processes [33-37]. However, these assessments are manufacturer-focused and do not allow the comparison of products or batches from different manufacturers. Since product or batch divergence may occur transiently following post-approval MCs, which might result in an unnoticed shift or drift of clinically relevant QAs from the acceptable variability range or limit, and potentially impact clinical outcomes [30-32]. The study finding highlights the importance of ensuring consistency in clinically relevant QAs, for example, glycosylation and potency between originators and biosimilars, for which, theoretically at least, the potential risk of divergence between products or batches (horizontally) or over extended periods (longitudinally) cannot be excluded.



Our findings confirm the new regulatory challenge of ensuring consistency of clinically relevant QAs (i.e., critical quality attributes) in products and batches after approval, as highlighted by Prior et al. [30]. The comparability exercise is a powerful regulatory tool to assess the biosimilarity of biosimilars at the time of approval and ensures consistency in products or batches of the same manufacturer after approval. However, it cannot be used to guarantee consistency in clinically relevant QAs between products of different manufacturers since each has a separate lifecycle. Although not all post-approval MCs cause shifts or drifts in QAs and not all shifts and drifts in QAs have clinical consequences, it is assumed that the risk of product divergence only increases with time, the number of products, and post-approval (high-risk) MCs [30-32]. Therefore, there is a need to develop a tool to address the challenge of potential product divergence that regulators and manufactures are likely to encounter in the future. One ideal solution is to develop and promote reference standards for clinically relevant QAs such as biological activity (potency), as proposed and extensively explained by Prior et al. [30]. Consistency in potency is critical to ensure that patients receive comparable product and harmonized doses, especially when considering interchangeability or switching of biosimilars and originators [38-41]. This may require the development of relevant potency assays that correlate with the size of the clinical effect. Recently, the Expert Committee on Biological Standardization established the first World Health Organization reference standards for several mAbs [42-46]. These reference standards would allow regulators and manufacturers to detect potential product or batch divergences and prevent undesirable clinical events for both originators and biosimilars over their lifecycle. Moreover, reference standards may help to standardize and harmonize potency estimates and clinical monitoring assays that would be useful for clinical decision making and treatment strategies in medical practice.

This study is the first to provides insights into characterization of the type and risk level of MCs implemented for TNF-α-i products over a period of 20 years. Nevertheless, this study is not without limitations. First, the findings are limited to post-approval MCs of TNF-α-i products and may not be generalizable to other groups of biopharmaceuticals. Although post-approval MCs are product-, company-, and time-dependent, comparable findings are expected for other biopharmaceuticals as they share the same degree of complexity in the manufacturing process. Second, the rating of MC risk levels may be subjective and prone to misclassification bias. However, the classification of the risk levels was validated by an expert in quality and manufacture of biopharmaceuticals to reduce the effect of misclassification, and the classification can be used in future studies. Third, it was not possible to identify the QAs relevant to the MCs and determine to which extent the high-risk MCs influenced the clinically relevant QAs, because pertinent data are not available in publicly accessible regulatory documents. Such information on comparability of QAs would be very helpful in identifying the clinically relevant QAs and their margins and assess the potential impact of MCs on QAs. And lastly, the data we used in our study does not allow us to assess or conclude on the impact of these MC on clinical outcomes. However, with the retrospective nature of the present study there are no signals of a negative impact of the MCs on clinical practice.

## Conclusion

To conclude, many post-approval MCs were implemented for TNF-α-i products introduced to the European market during the last two decades, with a comparable overall incidence rate for both originators and biosimilars. Most of MCs were related to manufacturing and quality control which reflects that the modernization process and optimization of quality of originators and biosimilars are never finished. Differences in the type of MCs between originators and biosimilars were limited to the DS manufacturing process and the DP manufacturing site, which may be explained by the development within the technological space to enhance product quality, manufacture upscaling to meet market demands, and bring innovative solutions for patient care. As far as our data shows there is no reasons to assume that post-approval MCs will lead to differences between TNF-α-i originators and biosimilars in clinical practice.

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# Supplementary data

**Supplementary table S1:** Details on the nature of high-risk manufacturing changes for each type of manufacturing changes that were introduced to tumor necrosis TNF-α inhibitors between August 1999 and May 2021.

| Type of MCs | Description of the nature of high-risk MCs | Originators (year(s)) | Biosimilars (year(s)) |
|---|---|---|---|
| Drug substance (DS) | | | |
| DS Manufacturing | | | |
| DS Manufacturing site | - Add a new manufacturing site. The proposed manufacturer uses a substantially different route of synthesis or manufacturing conditions. | Enbrel (2003, 2005, 2008, 2009) | SB4 -BS1 (2018) |
| DS Manufacturing process | - Substantial change to the manufacturing process of DS may have a significant impact on the quality, safety or efficacy of the medicinal product. | -- | CTP13-BS1 (2019) |
| | - Change refers to a [-] in the manufacturer of biological/immunological substance which may have a significant impact on the medicinal product and is not related to a protocol. | Remicade (2010), Humira (2013, 2015), Enbrel (2018) | SB2-BS2 (2016, 2017, 2018), SB4-BS1 (2017), SB5-BS2 (2019), CTP13-BS1 (2021) |
| | - Change in the purification process. | Remicade (2009) | -- |
| | - Change in the growth media components. | Remicade (2009) | -- |
| | - Change in the chromatography columns. | Remicade (2008) | -- |
| | - Change in the downstream process. | Enbrel (2010) | -- |
| DS Batch size | - Change in the batch size that requires assessment of the comparability of a biological drug substance. | Humira (2013) | CTP13-BS1 (2021) |
| DS In-process test or limits | - Addition or replacement of in-process tests or limits as a result of safety or quality issues. | Remicade (2011) | -- |
| | - Widening of the approved in-process test limits, which may have a significant effect on the overall quality of DS. | Remicade (2011) | CTP13-BS1 (2015, 2020) |
| DS Quality control | | | |
| DS Specification parameters or limits | - Change to the specification parameters or limits of DS. | Remicade (2003, 2005, 2007), Humira (2003, 2004, 2005, 2006, 2007, 2008), Enbrel (2006, 2008, 2009) | -- |
| | - Widening of the approved specification limits, which may have a significant effect on the overall quality of DS or DP. | -- | CTP13-BS1 (2015) |

**Supplementary table S1 (continued)**: Details on the nature of high-risk manufacturing changes for each type of manufacturing changes that were introduced to tumor necrosis TNF-α inhibitors between August 1999 and May 2021.

| Type of MCs | Description of the nature of high-risk MCs | Originators (year(s)) | Biosimilars (year(s)) |
|---|---|---|---|
| | - Deletion of specification parameter which may have a significant effect on the quality of the DS or DP. | Enbrel (2018) | CTP13-BS1 (2020) |
| | - Change outside the approved specification limits or range of DS. | Enbrel (2018) | CTP13-BS1 (2016, 2019, 2020), SB2-BS2 (2017, 2019) |
| DS Analytical test procedures | -- | -- | -- |
| DS Packaging system | | | |
| DS Primary (immediate) packaging | -- | -- | -- |
| DS Stability | | | |
| DS Shelf-life | -- | -- | -- |
| DS Storage conditions | -- | -- | -- |
| DS Stability protocol | -- | -- | -- |
| Drug product (DP) | | | |
| DP Composition | | | |
| DP Strength | - Change or addition of a new strength/ potency | Enbrel (2005, 2011), Humira (2017) | SB4-BS1 (2017), SB5-BS2 (2019), CTP13-BS1 (2019), FKB327-BS5 (2020), GP2017-BS4 (2020), |
| DP Formulation | - Replacement of excipient with a comparable excipient. | Remicade (2001, 2003) | -- |
| | - Change or addition of a new pharmaceutical form. | Enbrel (2006) | CTP13-BS1 (2019) |
| DP Manufacturing | | | |
| DP Manufacturing site | -- | -- | -- |
| DP Manufacturing process | - Substantial change to the manufacturing process of the DP may have a significant impact on the quality, safety, or efficacy of the medicinal product. | Humira (2010) | -- |
| | - Change in the manufacturing process requires to require an assessment of comparability. | Remicade (2011, 2014), Humira (2011, 2015), Enbrel (2015), | CTP13-BS1 (2018) |
| DP Batch size | -- | | |

**Supplementary table S1 (continued)**: Details on the nature of high-risk manufacturing changes for each type of manufacturing changes that were introduced to tumor necrosis TNF-α inhibitors between August 1999 and May 2021.

| Type of MCs | Description of the nature of high-risk MCs | Originators (year(s)) | Biosimilars (year(s)) |
|---|---|---|---|
| DP In-process test or limits | - Widening of the approved in-process test limits, which may have a significant effect on the overall quality of DP. | -- | CTP13-BS1 (2020), SB2-BS2 (2020) |
| Excipients Quality control | | | |
| Excipients Specification parameters or limits | -- | -- | -- |
| Excipients Analytical test procedures | -- | -- | -- |
| DP Quality control | | | |
| DP Specification parameters or limits | - Change outside the approved specification limits or range of DP. | -- | CTP13-BS1 (2015, 2020), SB2-BS2 (2017). FKB327-BS5 (2020) |
| | - Deletion of a specification parameter, which may have a significant effect on the overall quality of DP. | Remicade (2020) | -- |
| DP Analytical test procedures | - Replacement of biological test methods or a method using a biological substance. | Remicade (2013) | -- |
| DP Packaging system | | | |
| DP Primary (immediate) packaging | - Change in shape or dimensions concerns fundamental part of immediate packaging, which may have a significant impact on the delivery, use, safety, or stability of the DP. | Enbrel (2015) | ABP501-BS1 (2019) |
| DP Secondary (nonfunctional) packaging | -- | -- | -- |
| DP Stability | | | |
| DP Shelf-life | -- | -- | -- |
| DP Storage conditions | -- | -- | -- |
| DP Stability protocol | -- | -- | -- |

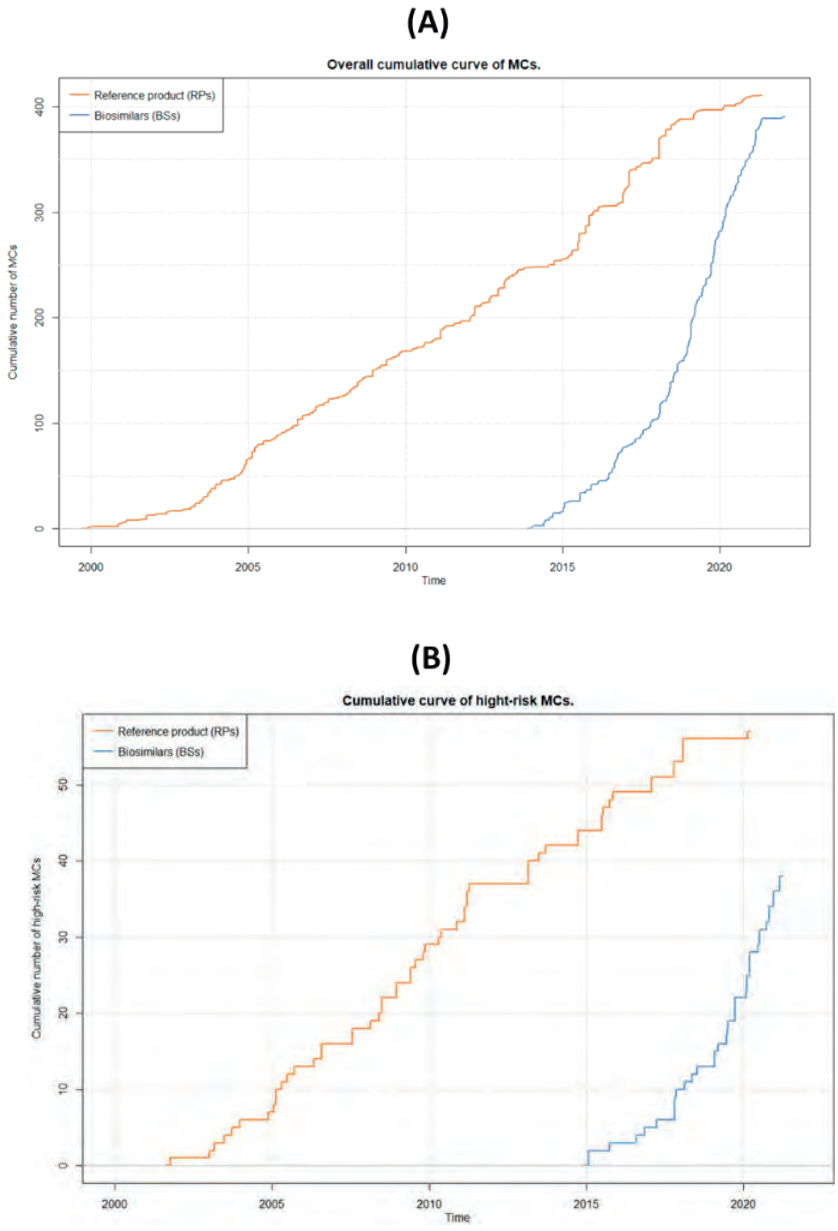
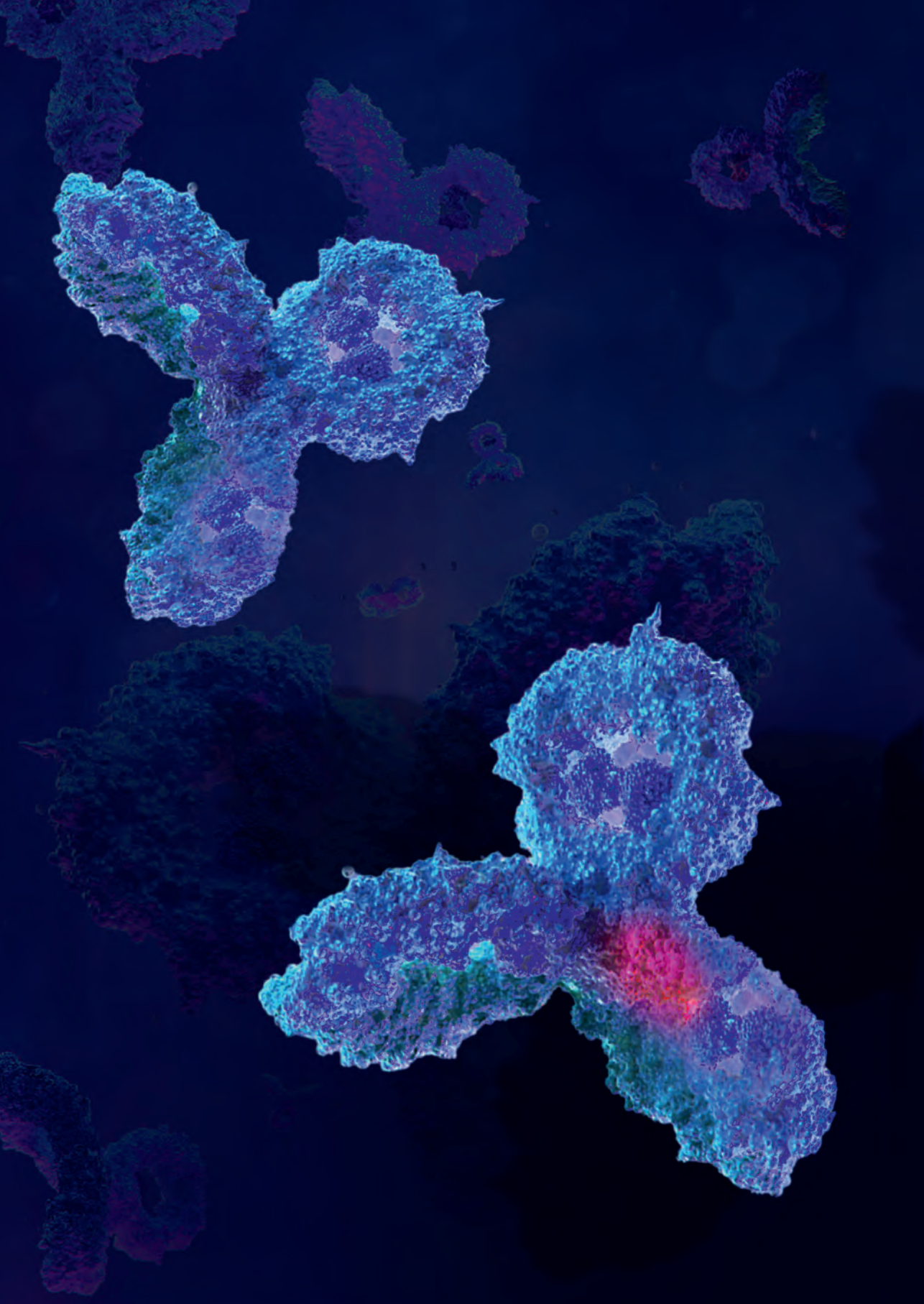



**Figure S1:** Cumulative curves of post-approval manufacturing changes since the date of marketing authorization, originators (Reference products; RPs) and biosimilars (BSs). Overall cumulative curve of manufacturing changes (A), and cumulative curve of high-risk manufacturing changes (B).



# Chapter 3.2

## Post-approval quality-related regulatory actions of biopharmaceuticals authorized in the United States and European Union between 1995 and 2019

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(Submitted)

Author's contribution: AMA designed the study, performed data management, conducted data curation, analysis, and validation, prepared the first- draft of the manuscript, and implemented significant contribution from co-authors up to the final version. Throughout the process, AMA asked for and implemented input and feedback from supervision team and co-authors, who performed critical review of the manuscript and provided significant contributions to the study.

## Abstract

The quality of biopharmaceuticals is carefully monitored to ensure safety and efficacy throughout the entire life cycle. Quality defects can lead to regulatory actions (RAs). We conducted a retrospective analysis to determine the type (letters, recalls, marketing withdrawals), content and frequency of quality-related RAs for recombinant DNA biopharmaceuticals that had been approved in the EU and the US between January 1995 and December 2019, from their market authorization date up to August 2021. We identified 67 quality-related RAs for 41 (12.5%) of the 324 biopharmaceuticals, all for originators and none for biosimilars. Two-third were letters that had been mainly issued for manufacturing issues, such as good manufacturing practice deviations that affected the product in general, and one-third were recalls that had been mainly issued for specification issues, such as particulate matters that affected specific batches. The type of actions that had to be taken by healthcare professionals (HCPs) depended on the nature of the quality defects. Regulatory letters often specify actions such as restrict, monitor, switch, and inform at patient level to counter the potential shortage, whereas regulatory recalls often specify HCP actions such as check, handle and recall at product level to avoid negative implications for patient care. Manufacturers and regulators should continue efforts that reduce the occurrence of any quality defects that may impact patient care. Further studies are needed to assess the effectiveness and impact of the recommended HCP actions on clinical practice and patient care.

## Introduction

Biopharmaceuticals have changed the prognosis of many difficult-to-treat or incurable diseases and have, thus, became an integral part of the therapeutic arsenal [1, 2]. Since the first recombinant insulin was approved in 1982 [3], more than 300 biopharmaceuticals, including hormones, growth factors, enzymes, clotting factors, monoclonal antibodies and fusion proteins, have entered the markets of the European Union (EU) and the United States (US) [4-10]. Biopharmaceuticals currently account for approximately 30% of all approvals of drugs that contain a novel active substance [11]. The currently approved biopharmaceuticals include more than 100 biosimilars that have been approved in the EU and/or the US after the expiration of the patent and exclusivity rights of 17 biopharmaceuticals.

Biopharmaceuticals have complex production processes and small changes in the manufacturing process can affect the quality characteristics of the drug substance, for example, changes in the quality attributes (QAs) of the active substance and/ or drug product (DP), including changes in the formulation, and primary and secondary packaging. Changes in quality characteristics do not necessarily have an impact on the clinical performance of a biopharmaceutical. However, changes in so-called critical quality attributes (CQAs) could potentially influence safety, immunogenicity and/or efficacy [12, 13]. This is illustrated, for example, by the increased number of incidences of pure red cell aplasia (PRCA) in patients who received batches of Eprex® after a change in formulation in 1998, when human serum was replaced by polysorbate 80 and glycine to reduce the risk of contamination with viral infections associated with human serum [14, 15]. It was found that the new formulation was less stable, resulting in aggregates that induced PRCA. Therefore, biopharmaceuticals have to be careful controlled, and monitoring for product quality and manufacturing consistency is critical to ensure the safety and efficacy of the products throughout their lifecycle [17, 24].

An approved biopharmaceutical with a quality defect is an authorized medicine that fails to meet the quality standards because of an unintentional or inattentive quality defect that may lead to undesirable clinical problems. Studies have shown that, in general, the incidences of quality defects in medicines varies between countries and are less often reported in highly regulated markets [16-22]. Quality defects can be identified by the internal quality management system of the manufacturer during batch release testing [24], through Good Manufacturing Practices (GMP) inspections [25, 26], through assessment of manufacturing changes or deviations [27], and through post-marketing surveillance monitoring [28]. It is the responsibility of the manufacturer to report a quality defect to the regulatory authorities as soon as it is identified, which may occur before or after the product or specific batch has been released to the market. In turn, regulators can issue post-approval regulatory actions (RAs) to inform health care professionals

(HCPs) of a quality defect along with the recommended actions to protect patients. The RAs can range from regulatory letters that inform HCPs of a quality defect to regulatory recalls or marketing withdrawals, depending on the potential clinical consequences of the quality defects.

The majority of studies that have explored the outcome of post-approval RAs mainly focused on the clinical benefits and risks of the safety and efficacy of the medicines, including small molecule drugs and biopharmaceuticals [29-36]. Studies that explored RAs related to quality defects of biopharmaceuticals are scarce in the literature. Ebbers et al. assessed the number of and reasons for regulatory recalls issued in the US between 2003 and 2013 for biopharmaceuticals and small molecule drugs [22]. In contrast to 1,751 recalls for small molecule drugs, only 41 recalls were related to biopharmaceuticals, and none of these were associated with unexpected clinical problems. The recalls were mostly related to defective devices and containers and packaging and labeling errors, which were unrelated to the complexity of the manufacturing process for biopharmaceuticals. The study by Ebbers et al. focused on a specific region and assessed a single type of RA (recalls) and, most importantly, could not identify the product associated with the RA because they did not know what they were. Information on the specific biopharmaceuticals for which a quality-related RA was issued will add to the knowledge of the complexity of the biopharmaceutical and the potential for a quality defect. In addition, Ebbers et al. did not assess the recommendations and actions required from the HCPs to protect the patients. Other studies that assessed the quality and applicability of information on patient monitoring in clinical practice have reported that this information was insufficient in various regulatory documents, such as direct healthcare professional communications (DHPCs) and summary of product characteristics documents (SPCs) [23, 37, 38]. However, these studies do not provide information on recommendations or actions for HCPs in the event of post-approval quality-related RAs.

Therefore, our study aims to describe the type, content, frequency and timing of post-approval quality-related RAs, including the nature of the underlying quality defects and the type of HCP actions required for biopharmaceuticals approved in the EU and the US from 1995 to 2019.

## Methods

### Study design

A retrospective study was performed using two cohorts of biopharmaceuticals that were approved in the EU (cohort EU) and the US (cohort US) between 1 January 1995 and 31 December 2019. This study period was selected to coincide with the establishment of the European Medicine Agency (EMA) in 1995 as well as the Food and Drug Administration

(FDA) making information on RAs publicly accessible from 1995 onwards. The follow-up of each biopharmaceutical was from the date of marketing authorization in the region until 31 August 2021 or the date of market withdrawal, whichever came first. In this study, we defined biopharmaceuticals as a biological medicine that contain a therapeutic protein that was produced by recombinant DNA or hybridoma technology as an active biological substance. Vaccines and naturally extracted biological medicines, such as plasma-derived (blood) and urine-derived products; products for further manufacturing and transfusion and transplantation; allergenic products; advanced therapy medicinal products (ATMPs), including cell and gene-based products; and biopharmaceuticals used for diagnostic testing were excluded.

Information on biopharmaceuticals that had been approved in the EU and the US were retrieved from the EMA website (https://www.ema.europa.eu/en: access date September 2021) and the Purple Book on the FDA website (https://purplebooksearch.fda.gov/: access date September 2021), respectively. Biosimilars that contain the same active biological substance as the corresponding originator and are marketed under different trade names by the same or different pharmaceutical companies were all included separately (for a list of included biopharmaceuticals, see supplementary file). In the EU, the EMA publishes the European Public Assessment Reports (EPARs) that contain a list of EU-approved human medicines, including biopharmaceuticals that have been approved via the centralized procedure at the EMA and granted marketing authorization from the European Commission (EC). In the US, the FDA publishes a Purple Book that contains a list of biopharmaceuticals that have been approved by the Center for Biologics Evaluation and Research (CBER) and the Center for Drug Evaluation and Research (CDER) in the US.

Biopharmaceuticals have been classified into classes such as monoclonal antibodies, fusion proteins, clotting factors, enzymes, hormones and growth factors. Additional product characteristics include the product type (originator or biosimilar), the approval region (EU only, US only, both EU and US), approval period (1995 – 2004, 2005 – 2012, 2013 – 2016, 2017 – 2019), protein type (glycoprotein or non-glycoproteins) and pharmaceutical dosage forms (powder, a solution, a combination of powder and solution or others, for example, gel).

### Identification of quality-related regulatory actions

A quality-related RA is defined as a regulatory communication that is issued by regulators due to a quality defect that could either affect the drug in general or only one or more specific batches of a biopharmaceutical. The quality-related RAs can be communicated through regulatory letters, including direct healthcare professional communications (DHPCs), dear healthcare professional letters (DHPLs), recalls or marketing withdrawals for both the EU and the US cohorts. For the EU cohort, the quality-related RAs were identified from the Medicines Healthcare Products Regulatory Agency (MHRA) data-

base in the United Kingdom (access date September 1, 2021) from 1995 until the end of follow-up. Information identified from the MHRA was crosschecked with data obtained from the medicine evaluation board (MEB) in the Netherlands, for the time period January 1997 to August 2021 by the (co)authors (AMA, TJG, and HG) as a validity check. This crosschecking resulted in the capture of two additional quality-related RAs that were missing from the MHRA database, both concerning follow-up letters for letters previously issued. For the US cohort, the quality-related RAs were identified from MedWatch, the Center for Biologics Evaluation and Research (CBER) and the Center for Drug Evaluation and Research (CDER) on the official FDA website (access date 1 September 2021). If a biopharmaceutical has been approved in the EU and the US and had received a quality-related RA only in one of these regions, a manual crosschecking with the use of the product brand name(s) was conducted by the first author (AMA) to avoid missing any quality-related RAs. This manual crosschecking did not produce any potentially missed quality-related RAs.

## Data extraction and validation

The first author (AMA) identified quality-related RAs and extracted information on the relevant biopharmaceutical(s). When it is unclear whether an RA was related to a quality issue and should be included, the issue was discussed by the (co)authors (AMA, TJG, TCE, and HG). The study outcomes were independently coded by two of the authors (AMA and TCE). Discrepancies were resolved by discussion to reach a consensus agreement (AMA and TCE).

## Outcomes

The outcomes that were assessed in this study were the type, content, frequency and timing of quality-related RAs, including the nature of the underlying quality defect and the type of required HCP action.

### *Type of quality-related regulatory actions*

The type of quality-related RA was categorized into regulatory letters, recalls, or marketing withdrawals issued by EU and US regulators due to a quality defect. In the EU and the US, the most relevant regulatory actions are communicated through 1) regulatory letters, including the DHPCs (EU) and DHPLs (US), 2) regulatory recalls (EU and US) or 3) marketing withdrawals (EU and US). In the EU, recalls fall under the remit of the national competent authorities that are responsible for their markets. The EMA does not have information regarding the recalls at individual EU member state level. DHPCs were classified as a regulatory recall if "recall product or batches" was explicitly mentioned. The type of quality-related RA was also categorized into product in general or specific batches, which was determined based on information provided in the RA. When the RA

provided information on specific batches (e.g., batch number(s)), the quality-related RA was defined as batch-related. In some cases, the mention of specific numbers of batches may be used as a way to identify a quality defect that may affect a product in general, such as counterfeiting. If this was the issue, the quality-related RA was categorized as product in general.

*Content of the quality-related regulatory action*

The content of quality-related RAs was assessed in terms of the nature of the underlying quality defects and the type of required HCP actions. The nature of the underlying quality defects was divided into seven main categories with 14 sub-categories (box 1). This classification was inspired by the categories of the quality defects reported by *Ebbers H et.al.,* but with some additions [22].

The type of required HCP actions was divided into three action levels: product level, patient level and other, all of which were further divided into different subtypes (box 2). This classification was developed by our team through initial screening of the statements in the identified quality-related RAs to describe the required actions to be taken by the HCPs. Each statement was assigned to a specific type of HCP action and was further developed to reflect the actions in clinical practice and pharmacy. The types of required HCP actions are not mutually exclusive, and a quality-related RA could include one or more types of required HCP action.



*Frequency and timing of quality-related regulatory action*

The frequency of quality-related RAs was defined as the number of the quality-related RAs stratified by the type of RA. If multiple quality-related RAs had been issued for the same biopharmaceutical at different moments during follow-up, these were defined as separate RAs. When a quality-related RA involved multiple biopharmaceuticals, it was regarded as a single RA, unless the action occurred at different time points. Multiple quality-related RAs issued for a biopharmaceutical due to the same or different defect or a follow up of previously communicated quality defects were defined as separate RAs. The timing of the quality-related RAs was defined according to the calendar date they were issued by regulators (event) relevant to the date of approval.

**Box 1:** The nature of underlying quality defects.

| Category of quality defects | The nature of underlying quality defects | Definition of the quality defects<br>*Examples of potential clinical consequences "Direct quotes."* |
|---|---|---|
| **Adulteration** | **Counterfeiting** | **A product or batch that does not meet the quality standard of the regulatory authorities or infringes the trademark law.**<br>*"It cannot be assumed that the counterfeit product is either safe or effective."* |
| | **Falsification** | **A fake product or batch that mimics the real medicine.**<br>*"The falsified product cannot be considered effective."* |
| **Contamination** | **Chemical contamination** | **A product or batch contaminated with any chemical substance(s) that is unrelated to the drug substance or drug product.**<br>*"No example in our dataset because there was no quality-related regulatory action due to chemical contamination."* |
| | **Microbial contamination** | **A product or batch that is accidently contaminated with (non) infectious microbes.**<br>*"The recall has been initiated due to concerns about potential microbial contamination of the alcohol products with Bacillus cereus, which could potentially lead to life-threatening infections."* |
| **Manufacturing** | **Good Manufacturing Practices (GMP) deviations** | **The occurrence of an unexpected event at a manufacturing site or non-compliance to procedures or specifications outlined in the current GMP.**<br>*"There is a risk of delays of fulfilling orders and of potential interruption "* |
| | **Capacity** | **An occurrence of a temporary event in the process or material that impacts production capacity**<br>"The supply shortage is related to a temporary production capacity issue." |
| | **Unclear manufacturing issue** | **The nature of the underlying manufacturing issues is unclear**<br>*"Due to unexpected delays in the release of three lots intended for global distribution product supply may continue to be restricted."* |
| **Product composition** | **Formulation** | **A change in the product composition, including drug substances, as a result of a new process or the introduction of different excipients or different dosage forms.**<br>*"ReFacto AF is contraindicated in patients with a known hypersensitivity to any of the constituents of the preparation or hamster proteins. As with any intravenous protein products, allergic type hypersensitivity reactions are possible. Manifestations of hypersensitivity reactions may include hives, generalized urticaria, anaphylaxis, hypotension, wheezing, and tightness of the chest."* |

**Box 1 (Continued):** The nature of underlying quality defects.

| Category of quality defects | The nature of underlying quality defects | Definition of the quality defects<br>*Examples of potential clinical consequences "Direct quotes."* |
|---|---|---|
| **Specification** | **Out-of-specification** | **A test result of a quality attribute of a drug substance or drug product that is outside the predefined specification or acceptance criteria set by manufacturer or the quality and regulatory standards.**<br>*"Injection of NovoMix®30, with a content of around 50% of the intended dosage, may lead to hyperglycemia to some degree in patients with Type 1 or Type 2 diabetes mellitus. Injection of NovoMix®30, with a content of 150% of the intended dosage, may in worse case lead to severe hypoglycemia."* |
| | **Particulate matters** | **Mobile, undissolved, small to subvisible particles, other than gas bubbles, unintentionally present in the drug product.**<br>*"Infused foreign particles would most likely remain close to the injection site. This could cause local venous damage or injection site reactions such as pain or local irritation."* |
| **Packaging** | **Defective primary packaging** | **Damage to part of the primary packaging with direct and immediate contact of the drug product.**<br>*"There is a risk that damaged vials may lead to a loss in sterility, which can cause infections in patients."* |
| | **Secondary packaging and labeling errors** | **Unintended errors in the packaging of a drug product in incorrect secondary packaging or errors such as missing information or an incorrect name or strength printed on the secondary packaging and labeling.**<br>*"If the patient did reconstitute Myalepta with 0.6 mL WFI, they would be at risk of administering a dose of metreleptin with a concentration higher than 5 mg/mL. This may cause adverse effects, such as injection site reactions. The patient may also administer a higher dose than intended, up to a dose of 5 mg. "* |
| | **Defective administration device** | **A technical or physical defect in the administration device.**<br>*"A technical or physical defect in the administration device could potentially result in shortage."* |
| **Stability** | **Stability and storage issues** | **The drug product was not stored in compliance with the recommended storage conditions**<br>*"Recall initiated by the manufacturer because products were stored at temperatures below the storage requirements. If product samples are exposed to temperatures below 32°F, it could cause a lack of efficacy and damage to the cartridge and pen-injectors. If product from an improperly stored vial, cartridge or pen-injector is used, there is a risk that the patient may not receive the correct dosage of medicine as intended, which may lead to hyperglycemia or hypoglycemia, resulting in adverse health consequences ranging from limited to life-threatening. "* |

**Box 2:** Types of healthcare professional actions.

| Action level | Type of action | When the required actions to be taken by the HCPs are stated in the communication |
|---|---|---|
| **Product level** | **Check** | Visual inspection and check |
| | **Handle** | Use filter to remove particulate matters |
| | **Recall** | Quarantine and return affected product or batch(es) |
| | | Recall affected batches from pharmacies before dispensing or from patients if already dispensed |
| **Patient level** | **Ensure** | Ensure that the correct strength is prescribed and dispensed |
| | | Check dispensing records to identify patients to whom the affected product has been dispensed |
| | | Refer to (the corrected) prescription information |
| | **Inform** | Educate, train and inform patients to minimize potential clinical consequences of the quality defect |
| | **Monitor** | The recommendation to closely monitor patients for changes in hemoglobin, platelets and chitotriosidase levels, as appropriate, at baseline and once every two months thereafter, remains. |
| | **Switch** | Switch to alternative presentations or other treatments |
| | **Restrict** | Reduce treatment frequency or adjust dose and preparation |
| | | Follow (temporary) treatment recommendation and restriction or instruction |
| | | Limit available treatment for patients already started on the treatment |
| | | No new patient should start treatment (restriction use for new patients) |
| **Others** | **Report** | Report suspected adverse drug reactions or quality problems |
| | | Contact regulatory authority |
| | **No actions** | The regulatory action did not require any actions to be taken by HCPs |
| | **Unclear action** | The regulatory action did not report on whether there are required actions to be taken by HCPs |

### Data analysis

The number of biopharmaceuticals stratified by product characteristics in the EU and US cohorts was compared with descriptive statistics. The number of biopharmaceuticals that received quality-related RAs were counted, and the associated products were identified. The incidences of quality-related RAs were calculated as a simple frequency. The frequency of quality-related RAs was compared with the type of RA (letters versus recalls, product in general versus specific batches) to assess the content of the RA in relation to the nature of the underlying quality defects and type of required HCP actions. The mean time to the (first) quality-related RA was calculated by summing the time between the date of regulatory approval and an RA, divided by the number of quality-related RAs issued for a biopharmaceutical. The statistical analyses were conducted with the statistical software package SPSS version 27 (SPSS Inc, Chicago, Illinois).

## Results



### Characteristics of biopharmaceuticals

A total of 511 biopharmaceuticals were approved between January 1995 and 31 December 2019 (275 in the EU, 236 in the US), of which 187 were approved in both regions during the study period, which means that there was a total of 324 unique biopharmaceuticals. The median follow-up time was 7.2 years (range = 0.5–26.3). The product characteristics in the two cohorts were similar, except for the proportion of approved biosimilars, which was higher in the EU (22%) than in the US (12%) (Table 1).

During the study follow-up period, 67 quality-related RAs were issued for 41 (34 approved in both regions, four in the EU only and three in the US only) of the 324 unique biopharmaceuticals (Figure S1). The 41 unique biopharmaceuticals were all originator products, and not a single biosimilar had received a quality-related RA. The therapeutic protein classes of the 41 unique biopharmaceuticals were hormones and growth factors (n = 24), mAbs and fusion proteins (n = 9) and enzymes and clotting factors (n = 6) (Table S1).

**Table 1:** Characteristics of 511 biopharmaceuticals approved in the EU and the US between 1995 and 2019.

| Product characteristics | EU cohort (N= 275) | US cohort (N= 236) |
|---|---|---|
| **Approval region** | | |
| Both regions | 187 (68.0%) | 187 (79.2%) |
| EU only | 88 (32.0%) | - |
| US only | - | 49 (20.8%) |
| **Approval period** | | |
| 1995–2004 | 68 (24.7%) | 65 (27.5%) |
| 2005–2012 | 62 (22.5%) | 54 (22.9%) |
| 2013–2016 | 61 (22.2%) | 60 (25.4%) |
| 2017–2019 | 84 (30.5%) | 57 (24.2%) |
| **Product type** | | |
| Originators | 214 (78.0%) | 208 (88.0%) |
| Biosimilars | 61 (22.0%) | 28 (12.0%) |
| **Therapeutic protein class** | | |
| Monoclonal antibodies | 108 (39.3%) | 98 (41.5%) |
| Growth factor | 58 (21.1%) | 39 (16.5%) |
| Hormones | 46 (16.7%) | 35 (14.8%) |
| Clotting factor | 30 (10.9%) | 29 (12.3%) |
| Enzymes | 23 (8.4%) | 23 (9.7%) |
| Fusion protein | 10 (3.6%) | 12 (5.1%) |
| **Protein type** | | |
| Glycosylated protein | 181 (65.8%) | 157 (66.5%) |
| Non-glycosylated protein | 94 (34.2%) | 79 (33.5%) |
| **Pharmaceutical dosage form** | | |
| Solution | 174 (63.3%) | 136 (57.6%) |
| Powder | 85 (30.9%) | 86 (36.4%) |
| Solution and powder | 15 (5.5%) | 12 (5.1%) |
| Others | 1 (0.4%) | 2 (0.8%) |

## Types of quality-related regulatory action

The type of quality-related RAs most often involved regulatory letters (n = 45; 67.0%, of which 37 EU and eight US) and, to a lesser extent, regulatory recalls (n = 22; 33.0%, of which 11 EU and 11 US). There were no market withdrawals due to quality defects. The quality-related RAs mostly concerned the product in general (60.0%) rather than specific batches (40.0%) (Table 2).

## Content of the quality-related regulatory action

*Nature of underlying quality defects*

The most frequent category of quality defects for biopharmaceuticals were related to manufacturing (40.4%), followed by specification (25.4%) and packaging (20.8%). The quality defects related to the categories of adulteration, contamination, product composition and stability were less frequently observed for biopharmaceuticals. The regulatory letters were mainly issued for manufacturing issues and, more specifically, GMP deviations. In a few cases, regulatory letters were issued because of particulate matters (5%). In contrast, the regulatory recalls were mostly issued because of specification issues (57.7%) that involved both out-of-specification and particulate matter. The quality-related RAs regarding product in general were often issued because of manufacturing issues (65.0%), while the quality-related RAs regarding specific batches were issued because of specification issues (51.8%). Interestingly, there were no quality-related RAs issued because of chemical contamination or formulation issues (Table 2).



*Types of required healthcare professional actions*

All quality-related RAs reported statements related to at least one action required by HCPs. None of those were classified as unclear actions and none indicated that "no action" was required from HCPs. A substantial variation in frequency was observed between the types of required HCP actions, ranging from 6% for the action "ensure" to 82.1% for the action "report." The regulatory letters often included HCP actions related to the patient level, whereas the regulatory recalls often included HCP actions related to the product level. The most frequent types of HCP actions in the regulatory letters were restrict (42.2%) and monitor (35.6%), followed by switch and inform (both 17.8%), which were often recommended to counter manufacturing issues. The most frequent type of required HCP action in regulatory recalls was the action "recall" (86.4%), which was often recommended to counter specification issues. In limited cases, there was no explicit statement for the HCP action "recall." In two regulatory recalls, one was issued because of microbial contamination of the alcohol prep pad supplied with drug product and the other was issued because of a defective administration device associated with slow or incomplete delivery of the content (Table 3).

**Table 2:** The nature of the underlying quality defects that resulted in quality-related regulatory actions of biopharmaceuticals approved in the European Union and the United States between 1995 and 2019.

| Nature of underlying quality defects | All (n = 67) | Regulatory letters (n= 45) | Regulatory recalls (n = 22) | Product in general (n = 40) | Specific batches (n = 27) |
|---|---|---|---|---|---|
| **Adulteration** | | | | | |
| Counterfeiting | 3 (4.5%) | 3 (6.7%) | 0 (0.0%) | 3 (7.5%) | 0 (0.0%) |
| Falsification | 1 (1.5%) | 0 (0.0%) | 1 (4.5%) | 0 (0.0%) | 1 (3.7%) |
| **Contamination** | | | | | |
| Chemical contamination | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| Microbial contamination | 2 (3.0%) | 0 (0.0%) | 2 (9.1%) | 2 (5.0%) | 0 (0.0%) |
| **Manufacturing** | | | | | |
| GMP deviations | 16 (23.9%) | 15 (33.3%) | 1 (4.5%) | 15 (37.5%) | 1 (3.7%) |
| Capacity | 5 (7.5%) | 5 (11.1%) | 0 (0.0%) | 5 (12.5%) | 0 (0.0%) |
| Unclear manufacturing issue | 6 (9.0%) | 6 (13.3%) | 0 (0.0%) | 6 (15.0%) | 0 (0.0%) |
| **Product composition** | | | | | |
| Formulation | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| **Specification** | | | | | |
| Out-of-specification | 4 (6.0%) | 0 (0.0%) | 4 (18.1%) | 0 (0.0%) | 4 (14.8%) |
| Particulate matters | 12 (17.9%) | 5 (11.1%) | 7 (31.8%) | 2 (5.0%) | 10 (37.0%) |
| **Packaging** | | | | | |
| Defective primary packaging | 7 (10.4%) | 4 (8.9%) | 3 (13.6%) | 4 (10.0%) | 3 (11.1%) |
| Secondary packaging and labeling errors | 3 (4.5%) | 3 (6.7%) | 0 (0.0%) | 1 (2.5%) | 2 (7.4%) |
| Defective administration device | 4 (6.0%) | 3 (6.7%) | 1 (4.5%) | 2 (5.0%) | 2 (7.4%) |
| **Stability** | | | | | |
| Stability and storage issue | 4 (6.0%) | 1 (2.2%) | 3 (13.6%) | 0 (0.0%) | 4 (14.8%) |

## Frequency and timing of quality-related regulatory action

Of the 41 unique biopharmaceuticals that received quality-related RAs, two thirds (n = 27, 65.0%) had a single quality-related RA (n= 27 RAs), and the remaining one-third (n= 14, 35.0%) had more than one quality-related RAs (n = 40 RAs) during the study follow-up period. Twenty-seven quality-related RAs were issued for 27 unique biopharmaceuticals for different underlying quality defects, mainly related to specification (n = 9), manufacturing (n = 6), packaging (n = 6), stability (n = 3), contamination (n = 2) and adulteration (n =1). Out of 40 quality-related RAs issued for 14 unique biopharmaceuticals, more than half (26 RAs) were follow-up RAs issued for the same quality defects, mainly related to manufacturing (n = 20), packaging (n = 4), and adulteration (n = 2) (Table S1). The mean time from marketing authorization to the issuing of a quality-related RAs was 9.5 years (SD = 6.7 years), and 60% of the quality-related RAs were issued within 10 years after approval.

Fifty-nine of the 67 quality-related RAs were issued for the 34 biopharmaceuticals that had been approved in both the EU and the US. Of the 59 quality-related RAs, 37 were only issued in the EU, 12 were only issued in the US and 10 were issued in both regions. More than half of the 37 RAs issued in the EU were follow-ups of previously issued RAs for the same quality defects and included the same types of required HCP actions. The 10 quality-related RAs issued in both regions concerned five out of 34 biopharmaceuticals approved in both regions and involved the same underlying quality defects in both regions, but the types of HCP actions required to deal with the quality defects differed slightly between the EU and the US. The majority of these 10 quality-related RAs were issued in both regions within the same 2 weeks (Table 4).

**Table 3:** Type of required healthcare professional actions described in the quality-related regulatory actions toward biopharmaceuticals approved in the European Union and United States between 1995 and 2019.

| Type of HCP actions | All (n = 67) | Regulatory letters (n= 45) | Regulatory recalls (n = 22) |
|---|---|---|---|
| **Product level** | | | |
| Check | 19 (28.4%) | 13 (28.9%) | 6 (27.3%) |
| Handle | 12 (17.9%) | 9 (20.0%) | 3 (13.6%) |
| Recall | 27 (40.3%) | 8 (17.8%) | 19 (86.4%) |
| **Patient level** | | | |
| Ensure | 4 (6.0%) | 4 (8.9%) | 0 (0.0%) |
| Inform | 10 (14.9%) | 8 (17.8%) | 2 (9.1%) |
| Monitor | 18 (26.9%) | 16 (35.6%) | 2 (9.1%) |
| Switch | 8 (11.9%) | 8 (17.8%) | 0 (0.0%) |
| Restrict | 19 (28.4%) | 19 (42.2%) | 0 (0.0%) |
| **Others** | | | |
| Report | 55 (82.1%) | 37 (82.2%) | 18 (81.1%) |
| No action required from HCPs | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| Unclear actions for HCPs | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |

**Table 4:** The quality defects and the type of required healthcare professional actions for biopharmaceuticals approved in both regions that received quality-related regulatory action in both regions.

| Product name (active substance) | Date of regulatory action (region) | Underlying nature of quality defects (region) | Type of required HCP actions (region) |
|---|---|---|---|
| Herceptin® (*trastuzumab*) | 21-11-2006 (EU)<br>28-6-2008 (US) | Defective primary packaging (both regions) | "check," "recall," "report" (EU)<br>"check," "report" (US) |
| Cerezyme® (imiglucerase), | 1-12-2009 (EU)<br>13-11-2009 (US) | Particulate matters (both regions) | "check," "handle," "recall," "report" (EU)<br>"check," "handle," "recall," "inform," "monitor," "report" (US) |
| Fabrazyme® (*agalsidase beta*), | 1-12-2009 (EU)<br>13-11-2009 (US) | Particulate matters (both regions) | "check," "handle," "recall," "report" (EU)<br>"check," "handle," "recall," "inform," "monitor," "report" (US) |
| Thyrogen® (*thyrotropin alfa*) | 1-12-2009 (EU)<br>13-11-2009 (US) | Particulate matters (both regions) | "check," "handle," "recall," "report" (EU)<br>"check," "handle," "recall," "inform," "monitor," "report" (US) |
| | 28-7-2011(EU)<br>24-5-2010 (US) | Unclear manufacturing issue (both regions) | "restrict" (EU)<br>"restrict" (US) |
| Helixate NexGen® (*octocog alfa*), | 11-8-2016 (EU)<br>10-8-2016 (US) | Stability and storage condition (both region) | "recall" (EU)<br>"recall," "report" (US) |

## Discussion

The present study identified 67 quality-related RAs issued for 41 (12.5%) out of 324 unique biopharmaceuticals approved between 1995 and 2019 in the EU and the US. All the quality-related RAs were issued for originators, and more than half involved (26 out of 41) hormones and growth factors. The type of quality-related RAs mainly involved letters related to products in general and recalls of specific batches to a lesser extent. None of the quality-related RAs were related to marketing withdrawals. The regulatory letters were often issued for manufacturing issues, while regulatory recalls were mainly issued due to specification issues. The HPC required actions in regulatory letters were most often related to restrict and monitor, followed by switch and inform at patient level, whereas the action recall was most frequently required in regulatory recalls. Two thirds of the 41 unique biopharmaceuticals (n = 27, 65.0%) received a single quality-related RA during the study follow-up period, and the remaining one third (n = 14, 35.0%) received more than one RA, but more than half of these were follow-up RAs issued for the same quality defects and included the same type of required HCP actions. The majority (60.0%) of the quality-related RAs were issued within 10 years after approval of the biopharma-

ceuticals, which may suggest that the experience on manufacturing may have a partial role in the occurrence of a quality defect.

Our finding that quality-related RAs were issued for one out of every eight biopharmaceuticals during their lifecycle is lower than what was reported by Giezen et. al., who reported that RAs were issued for a quarter of the all biopharmaceuticals due to safety concerns [30]. Knowledge of the safety and efficacy increase as the use in clinical practice increases, and this aligns with the way the pharmacovigilance systems operate. Our result shows that quality-related RAs were only issued for originators and not for biosimilars (at least during our follow up), which does not mean that this is always the case, and further investigation may be needed for a solid conclusion. However, the goal of the manufacturer, whether originator or biosimilar, is always to ensure consistency and prevent quality defects, because the cost of quality defects of biopharmaceuticals can be catastrophic in different dimensions of patient care, and it can also indicate a disastrous failure of the manufacturer's quality plan, leading to economical and reputational damage.



Quality defects can also compromise critical quality attributes of biopharmaceuticals and potentially impact clinical outcomes and patient care. Predicting the impact that a quality defect may have on clinical outcomes and patient care is challenging and prevent quality defects remains a key regulatory strategy. Our study shows that the number of quality-related RAs for biopharmaceuticals is low, which could be partly related to post-marketing quality surveillance and manufacturing control of biopharmaceuticals [39]. Over recent decades, several regulatory strategies have been developed for in-process control, quality by design and quality indicators to identify quality defects and prevent biopharmaceuticals with quality defects from being released to the market [40-44]. Moreover, the advancement of analytical methods and instrumentation allow for more precise characterization and control of the quality of biopharmaceuticals; for example, the current generation of mass spectrometry is one million times more sensitive than previously used analytical techniques to characterize complex structural and functional attributes [45, 46]. The advancements in analytical technology together with regulatory efforts may increase the possibility of detecting quality defects before the products reach the patients. The high cost of the manufacture of biopharmaceuticals could translate to more careful control and monitoring to avoid the loss of batches and products. However, the findings of this study cannot be extrapolated to biologicals extracted from natural sources, such as blood products from human plasma, where manufacturing relies significantly on ensuring the quality and safety of the raw material (e.g., human plasma). Future studies could explore whether there are differences between quality-related RAs for biopharmaceuticals and extracted biologicals.

Previous studies that assessed quality-related RAs for medicines in general mainly focused on the number of and underlying reasons for recalls [16-22]. However, we have

shown that quality-related RAs often included letters sent to HCPs (n = 45) and are less frequently concerned with recalls (n= 22), which shows that consideration of only recalls could lead to an underestimation of the quality-related RAs for biopharmaceuticals. Quality-related RAs issued as follow up for the same quality defect accounted for more than half of the regulatory letters (26 out of 45 for seven biopharmaceuticals), which may explain why there are more regulatory letters than recalls. This finding suggests that some quality defects may take a while to address and solve, and it shows the willingness of the regulators to inform HCPs, who continuously have to make informed decisions based on the most recent information.

In the present study, we identified 11 recalls for biopharmaceuticals in the US, which is lower than the 41 recalls found by Ebbers et al., despite the use of the same definition for biopharmaceuticals in the two studies. This disparity could be partially attributed to the differences in the cohort and recall definitions and the data collection. First, our study identified recalls for biopharmaceuticals approved between 1995 and 2019, while Ebbers H. *et. al.* were unable to link recalls with specific products. Thus, it could be that Ebbers H. *et. al* included recalls of products that had been approved outside the defined period in our study. Second, the definition of a recall differed between the two studies, which may explain the differences in the number of recalls. For example, recalls due to defective device, packaging and labeling issues, which accounted for half of the recalls in the study by Ebbers *et. al.*, were not considered as quality-related RAs in our study, because they were not directly related to the quality of the drug product. Furthermore, although our study collected data directly from the official FDA website and Ebbers *et. al* obtained data from the FDA through a Freedom of Information Act (FOIA) request, we do not believe that this would influence our findings, because the FDA has a strict policy regarding communicating information on recalls to the public [47].

The differences in the content of the types of quality-related RAs is likely explained by the different natures of the underlying quality defects that require different types of HCP actions to counter the potential risk to clinical outcomes and patient care. This difference can be illustrated by the regulatory letters that often issued due to manufacturing issues, which are mainly GMP deviations. On the other hand, the regulatory recalls often issued due to specification issues, including out-of-specification and particulate matters, which require different types of HCP actions. The manufacturing issues often affect the product in general and, sometimes, multiple products from the same manufacturer. As manufacturing issues could potentially result in shortages, HCPs are often required to take actions such as “restrict,” “monitor,” “switch” and “inform” at patient level. In contrast, the specification issues, including out-of-specification (OOS) (e.g., OOS in volume, potency, strength and preservative) and particulate matters, were often associated with specific batches. The potential impact of OOS and particulate matters may not be known at the time the regulatory recall is issued, but, at least theoretically,

they may potentially affect patient safety, immunogenicity and product efficacy, which require actions such as "check," "handle" and "recall" at the product level. It is important to note that particulate matters are a common challenge for all injectable drugs and not specific to biopharmaceuticals. In some cases, particulate matters not lead to regulatory recalls, especially where there are no alternatives, and regulators may recommend the HCPs should administer the product through a 0.2 µm filter to remove particulates, which is also described in the SPCs for some biopharmaceuticals to minimize the potential occurrence of infusion-associated reactions. Nevertheless, regulators acknowledge that, according to pharmacopeia, the test for particulate matters may be insufficient to detect particulates during lot-release testing. In response to this, the FDA recently published a draft guidance for an inspection program for injectables that provides a risk-based approach to control, assess, correct and prevent the risk of particulates and could contribute to improving early detection and prevention of particulate matters before the affected product and batch reach patients [48].



Previous studies have assessed the quality and applicability of information on the monitoring of psychiatric drugs and a selection of DHPCs in the SPCs and found that the information is insufficient for HCPs in clinical practice [23, 37, 38]. Since the methodology applied in previous studies only assessed a single type of HCP action, "monitor," we were unable to assess the quality and applicability of the various types of HCP actions that may be associated with quality-related RAs. However, our study observed that the nature of the underlying quality defects for some quality-related RAs was unclear, which could be a sign of incompleteness or a variation in the extent of the details in the quality-related RAs. For example, the Genzyme company reported in the literature that a bioreactor had been contaminated with the virus "Vesivirus 2117," which does not cause human infections but impairs the growth of the producing cell line, and they provided a clear description of the underlying quality defects, which could not be deduced from the corresponding RAs [49]. This observation emphasizes the need for improvement to enhance the clarity and complexity of the quality-related RAs, so the quality and applicability of presented information for clinical practice can be explored in future studies. A clear example is a quality-related RA (recall) issued in the EU for Novomix® (2013), which contained components such as the nature of the underlying quality defect (OOS in insulin strength) that were illustrated with pictures to enable the HCPs to easily recognize the affected batches and to understand the type of HCP actions required and the potential implications for patient care, which is useful for HCPs to make informed decisions to protect the patients.

Although the aim of our study was not to compare the regulatory interventions in the EU and the US, we did identify only slight differences in the type and content of the quality-related RAs. For the type of quality-related RAs, more regulatory letters were issued in the EU than in the US. This finding can be partially attributed to the follow-up

letters issued for the same quality defects, which accounted for more than half of the regulatory letters issued in the EU. This finding is in contrast to previous observations by Giezen et. al., who found that there were more letters sent in the US than in the EU. This was attributed to the fact that the US is able to issue letters to correct previous advertising and labeling updates, while the EU appeared to favor incorporating safety communication in the labeling, even though the dissemination of letters in both the EU and US is initiated by both the companies and the regulators when new information is obtained [30]. Another difference is that only five out of 34 biopharmaceuticals approved in both the EU and the US received quality-related RAs due to the same quality defects, which suggests that quality-related RAs could be country- or region-specific since the manufacturers that supply a country or regions can be in different sites. This is reflected in the biosimilar regulations, where reference product batches derived from different regions (i.e., EU or US markets) can be used to establish the quality target profile and to conduct comparability exercises for biosimilars [50]. Regarding the biopharmaceuticals that had been approved in both regions and received quality-related RAs for the same quality defect, we noted that the types of HCP actions recommended in the EU and the US were consistent at the product level but differed slightly at the patient level. This observation suggests that the US regulators put more emphasis on the type of HCP actions at the patient level, such as "inform" and "monitor."

The different types of RAs; the length of the study period, which included 25 years of follow up; the large sample size of biopharmaceuticals that had been approved in the EU and the US, the largest global pharmaceutical markets; and the identification of biopharmaceuticals that had received quality-related RAs were important strengths of this current study. The study also provided a classification of both the nature of the underlying quality defects and the type of HCP actions, which can be used as frameworks for HCPs in clinical practice to understand the nature of quality defects and the type of actions required to prevent potential clinical consequences of the (future) quality problems.

However, the study also encountered some limitations that should be mentioned. First, the classification of the nature of the underlying quality defects and the type of HCP actions may be subjective, and, to minimize this, the classification was performed in duplicate. Second, some quality-related RAs may have been missed. The EMA started to publish regulatory letters on its website in 2020 and only provides information on the number of recalls, which fall in the remit of national regulatory authorities. To reduce the effect of this limitation, quality-related RAs were collected from the public website of the MHRA in the UK, which has been publishing the data since 1986. We may have missed RAs that were not issued in the UK but consider the MHRA to be a valid data source for the EU cohort for the period 1995 to 2020. A sample of the data collected from MHRA between 2007 and 2020 was additionally crosschecked with the data obtained

from the MEB since the majority of the quality-related RAs (63 out of 67 RAs, 95%) were issued after 2007, and it was found that only two (follow-up) RAs had been missed in the MHRA search, which reflects the robustness of data collection. The same applies for the US cohort, where data was collected from the FDA public website, which archives data older than three years. To overcome this limitation, we manually searched all the FDA archives to minimize the probability of missing a quality-related RA. In addition, for all biopharmaceuticals that had been approved in both the EU and the US and had received a quality-related RA in one region only (either EU or US), a manual search was conducted with the product brand name in the other jurisdiction. This manual cross-checking showed that no quality-related RAs had been missed in the initial search. Finally, the study could not explore the root cause or the preventive actions taken by the companies to resolve the quality defects, because the pertinent information submitted by companies to the regulatory authorities is not publicly available.



## Conclusion

Although the EU and the US have established highly advanced regulatory systems, quality-related RAs were issued for one out of every eight biopharmaceuticals, all for originators and none for biosimilars, during their lifecycle. This is considerably lower than the occurrence of safety-related RAs that has been reported for biopharmaceuticals. The majority of the quality-related RAs involved regulatory letters rather than recalls, which shows that considering recalls as a proxy for quality defects may lead to an underestimation of the number of quality-related RAs for biopharmaceuticals. The regulatory letters mainly reported on manufacturing issues and required HCP actions at the patient level that would counter the potential risk of shortages. The regulatory recalls mainly involved specification issues and required HCP actions at product level to avoid negative implications for patient care. Manufacturers and regulators should continue their efforts to reduce the occurrence of any quality defect that may impact patient care. This study provides insight into recommendations to HCPs in relation to quality-related Ras; however, further studies are needed to assess their effectiveness and the impact of these on clinical practice and patient care.

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## Supplementary data




**Supplementary figure S1:** Flowchart of data collection of quality-related regulatory actions issued in the EU and the US for biopharmaceuticals approved in the EU and US between 1995 and 2019, FDA, Food and Drug Administration, MHRA, Medicines and Healthcare products Regulatory Agency, MEB, Medicine Evaluation Board.

**Supplementary Table S1:** Information on the nature of underlying quality defects for 41 biopharmaceuticals approved in the EU and the US between 1995 and 2019 and received at least one quality-related regulatory actions during the study follow up from 1995 till 2021.

| | Product class/ brand name | Active substance | Single or multiple regulatory actions (follow-up) | The category / nature of underlying quality defects (region) |
|---|---|---|---|---|
| | **Hormones** | | | |
| 1 | Apidra® | Insulin glulisine | Single | Manufacturing issues / GMP deviation (EU) |
| 2 | Avonex® | interferon beta-1a | Single | Manufacturing issues / GMP deviation (EU) |
| 3 | Extavia® | interferon beta-1b | Single | Contamination / Microbial contamination (US) |
| 4 | Fiasp® | insulin aspart | Multiple | Specification / particulate matters (EU),<br>Stability / stability and storage issues (US) |
| 5 | Forsteo® | teriparatide | Single | Contamination / Microbial contamination (US) |
| 6 | Glucagen® | glucagon | Single | Packaging/Defective primary packaging (US) |
| 7 | Gonal F® | follitropin alfa | Multiple | Packaging /Secondary packaging and labeling errors (EU)* |
| 8 | Insuman® | insulin human | Single | Manufacturing issues/Unclear quality defect (EU) |
| 9 | Levemir® | insulin detemir | Multiple | Stability /Stability and storage issues (US)* |
| 10 | Myalepta® | metreleptin | Single | Packaging/Secondary packaging and labeling errors (EU) |
| 11 | Natpara® | parathyroid hormone | Single | Specification/ Particulate matters (US) |
| 12 | Novolog® | insulin aspart | Single | Stability/Stability and storage issues (US) |
| 13 | Tresiba® | insulin degludec | Single | Stability/Stability and storage issues (US) |
| 14 | Xultophy® | insulin degludec / liraglutide | Single | Stability /Stability and storage issues (US) |
| 15 | Novomix® | insulin aspart | Single | Specification/Out of specification (EU) |

*Quality-related regulatory action issued due to the same nature of underly quality defects occurred at different time points

**Supplementary Table S1 (Continued):** Information on the nature of underlying quality defects for 41 biopharmaceuticals approved in the EU and the US between 1995 and 2019 and received at least one quality-related regulatory actions during the study follow up from 1995 till 2021.

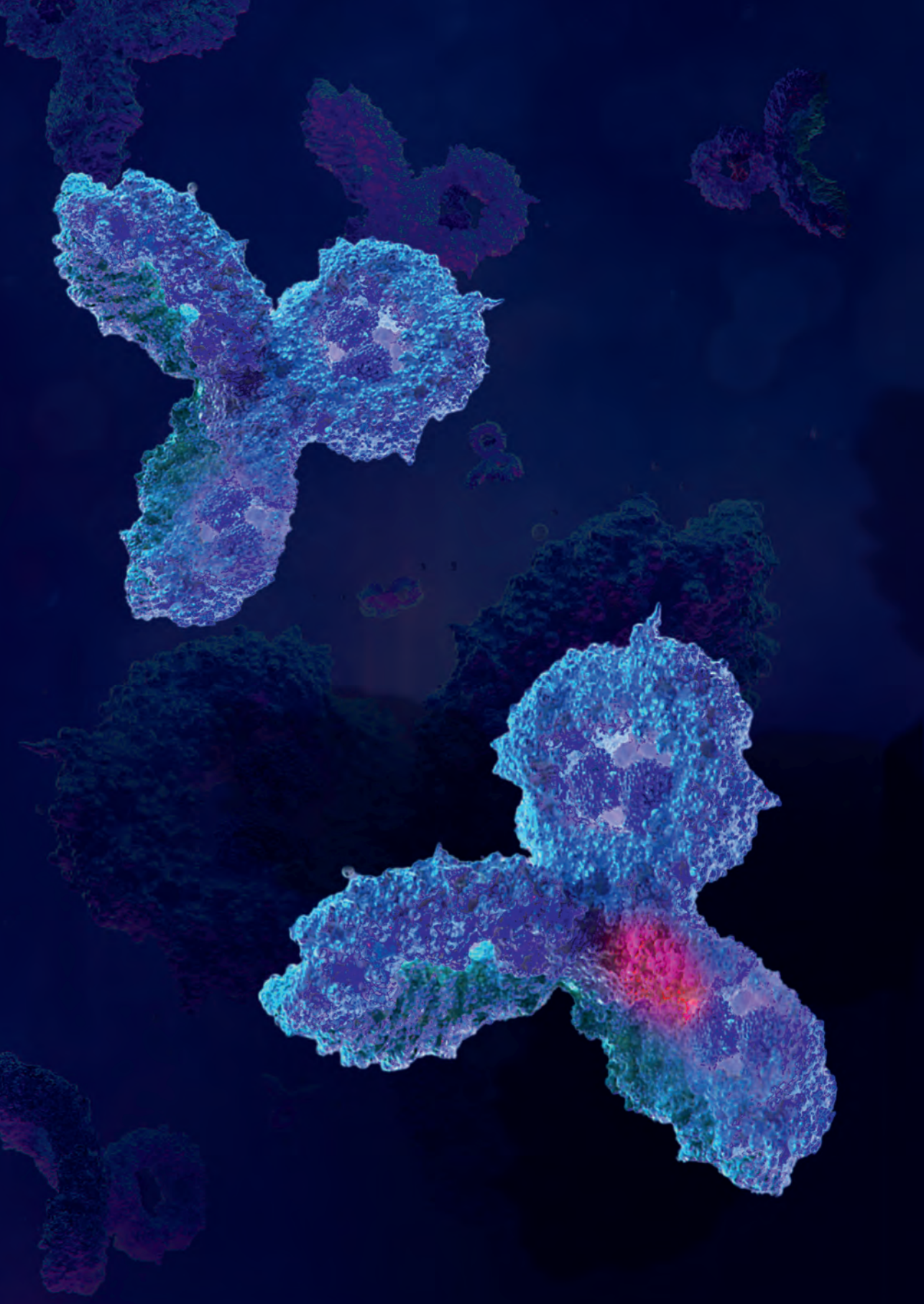
| | Product class/ brand name | Active substance | Single or multiple regulatory actions (follow-up) | The category / nature of underlying quality defects (region) |
|---|---|---|---|---|
| 16 | Thyrogen® | thyrotropin alfa | Multiple | Specification/Particulate matters (EU and US), Manufacturing issues / Unclear quality defect (EU and US) |
| 17 | ViraferonPeg® | peginterferon-alfa-2b | Single | Packaging /Defective primary packaging (EU) |
| | **Growth factor** | | | |
| 18 | Aranesp® | darbepoetin alfa | Single | Specification/Particulate matters (US) |
| 19 | Increlex® | mecasermin | Single | Manufacturing issues/GMP deviation (EU) |
| 20 | Inductos® | dibotermin alfa | Multiple (follow up) | Manufacturing issues/GMP deviation (EU) |
| 21 | Mircera® | methoxy polyethylene glycol-epoetin beta | Single | Manufacturing issues/Unclear quality defect (EU) |
| 22 | Neupopeg® | pegfilgrastim | Single | Packaging/Defective administration device (EU) |
| 23 | Serostim® | somatropin | Multiple (follow up) | Adulteration /Counterfeiting(US) |
| 24 | Zomacton® | somatropin | Single | Specification /Out of specification (EU) |
| | **Enzyme** | | | |
| 25 | Aldurazyme® | laronidase | Single | Specification/Particulate matters (US) |
| 26 | Cerezyme® | imiglucerase | Multiple (follow up) | Manufacturing issues / GMP deviation (EU), Specification / particulate matters (EU and US) |
| 27 | Fabrazyme® | agalsidase beta | Multiple (follow up) | Manufacturing issues/GMP deviation (EU), Specification/Out-of-specification (EU) Specification/ Particulate matters (EU and US) |
| 28 | Hylenex® | hyaluronidase | Single | Specification/Particulate matters (US) |

*Quality-related regulatory action issued due to the same nature of underly quality defects occurred at different time points

**Supplementary Table S1 (Continued):** Information on the nature of underlying quality defects for 41 biopharmaceuticals approved in the EU and the US between 1995 and 2019 and received at least one quality-related regulatory actions during the study follow up from 1995 till 2021.

| | Product class/ brand name | Active substance | Single or multiple regulatory actions (follow-up) | The category / nature of underlying quality defects (region) |
|---|---|---|---|---|
| 29 | Myozyme® | alglucosidase alfa | Single | Specification/Particulate matters (US) |
| 30 | VPRIV® | velaglucerase alfa | Single | Specification/Particulate matters (US) |
| | **Monoclonal antibodies and fusion protien** | | | |
| 31 | Avastin® | bevacizumab | Single | Adulteration / counterfeiting (US) |
| 32 | Enbrel® | etanercept | Single | Manufacturing issues /Capacity (EU) |
| 33 | Herceptin® | trastuzumab | Multiple | Adulteration/falsification (EU), Packaging/Defective primary packaging (EU and US) |
| 34 | Lucentis® | ranibizumab | Multiple (follow up) | Packaging/Defective primary packaging (EU), Packaging/Defective administration device (EU) |
| 35 | Nucala® | mepolizumab | Single | Packaging /Defective administration device (EU) |
| 36 | Nulojix® | belatacept | Multiple (follow up) | Manufacturing issues/Capacity (EU) |
| 37 | ReoPro® | abciximab | Multiple (follow up) | Manufacturing issues/Unclear quality defect (EU) |
| 38 | Soliris® | eculizumab | Multiple | Specification/Particulate matters (US)* |
| 39 | Vectibix® | panitumumab | Single | Packaging /Defective primary packaging (EU) |
| **Clotting factors** | | | | |
| 40 | Helixate NexGen® | octocog alfa | Multiple | Stability/stability and storage issues (US and EU) |
| 41 | Kogenate FS® | octocog alfa | Single | Specification/Out of specification (EU) |

*Quality-related regulatory action issued due to the same nature of underly quality defects occurred at different time points



# Chapter 4

## General discussion and future directions

Author's contribution: AMA conceived the idea and set-up of the general discussion. AMA conducted literature review, outlined and wrote down the general discussion. Throughout the process, AMA asked for and implemented input and feedback from the supervision team.

## General discussion

Biopharmaceuticals are defined as a class of biologicals where the active substance is produced in living cells (e.g., Escherichia coli) through recombinant DNA technology. Biopharmaceuticals are distinguished from biologicals extracted from natural sources as well as from chemically synthesized small molecule drugs [1]. Biopharmaceuticals have been used in clinical practice since the discovery and approval of the first recombinant human insulin in 1982, which resulted from breakthrough discoveries in basic sciences that unlocked DNA and protein structures and from mechanistic unraveling of many diseases. Since then, multiple generations of biopharmaceuticals have been developed and approved and have provided novel and innovative ways to treat various diseases [2, 3]. However, these valuable products are often extremely expensive. The expiration of patents and exclusivity rights of biopharmaceuticals allows for the introduction of biosimilars, which are defined as highly similar versions of the reference products or originators. The introduction of biosimilars provided alternative and more affordable treatment options to alleviate the pressure on healthcare budgets and to improve patient access to important biopharmaceuticals.

Biopharmaceuticals, both originators and biosimilars, are associated with more complex structures, functions and manufacturing processes when compared to the small molecule pharmaceuticals [4]. As a result, manufactured biopharmaceuticals often demonstrate an inherent variability and (minor) differences, even between batches from the same process; therefore, careful control and monitoring are required to ensure the quality of biopharmaceuticals [5, 6]. For this reason, quality, currently defined by the so-called quality attributes (QAs), is a key regulatory aspect of (bio)pharmaceuticals. These QAs are physical, chemical, biological or microbiological properties that define the structure and functions of the drug substance (DS) and the drug product (DP) [7, 8]. QAs usually reflect the consistency of the manufacturing process and the quality characteristics of the DS and the DP, although the molecular and manufacturing complexity make it difficult to fully characterize and (re)produce biopharmaceuticals [9]. The critical quality attributes (CQAs) are a subset of QAs. These CQAs are considered to potentially influence clinical outcomes if they are outside the acceptable range or limit, although meaningful differences are largely unknown. The CQAs has become ever more important for comparability assessments since modern analytical methods have high precision and are able to detect increasingly smaller differences in (C)QAs.

Similar to all other medicines, biopharmaceuticals are required to obtain regulatory approval before being introduced into daily clinical practice. The regulatory process aims to ensure that patients and health care professionals can use the treatments with a positive benefit-risk profile in clinical practice. Regulatory authorities, such as the European Medicines Agency (EMA) in the EU and the Food and Drug Administration (FDA) in the

US, have established several regulatory pathways for biopharmaceuticals, differentiating between reference products and biosimilars. A reference product is a biopharmaceutical that contains a novel active biological substance, while a biosimilar is a follow-on biopharmaceutical that contains a highly similar version of the active biological substance of an already-approved reference product. The regulatory approval of reference products relies on the full assessment of the quality, safety, and efficacy of the product, including the outcomes of clinical trials that show that the benefits outweigh the risks, while the approval of biosimilars relies mainly on the comparability exercises that demonstrate biosimilarity based on the comparability of QAs, rather than re-establishing the safety and efficacy of the product.

Even though biosimilars have been released in the EU and, later, the US markets, the uptake and acceptance of biosimilars in clinical practice vary between European countries and is still low in the US. The low uptake and acceptance in the US has been attributed to, amongst others, budget and reimbursement factors and a lack of understanding and acceptance or trust in the science behind biosimilar approval that relies on the comparability of QAs [10, 11]. Information on the comparability of QAs is available via various sources from regulatory and scientific communities. Regulators publish documents such as the European public assessment reports (EPARs) from the EMA and the review reports from the FDA. These documents reflect on the regulatory assessment of the data submitted by the company seeking regulatory approval. In addition to the communications from the regulatory authorities, the biosimilar field has attracted significant scientific attention from academics and privately funded organizations, which has also resulted in publications (Chapter 2.1) [12]. Previous research on the comparability of QAs focused on demonstrating variability and consistency between products and batches of biopharmaceuticals sourced from different markets [13]. However, these studies investigated less complex proteins (e.g., filgrastim and epoetin) than those that are currently available (e.g., monoclonal antibodies and fusion proteins such as tumor necrosis factor-α inhibitor products (TNF-α-i)) and did not reflect on the comparability of QAs that should be assessed to support biosimilar approval.

Drug regulation must ensure the quality, safety and efficacy of (bio)pharmaceuticals at the time of approval and continuously control and monitor the substances throughout their life cycle through post-approval pharmacovigilance systems [14, 15]. Biopharmaceuticals are complex molecules and can be affected by changes in manufacturing, storage and transportation, which may influence the CQAs and could potentially affect clinical outcomes and patient care. Therefore, careful control and monitoring of the quality of biopharmaceuticals is important to ensure batch-to-batch consistency, so that patients will receive safe and effective treatment. Therefore, post-approval quality surveillance of biopharmaceuticals is relevant.

This thesis followed up on previous research projects from our group that focused on post-approval regulatory learnings for biopharmaceuticals [16-21]. This thesis aimed to study the quality of biopharmaceuticals by providing insight into (1) the comparability of QAs with an emphasis on the CQAs, and (2) post-approval quality-related surveillance and regulatory actions for biopharmaceuticals. Moreover, this thesis aimed to provide information regarding post-approval changes and defects in the quality of biopharmaceuticals that could potentially influence patient care. The current chapter provides a broader perspective of several key findings from previous chapters in two main themes. The two themes are 1) the comparability of (critical) quality attributes, and 2) post-approval quality surveillance of biopharmaceuticals. In addition, the discussion continues to reflect on the potential implications and future directions of comparability of (C)QAs and post-approval quality surveillance for drug regulation, policy making and patient care and wraps up with an overall conclusion.

## Comparability of (critical) quality attributes of biopharmaceuticals



The comparability exercise is a key regulatory principle for biopharmaceuticals that can be applied in two scenarios, namely "post-approval changes" and "biosimilar pathway" (Figure 1). The first scenario, "post-approval changes," relates to a quality change in the product design or manufacturing process introduced by a company, and the exercise illustrates the comparability between pre- and post-change batches, mainly from the same manufacturer. This exercise has been used for a long time to demonstrate batch-to-batch consistency of biopharmaceuticals before and/or after a manufacturing change. We show that changes in the manufacturing processes of biopharmaceuticals are often introduced after approval, usually initiated for regulatory compliance, technical advancement or upscaling or innovation in the process and the product (Chapter 3.1). The second scenario, "biosimilar pathway," relates to the development of a biosimilar, and the exercise is used to compare a candidate biosimilar with an already-licensed reference product. The biosimilar pathway was created because the well-established generic pathway for small molecules is not fit for this purpose since the generic pathway assumes that two products can be therapeutically equivalent if the generics have the same QAs and comparable bioavailability to a reference product. However, biosimilars are never exactly the same as the reference product because biopharmaceuticals are complex molecules that are sensitive to differences in the manufacturing process, which causes inherent variability in the molecules. This inherent variability results in minor differences in the QAs, which requires a more comprehensive comparability exercise for

biosimilars than for the generics to ensure that the minor differences do not influence clinical outcomes.

The two scenarios are based on the same scientific principles. A comparability exercise with a stepwise approach is conducted in both scenarios, and it starts with a comparison of the QAs followed by comparative non-clinical and clinical studies, as required. The comparability of QAs is a relevant first step because it shows the similarities and (minor) differences between the QAs of the structure and function of the active biological substance in two batches (pre- and post-change) or products (biosimilar and reference product) and determines the need for further comparative non-clinical studies and clinical trials (Figure 1) [22]. For example, comparative non-clinical in vivo studies are not preferable due to limitations in sensitivity and species specificity and are only necessary if no suitable in vitro assay is available for the assessment of the QAs related to the function of the DS. However, comparative non-clinical in vivo studies may be necessary if a biosimilar is produced in a new cell line or formulated with a novel excipient. The goal of comparative non-clinical and clinical studies is always to rule out negative impacts of minor differences in QAs on clinical outcomes and patient care. Furthermore, depending on the regulatory system, the results of a comparison of QAs may be the basis for the extrapolation of indications, for example, an assessment of safety and efficacy from a clinical trial for one indication can be extrapolated to other indications without repeating the clinical trials for all indications.

The QAs of an authorized biopharmaceutical are reflected in the specifications of the DS and the DP included in regulatory dossiers. These specifications typically include a list of QAs, the required tests for each QA, a reference to analytical procedures for each test and an appropriate quantitative or qualitative acceptance criterion for each QA test. These specifications are a mandatory regulatory requirement and have to be established according to the ICH guideline (Q6B) for the specification of biologicals and biopharmaceuticals [25]. The specifications have been established to assess the acceptability of a biopharmaceutical for the intended use (i.e., safe and effective treatment) and to ensure that the processes, materials and product quality are consistent. According to the ICH guidelines (Q6B), the acceptance criteria are established in each QA test based on prior knowledge and experience with molecules, together with information related to the manufacturing process and analytical procedure capabilities, stability data, non-clinical studies and clinical trials throughout the development life cycle. This knowledge is key to support the justification for the specification of the DS and the DP. This means that the specifications and acceptance criteria may evolve as more information regarding the product quality, safety and efficacy becomes available. Although biosimilar developers often have no direct information about the QAs and manufacturing of a reference product, biosimilar development requires an understanding of the QAs related to the

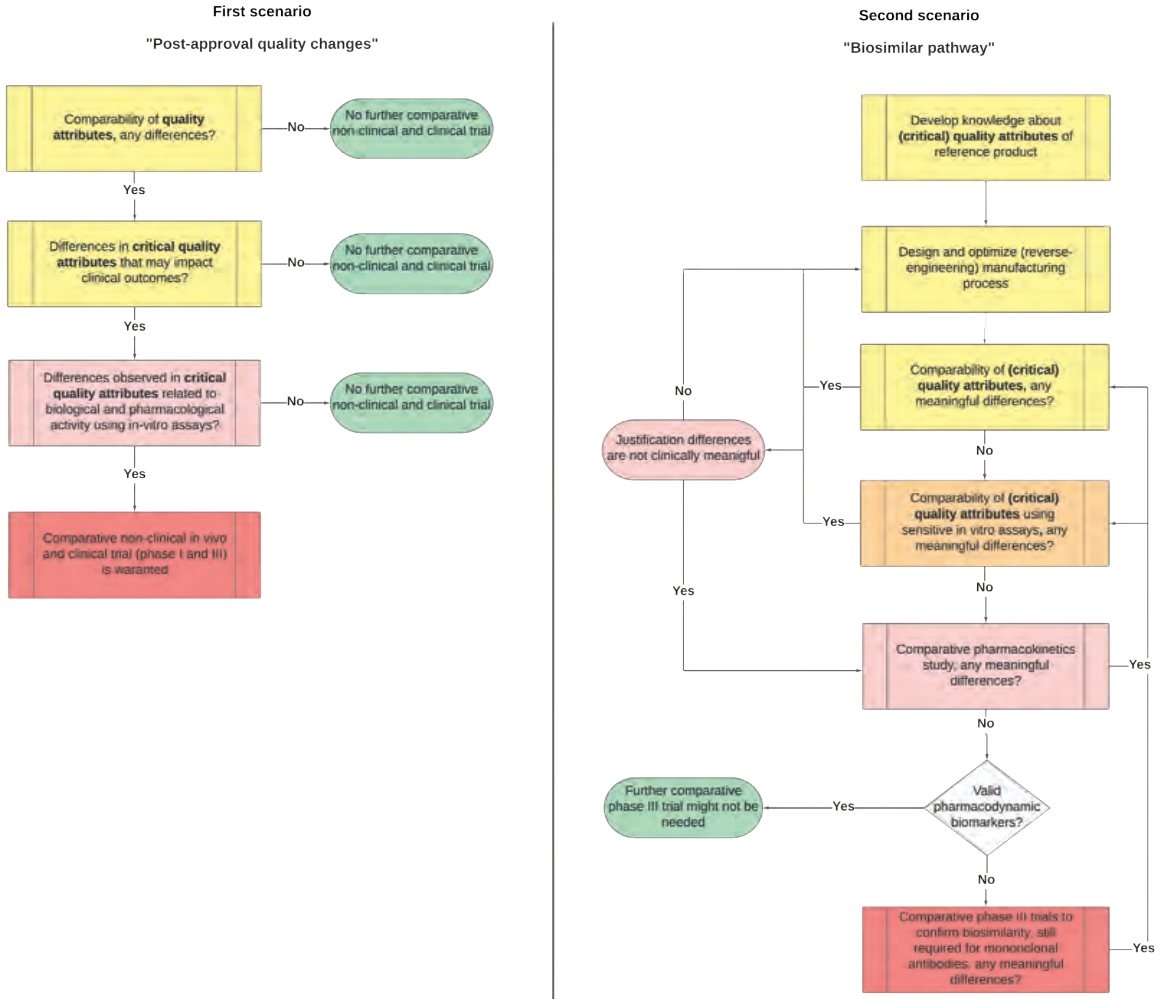



**Figure 1:** The possible outcomes of the comparability exercises for the two scenarios "post-approval quality change" and "biosimilar pathway" summarized from the ICH Q5E Comparability of Biotechnological Biological Products Subject to Changes in Their Manufacturing Processes and the EMA and FDA biosimilar guidelines [22-24].

structure and function of the active biological substance of the reference product. This step usually requires ample investment and development.

To facilitate the development and regulatory assessment of biosimilars, the EMA, FDA and other regulatory and health authorities have published several guidelines [23, 24]. These guidelines include general principles and specific requirements for the comparability of QAs, and comparative non-clinical in vitro and in vivo studies and clinical trials to support biosimilar approval. To some extent, the EMA and the FDA require similar regulatory requirements for the comparability of QAs of biosimilars, including the selection of the reference product, the types of QAs, the selection of the analytical procedures and the statistical tools used to calculate the acceptance criteria of the QAs. Various descriptive (e.g., min-max, x-sigma, tolerance intervals) and inferential (equivalence tests) statistical tools that can provide consistent decision rules, even though with some challenges for some tools such as equivalence tests, are mentioned in the regulatory guidelines. However, it has been recognized that the statistically significant differences only may not provide what differences mean for clinical outcomes without linking it to the CQAs [26]. Another key issue may be the need for a domestically licensed reference product, which may not always be feasible in some countries. If the reference product is not authorized in a country, regulators may require a reference product sourced from other jurisdictions. If a reference product is authorized locally but sourced from a foreign jurisdiction, such as ICH countries, regulators may require "bridging studies" that ensure that the local and foreign-sourced reference products are comparable, based on the comparability of the QAs and the comparative PK/PD trials. The bridging studies are required to allow biosimilar developers to use a foreign reference product in comparative non-clinical in vitro studies and clinical trials, which could facilitate global biosimilar development.

Because of the surge in biosimilars introduced into the European and global markets over the last 15 years, the need for comprehensive and reliable information on the comparability of the QAs for decision makers (e.g., clinicians, pharmacists, payers and regulators) becomes pertinent to understand the science behind biosimilar approval. Our systematic literature review found 79 scientific publications that present comparability exercises of QAs for (intended) biosimilars between 2006 and 2019, with a variation in the number of scientific publications per molecule and an increasing trend in publications over calendar time (Chapter 2.1). The variation in the number of publications per molecule was consistent with the findings reported in previous systematic reviews that assessed the availability of comparability exercises for quality and nonclinical and clinical data, suggesting an incomplete biosimilarity picture for certain molecules in the literature [27-29]. This increase in publications has accompanied the increase in approved biosimilars, which confirms a theory that the availability of publications on similar drugs can trigger industry to publish information related to the drugs [30]. This shows that bio-

similar development contributes to expand knowledge about the comparability of QAs, which is the core evidence required for biosimilar approval. However, this development is at odds with what is done for the generics of small molecule drugs. Given that generics are required to have the same active substances with identical QAs, the comparability of QAs becomes less relevant than the bioequivalence and bioavailability assessment in healthy volunteers. Clinical trials for new active substances, especially phase I trials, are often underreported or not published. The underlying reason may be that these are considered less interesting than patient data, which results in the first-in-human trials not being published for reasons such as non-significant results or negative clinical outcomes [31-34], which creates a bias towards positive results [35-38]. The underreporting of information on manufacturing and QAs for gene and cell therapies (GCTs), which are even more complex than biopharmaceuticals produced by recombinant DNA technology, was also reported by Coppens et al. [39]. The intellectual right protection for GCTs, biopharmaceuticals and other biomarkers could be the reason why information on the QAs of originators is not published. This is not the case for biosimilars once originators have become off-patent and intellectual rights are no longer relevant for the protection of information on the QAs. Furthermore, it seems that an increase in the evidence for the regulatory approval stimulates dissemination through scientific publications.



In Chapter 2.1, our study reported a variation in the reporting frequencies of the QAs related to the structure and function of the DS that are included in the comparability exercises for (intended) biosimilars. The most frequently reported types of QAs were biological activity (94%), followed by physicochemical properties (81%), post-translation modifications (79%), primary structure (77%) and purity and impurities (73%), which reflect the importance of these for the comparability assessment of biosimilars according to the EMA and the FDA guidelines. The analysis of QAs related to physicochemical properties, post-translation modifications, primary structure and purity and impurities can provide a first insight into the (dis)similarities in the structural attributes of the DS. A requirement is that these should be highly similar, except for the primary structure, which has to be identical because a different primary structure is, regulatory speaking, a different DS and not acceptable for the biosimilar pathway. The underlying reason why QAs related to biological activity are more frequently reported in scientific publications (Chapter 2.1) and EPARs (Chapter 2.2) is because these generate valuable information for comparability assessments. An analysis of biological activity can aid in the assessment of the impact of minor differences in structural QAs, confirm the higher-order structure, reflect the mechanism of action, predict the clinical activity and support the extrapolation of indication. Reporting of QA types has increased over time (2009 – 2019), with a sharp increase, from 0% to 47%, in the reporting of QAs related to immunochemical activities, probably driven by the publication of EMA guidelines for biosimilars of mAbs and fusion proteins in 2012 [40-42]. The development of regulatory guidelines resulted from early

interaction between regulators and the industry, where the publication of regulatory guidelines seems to be positively promoted to share knowledge about the QAs.

Our assessment of how the EU regulators report information on the QAs in the comparability exercise in a sample of EPARs for seven adalimumab biosimilars approved between 2017 and 2020 show that the regulators diligently placed more emphasis on CQAs (65–87%) compared to QAs (35–75%) (Chapter 2.3). The regulators emphasized the CQAs because these are considered to be relevant to the safety and efficacy of the DP. The CQAs provide a high level of assurance that structure and function of the DP are consistent between batches and stable during storage and transport. In some cases, a QA is considered a CQA because it has a direct influence on clinical outcome (e.g., potential impact of aggregates on safety and immunogenicity) or indirect influence on clinical outcomes (e.g., potential impact of glycosylation on aggregates formation and molecule functions that can potentially influence PK/PD, safety/immunogenicity and efficacy)[43]. Therefore, CQAs must fall within a defined range, limit or distribution to ensure that the DP will have the desired characteristics of quality, safety and efficacy when administered to patients.

The current practice of defining CQAs is based on a risk assessment of the criticality of the QAs as per the principles described in the ICH Q9 (quality risk management)[44]. The criticality is determined based on the probability of the occurrence of risk and the probable severity of that risk. The ICH Q9 guideline provides information on various risk assessment tools that vary based on the assessment of the QAs and risk factors (impact and uncertainty, occurrence severity and likelihood). This means that QAs that are considered as CQAs have to be measurable, sensitive to changes in the process and materials, relevant to the quality and stability of the DS and DP and critical to some extent for the biological and pharmacological activity (PK/PD) and clinical outcomes (safety, immunogenicity and/ or efficacy). Biosimilar companies conduct a risk assessment at an early stage of the development, based on previous knowledge of the molecule (or similar in class); the process, quality characterization and in vitro data; nonclinical in vivo studies and clinical experience. As a result, biosimilar development increases knowledge about CQAs in the public domain more than was the case before the biosimilar era.

Over recent years, the scientific and regulatory community witnessed rapid development and advancement in analytical technologies, such as mass spectrometry that enables precise quantification of minor differences in QAs, for example, glycosylation [45, 46]. As a result, detailed measurements are possible and, as minor differences in CQAs are inherent in all biopharmaceuticals, a precise quantification of the differences has to be combined with the clinical impact of the minor identified differences. It was also evident in our assessment of the EPARs that regulators often observe minor differences in CQAs, such as glycosylation and charge variants and biological activity (namely ADCC) between adalimumab biosimilars and the reference product (Humira®) (Chapter 2.3). The

glycosylation is the CQA with the most frequent notable differences between biosimilars and reference products [47-50]. Glycosylation is critical because of a potential impact on immunochemical activities (e.g., binding to FcγRIIIa, FcRn, and C1q), which may affect the biological activity (e.g., ADCC and CDC), PK (e.g., serum half-life), safety, immunogenicity and efficacy of the product [51-54]. These minor differences in glycosylation and charge variants have not been deemed clinically relevant, based on structural-activity studies and the lack of differences in the functions and PK profile. The same applies to minor differences in the ADCC detected in highly sensitive in vitro assays, which disappear in in-vitro assays with more physiological conditions (e.g., peripheral blood mononuclear cells). Although the result showed minor differences, it seems to be quantitative and does not preclude the overall conclusion for biosimilarity. The result reflects the importance of understanding and measuring CQAs, as these can determine the impact of minor differences in clinical outcomes and patient care.

Our study investigated the extent of the information of the test result and biosimilarity interpretation of reported QAs in the comparability exercises and found discordant variations between EPARs and scientific publications (Chapter 2.2), and between EPARs (Chapter 2.3) from regulatory and scientific communities. The EPARs often include only biosimilarity interpretations of the reported QAs, whereas scientific publications present both the test result and the biosimilarity interpretation of the reported QAs. This variation can possibly be attributed to the different objectives; EPARs reflect regulatory assessments whereas scientific publications reflect what authors find relevant to share with the scientific community. This finding may be influenced by the different requirements for publications, where presenting test results is mandatory. However. similar variations were also found in the reporting of safety and efficacy data described by the regulatory and scientific community, requiring users to combine the two sources for a complete overview to be able to make informed decision in clinical practice [55-60]. The variations in the reporting of information on QAs among EPARs was also found by Mielke et al. They analyzed clinical data on biosimilars in EPARs and linked it to the flexibility of clinical requirements for the regulatory approval of biosimilars (i.e., type and extent of clinical data is determined on a case-by-case basis) [61, 62]. This flexibility in regulatory requirement may not fit within QAs data because our study investigated adalimumab biosimilars that were compared with the reference product with same set of QAs.



## Post-approval quality surveillance of biopharmaceuticals

Post-approval surveillance is a key regulatory function to ensure that the quality, safety and efficacy of (bio)pharmaceuticals are consistent and fit for purpose throughout its life cycle. Regulators and the industry have developed post-approval surveillance tools,

including various pharmacovigilance activities, good manufacturing practices inspections and lot-release testing to facilitate the monitoring of the quality, safety and efficacy of biopharmaceuticals. Because biopharmaceuticals are complex molecules, they are vulnerable to changes or defects in product design, manufacturing, storage, and transportation. In general, two quality aspects that can occur for biopharmaceuticals after approval include (1) post-approval quality changes that can influence the QAs of the DS and DP and (2) post-approval quality-related regulatory actions (RAs) that may be related to a quality defect.

## Post-approval quality changes

Post-approval quality changes in biopharmaceuticals were assessed for TNF-α-i products, including originators and biosimilars available in the European market, and we found that approximately 800 changes had been implemented to the biosimilars and reference products during the last two decades (Chapter 3.1). Our study found that the majority of the post-approval quality changes were related to the manufacturing (50%) and quality control (20%) of the DS and DP, which shows that modernization and improvements in the quality and manufacturing of biopharmaceuticals is never finished. The proportion of low- and medium-risk changes in reference products and biosimilars of TNF-α-i products (89%) was consistent with the proportion reported by Vezér et al. (95%), which suggests that most post-approval changes are unlikely to influence the CQAs of the DS and DP [63].

Compared to Vezér et al., our study found a slightly higher number of high-risk changes (11%) (5%) (Chapter 3.1), which could be explained by the classification of risk for an extensive selection of quality changes. We performed a risk classification for more than 150 different quality changes, of which 25 were defined as high-risk compared to 15 different quality changes of which 5 were defined as high-risk in the Vezér et al. study. The low number of high-risk changes found in the two studies is likely related to the possible impact these can have on CQAs, which could pose a risk to safety and efficacy. We found a comparable rate of post-approval changes (seven changes per year) for reference products and biosimilars; this incidence rate was higher than what was reported by Vezér et al. (1.8 changes per year), who only included reference products of mAbs. This suggests that most of the post-approval changes for reference products were introduced after the publication of Vezér et al.'s study in 2014 and during the development of biosimilars for TNF-α-i products. Further research is warranted for a deeper understanding of these observed differences. Until 2020, it was estimated that global exposure to biosimilars of TNF-α-i was 1,286,578 patient-treatment years, with no adverse impacts on safety and efficacy [64]. To the best of our knowledge, no safety and efficacy concerns have been identified by the pharmacovigilance system for the studied products, despite the hundreds of post-approval quality changes that have been implemented. Thus, according to our data, there is no reason to assume that post-approval

quality changes will lead to differences between originators and biosimilars of TNF-α-i products in clinical practice.

Our study found that the type of post-approval quality changes to biosimilars were not related to those already implemented to originators of TNF-α-i products (Chapter 3.1), which reflects that biosimilars are standalone products after approval. The implementation of post-approval changes is likely to be initiated by several factors. First, post-approval changes to the quality of biopharmaceuticals may be implemented to comply with obligatory regulatory commitments and new standards. For example, post-approval changes related to stability occurred soon after approval because of commitments to complete long term stability studies to support or extend the shelf life of the DS and the DP [65, 66]. In addition, post-approval changes implemented to comply with a new regulatory standard, such as the use of serum-free medium in the downstream process and the formulation of biopharmaceuticals to reduce potential contamination with infectious diseases, was associated with the use of raw materials such as human or bovine serum [67, 68]. Second, as advancements in science and technology in the field of manufacturing scale up, protein characterization and purification are likely to play a role in the introduction of changes in the quality and manufacturing after approval [69-74]. These advancements increase capacity and efficiency and allows for the reduction of costs, processing time and loss in yield. For example, the optimization of mass spectrometry allows up to one million times resolution, which reveals complex molecular structures. Another example is the adoption of new purification technologies that enhances the productivity (i.e., 5–20 g/L protein titer can be achieved today compared to 0.05 g/L in 1980s), optimizes the post-translation modifications (e.g., glycosylation), and reduces process and product-related impurities (e.g., host-cell proteins, DNA and aggregates). The latter can be observed for biosimilars that have been developed decades after the reference products, and the biosimilar regulation allows the development of products with lower aggregates and optimized glycosylation, which are CQAs of biopharmaceuticals. These technical advancements may provide an understanding of why post-approval changes to manufacturing processes of the DS were more frequent in our study. Third, companies may introduce changes in quality to upscale the production lines and produce enough stock to meet market demands. Finally, companies introduce post-approval changes to provide alternative options for patient care, such as the two examples of a reference product (Humira®, adalimumab) and a biosimilar (Remsima®/Inflectra®, infliximab). While a new citrate-free formulation of Humira® was developed to reduce the pain associated with an injection-site reaction and improve the comfort and adherence of patients [75], the first infliximab for subcutaneous use was developed to enable self-administration and reduce the time associated with intravenous infusions to improve patient compliance and adherence [76, 77].

## Post-approval quality-related regulatory actions

Information on the occurrence of quality-related RAs for biopharmaceuticals is limited in literature. Our study found that, between 1995 and 2019, post-approval quality-related RAs were issued for 41 (12.5%) of 324 biopharmaceuticals approved in the EU and the US (Chapter 3.2.). This number is lower than what was reported by Giezen et. al. (23.6%) regarding biopharmaceuticals that had RAs due to safety concerns [78]. The difference may be attributed to the different aspects (quality defects versus safety concerns) investigated in the two studies. After approval, knowledge regarding safety issues increases as product use increases in clinical practice. This increase in knowledge aligns with the function of the pharmacovigilance system, and newly identified safety concerns can result in RAs. The majority (60%) of the quality-related RAs were issued within 10 years after approval of the corresponding biopharmaceuticals, which may suggest that experience of ongoing manufacturing could play a partial role in the identification of a quality defect. All quality-related RAs were issued for originators and none for biosimilars (at least during the study follow up), which is difficult to explain because originators and biosimilars share the same manufacturing complexity and are subject to the same manufacturing control and regulatory oversight after approval. The manufacturing control and regulatory oversight are in place to ensure that biopharmaceuticals retain a consistent quality and to prevent quality defects that may affect patients.

The finding that quality defects occurred during the life cycle of one out of every eight biopharmaceuticals approved in the EU and the US suggests that the probability that a biopharmaceutical will be subjected to a quality-related RA is rather small. This low incidence could be related to several factors, such as (1) the implementation of post-approval surveillance, pharmacovigilance and manufacturing control, including manufacturing site inspections and mandatory lot-release testing for biopharmaceuticals [79]; (2) the advancement in analytical methods and instrumentation for more precise characterization of biopharmaceuticals [54, 69]; (3) the development of several regulatory strategies for in-process quality control, quality by design, and quality indicators [80-84]; and (4) the prevention of loss of batches due to the high manufacturing costs of biopharmaceuticals. These factors could play a role in the early identification and careful control and monitoring to prevent biopharmaceuticals with quality defects from being released and affecting patients. Since the study only investigated biopharmaceuticals, this finding cannot be generalized to biologicals extracted from natural sources, such as human plasma and bovine serum or nonrecombinant vaccines, which have different manufacturing complexities compared to biopharmaceuticals.

Our study found that the type of quality-related RAs were often letters sent to HCPs (n = 45) and less frequently involved recalls (n = 22) (Chapter 3.2.). This finding shows that previous studies that only considered recalls could underestimate the number of quality-related RAs for biopharmaceuticals [85-91]. The high number of follow-up letters

that were issued for the same quality defects accounted for more than half of regulatory letters (26 out of 45 for seven biopharmaceuticals). This finding explains why the number of regulatory letters was higher than the number of recalls for biopharmaceuticals and suggests that it may take time to resolve quality defects. The follow-up letters reflect the willingness of regulators and the industry to keep HCPs updated, so that they can make informed decision based on the most recent information. Our study identified a lower number of recalls (n = 11) for biopharmaceuticals approved in the US compared to what was found in a study by Ebbers et. al. (n = 41) [91], despite both studies applying the same definition for biopharmaceuticals. The different numbers in the studies could be partially attributed to the differences in the methods, including the definitions of cohort and recall and the data collection. Ebbers et. al may have identified recalls for biopharmaceuticals approved before 1995, which were not included in our study. In addition, half of the recalls identified in the study by Ebbers et. al. were unrelated to the manufacturing and quality of biopharmaceuticals, and were therefore not considered as quality-related RAs in our study. Ebbers et. al obtained data on recalls through a direct request to the FDA, whereas we retrieved information on recalls from the public FDA website. However, this is not expected to influence our findings because the FDA has strict policies regarding communicating the recalls to the public [92].



Our study shows that the content of the quality-related RAs differed (Chapter 3.2), which can be explained by the different natures of the underlying quality defects, which requires different types of HCP actions to counter the potential risk to clinical outcomes and patient care. These differences are clear from the most frequent quality defects noted in regulatory letters and regulatory recalls. The regulatory letters were often issued for manufacturing issues (57.7%), mainly GMP deviation, which often affects products in general and sometimes multiple products from the same manufacturer. The regulatory letters often included required HCP actions at the patient level, such as "restrict" (42.2%), "monitor" (35.6%), "switch" (17.8%), and "inform" (17.8%), to overcome the potential risk of shortages associated with manufacturing issues. In contrast, the regulatory recalls were often issued for specification issues (49.9%), mainly particulate matters followed by out-of-specification (OOS) (e.g., OOS in volume, potency, strength, and preservative) that affected specific batches. The regulatory recalls that we could identify included HCP actions at product level, such as "check," "handle," and "recall" to, at least theoretically, counter the potential risk to patient safety, immunogenicity and product efficacy. These findings show that the types of HCP actions depend on the nature of the underlying quality defects and the information available to regulators prior to issuing the RAs.

The quality and applicability of the information on the RAs is important to enable HCPs to understand the nature of the underlying quality defects, potential clinical consequences and the required actions to counter the potential risk of quality defects in

patient care. We observed that the nature of the underlying quality defects in some quality-related RAs was unclear. For example, the Genzyme company reported in the literature that a bioreactor was contaminated with the virus "Vesivirus 2117," which does not cause human infections but impairs the growth of the producing cell line, and they provided a clear description of the underlying quality defects that could not be deduced from the RA [93]. The potential clinical consequences may not always be known to the developer and regulators before communicating the RAs, but the inclusion of such information regarding the clinical consequences, whenever possible, will assist HCPs to be aware of the potential implications for patient care. Previous studies have assessed the quality and applicability of information on monitoring in the summary of product characteristics for psychiatric drugs and a selection of DHPCs and found that the information is insufficient for HCPs in clinical practice [94 - 96]. Since the methodology applied in previous studies only assessed a single HCP action, "monitor," we were unable to assess the quality and applicability of the various types of HCP actions associated with quality-related RAs. This observation emphasizes the need for improvement to enhance the clarity and completeness of the quality-related RAs, so that the quality and applicability of the presented information for clinical practice can be explored in future studies.

## Potential implications of the comparability of (critical) quality attributes of biopharmaceuticals

### Implications for regulation

The studies presented in this thesis show that comparisons of CQAs has become increasingly available in the public domain in line with the increase in the development and approval of biosimilars. This availability has increased knowledge about CQAs, which could have potential implications for the regulation of biopharmaceuticals, especially the regulatory requirements for biosimilar approval. Since the approval of the first biosimilar (Somatropin) in 2006, the EMA has received 121 marketing authorization applications for biosimilars, of which 109 applications have been reviewed and 15 are currently (June 2022) under review. Of the 109 reviewed applications, 86 received positive opinions, two received negative opinions, and the remaining 22 were withdrawn by the companies during the regulatory review process. The negative opinions and application withdrawals for biosimilars were attributed to a lack of comparability to the reference product and selection of reference product and other quality aspects, such as process validation, the DP quality and stability and GMP/GCP compliance and also, in most cases, for commercial reasons [97]. It is not surprising that failure to demonstrate comparability of QAs and safety and efficacy were the main reasons for the unsuccessful regulatory filings of the biosimilars. For example, Alpheon® (interferon alpha 2a) received a negative opinion

from the EMA in 2006 because of large quantitative and qualitative differences in QAs related to impurities and significant differences in the rate of adverse events and the virologic relapse rate compared to the reference product, as well as a failure to demonstrate comparability between batches used in clinical trials and batches of biosimilars produced on a commercial scale.

In recent years, regulators have revisited the regulatory requirements for biosimilars, which was triggered by the advances in scientific knowledge and experience. Such efforts have already led to the omitting of value-limited requirements, such as a reduction in the comparative non-clinical in vivo studies and comparative efficacy trial (phase III). The reduction in non-clinical in vivo studies originated from the inability to detect and assess the impact of the differences in the QAs; the variability and lack of predictability of animal models for humans; and compliance with the principle of the 3Rs (replace, reduce, refine) for animal experiments. The comparative phase III clinical trials are no longer required for the regulatory approval of some (less complex) product classes (e.g., somatotropin, insulin, and filgrastim), if the PD biomarker as a surrogate for efficacy is available and the mechanism of action is clearly understood. However, a comparative efficacy trial is still required for the approval of biosimilars of complex and multifunctional molecules, such as mAbs and fusion proteins. There are ongoing debates among regulators on whether comparative efficacy and safety trials are necessary. The accumulated scientific knowledge and experience of the regulatory assessment of biosimilars have shown that comparative efficacy trials are not sensitive enough compared to a well-designed PK trial to detect differences in QAs, and their role in assessing the impact of minor differences in QAs on functions and clinical outcomes is limited [47-50]. This limitation was clearly demonstrated in two biosimilar applications for Alpheon® (interferon alpha 2a) that were refused in 2006 and a rituximab (from Mabion) application that was withdrawn in 2019 during regulatory review, where the efficacy trials met the primary and secondary endpoints despite major manufacturing issues and QA differences that were detected in comparability exercises of the QAs [98, 99]. In addition, the comparative phase III trials may be associated with ethical and time issues as well as financial burdens for sustainable biosimilar development, which are reasons why the regulators could potentially eliminate (unnecessary) comparative phase III trials and, instead, focus on the comparability of the QAs and comparative PK/PD studies.

In response to the ongoing debate regarding the need for comparative phase III trials, the EMA conducted a pilot study from 2017 to 2020 that aimed to advise manufacturers on how to minimize or avoid comparative clinical trials based on the outcome of the comparability of the QAs. The outcome of this pilot study was published by EMA in September 2021 and indicates that the immaturity of the comparability of QAs submitted by participants did not allow regulators to determine the extent and type of comparative clinical data that is required [100]. Despite this limitation, the initiative has

brought the development of biosimilars a step closer to transitioning to a more tailored regulatory requirement approach. The UK regulators have already made that transition and highlight in their recent guidelines that the outcome of the comparability of QAs and a well-designed comparative PK trial carry significant weight in the approval of biosimilars [48]. The rationale behind this transition is in-depth knowledge of the clinical profile of the reference product, increased knowledge of CQAs, the advancement in analytical technology to detect subtle differences in (C)QAs, the confirmatory PK trial and the robustness of the pharmacovigilance system. Conducting comparative efficacy trials may not be feasible for the biosimilars of, for example, orphan drugs, because the number of patients is limited. This translation is important to reduce (unnecessary) regulatory burdens and promote sustainable biosimilar development.

Currently, the regulatory assessment of post-approval quality changes and biosimilars is rather qualitative, such as looking at "pictures," interpreting and weighing results from comparability exercises of QAs, non-clinical studies, and comparative phase III clinical trials, where needed [101]. In small molecules generics, the regulatory requirements for PK trials to assess bioequivalence have made a quantitative journey over recent decades, enabling significant productivity in generic development. Biosimilar development enables the identification of CQAs for several biopharmaceuticals, and the advancement in analytical technologies has enabled the detection of minor differences between batches and products. However, the follow-up questions involve the effect of a minor difference on clinical outcomes and patient care and to what extent minor differences in CQAs are deemed acceptable. Quantification of the acceptable minor differences in CQAs is based on accumulated regulatory experience with the evolution of post-approval changes, and biosimilars are usually on a path for adoption and innovation, which could lead to early predication of comparability and reshape the regulatory requirements for biosimilars.

The relevance of the comparability of CQAs will increase if regulators decide to reduce unnecessary comparative phase III clinical trials. A critical component in this transition would be the identification and understanding of CQAs and how minor differences in CQAs would affect clinical outcomes. CQAs of biopharmaceuticals are molecule- and process-dependent, but the acceptance criteria for minor differences are not clear. Pharmacopeia organizations can play a crucial role in defining the CQAs and the acceptance criteria when they develop a reference standard for a specific molecule of a biopharmaceutical. Regulators have already gained extensive experience with the regulation of post-approval quality changes in biopharmaceuticals, including biosimilars. Minor differences in CQAs observed in previous applications of post-approval quality changes and biosimilars can be useful for the regulatory assessment of the comparability exercise for (future) applications. As knowledge regarding the CQAs and acceptance criteria may evolve over time with an increase in the knowledge and experience of the

product and process, regulators could develop a tool to monitor the CQAs throughout the life cycle. Developing a tool such as the risk management plan that is currently available to monitor safety concerns of drugs after approval would stimulate continuous regulatory learning about CQAs and the acceptance criteria for biopharmaceuticals.

Our study (Chapters 2.2 and 2.3) shows that the test result of QAs (i.e., actual results in quantitative or qualitative ways) in the comparability exercise are lacking in the EPARs, which may hamper the understanding of the biosimilarity interpretation. For example, it is debatable whether anyone would be able to understand a conclusion on safety and efficacy of a medicine without access to the actual results and outcome of a clinical trial (e.g., adverse events, quality of life, survival, etc.). The same is true for the test results of QAs, which can help readers of the EPARs to make informed decisions (Chapter 2.3). As EPARs are considered to be an unbiased information source, there is great value in providing insight into the comparability of QAs for various stakeholders, such as (future) biosimilar developers and non-European regulators. Biosimilar developers can use EPARs to learn from past success and failures and to predict the regulatory process [102]. Non-European regulators can use EPARs to support their own decision-making processes, relying on the regulatory assessment undertaken by competent authorities that are based on regulatory reliance models. Greater transparency of information on the EPARs can contribute to reduce the timing and cost of biosimilar development and assist non-European regulators to speed up regulatory reviews based on regulatory reliance and convergence to preserve limited resources [103-107].



**Implications for patient care**

The acceptance of biosimilars in clinical practice is hampered by, among other economic and tendering factors, a lack of understanding of the science of biosimilar approval [10, 11]. The benefits of the adoption of biosimilars into clinical practice are not restricted to the reduction in the cost of the treatment for patients and healthcare systems. Biosimilars have the potential to enhance patient access/ease of use/adherence to important biopharmaceutical-based treatments, provide more options in the same therapeutic classes for clinicians, and stimulate therapeutic innovations [108]. Clinicians are always searching for clinical trials that play a pivotal role in the regulatory approval of new medicines. However, biosimilar approval is based on demonstrating comparability rather than reestablishing the safety and efficacy of the reference product. Since biosimilars are biopharmaceuticals, there can be minor differences between the biosimilar and the reference product, which also occurs between batches of the reference product. To illustrate this, if a patient has used the reference product of etanercept for 10 years, compared to the "original batch" produced 10 years ago. the "biosimilar batch" they are current using might have minor differences that are not clinically meaningful. To achieve the benefits of biosimilars for patient care, there is a need for continuing education to

increase HCPs' understanding of the prominent role of the comparability of CQAs and the reduced weight of the evidence from comparative clinical trials in biosimilar approval. Among HCPs, pharmacists are uniquely positioned to take a leading role in the development of educational materials that can aid other HCPs (e.g., clinicians and nurses) and patients to understand the scientific evidence that underpins biosimilar approval. Such efforts could increase confidence in and acceptance of biosimilars in clinical practice to gain the benefits to society and patients offered by biosimilars. However, more consistent and comprehensive information on the comparability of CQAs in EPARs can help pharmacists to explain the clinical meaning of the minor differences between the biosimilar and the reference product. Therefore, pharmacists should be at the forefront of the successful adoption of biosimilars in clinical practice, and clinicians will be in a unique position in the successful utilization of biosimilars for patient care.

## Potential implications for post-approval quality surveillance of biopharmaceuticals

### Implications for regulation

Post-approval surveillance is an important regulatory tool to continue to monitor the safety, efficacy and quality of (bio)pharmaceuticals throughout their life cycle. Various regulatory tools have been developed to specifically monitor safety and efficacy after approval, where the quality surveillance rely on inspections and lot-release testing. It seems that post-approval quality surveillance and post-approval benefit-risk are two separate silos, which probably originated from the pre-approval system. However, we have learned that quality, safety and efficacy are more linked for biopharmaceuticals than for small molecule drugs. This can be illustrated by the Eprex® tragedy in 1998-2004, where a change in the formulation (replacing human serum albumin with polysorbate 80 and glycine) increased the immunogenicity of the DP, resulting in an unexpected increased incidence of pure red cell aplasia in patients who received the new formulation. Several explanations reported that the polysorbate 80 induced immunogenicity by the formation of epoetin-containing micelles, leachable interaction or aggregate formation. Although the Eprex® tragedy never occurred again (June 2022), it demonstrated how the quality of a biopharmaceutical is linked to clinical outcomes, including safety, immunogenicity and efficacy. This relationship poses the question of whether post-approval quality surveillance and post-approval safety and efficacy can also be linked and integrated into a single regulatory tool. This may require a change in the current approach in the regulation, which includes a separate benefit-risk balance and quality surveillance, to a combined approach of benefit-quality-risk balance for biopharmaceuticals. One proposal would be integration of post-approval quality changes and quality-related regulatory actions by expanding the scope of current tools such as the

Periodic Safety Update Reports to accommodate quality aspects of biopharmaceuticals. Moreover, since the current classification of post-approval quality changes is based on regulatory procedures (type IA, IB, II) rather than the potential risk of a change in the quality of biopharmaceuticals, there is a need for a consensus risk classification among regulators. Such a risk classification would be a useful tool for developers and regulators to harmonize regulatory requirements, especially regarding the extent and type of comparability exercises that are required to support post-approval quality changes.

**Implications for patient care**

Post-approval quality changes may impact CQAs, which could affect clinical outcomes and patient care. The CQAs of biopharmaceuticals are required to be consistent to ensure that the therapeutic biological function and clinical outcomes are not affected by post-approval changes and patients are not harmed by quality defects. The consistency of CQAs is important to ensure that patients receive comparable products/batches and harmonized doses of a biopharmaceutical. As a part of the quality management system, companies consistently monitor batches to show robustness and the capability of the manufacturing process of the biopharmaceuticals. Only a few companies report results that show batch-to-batch consistency, which are manufacture-focused and cannot address consistency between reference products and biosimilars [109-112]. Post-approval quality changes can result in a shift or drift in the CQAs, some of which could affect manufacturing consistency and result in product divergence. Not all shift or drift in CQAs is clinically relevant. This has been illustrated by two CQAs (glycosylation and potency), where a small shift was associated with breast pathological complete response (bpCR from 44.1 to 40.1 %) seen in a 3-year follow up phase III trial [113 - 115]. Although this small shift in efficacy was not confirmed in the 5-year follow-up study (no impact on efficacy endpoints i.e., response rate and long-term survival) [116], it does highlight a potential impact, at least theoretical, of product divergence. So far, product divergence has not been reported between reference products and biosimilars or between biosimilars of the same reference product. However, the risk of product divergence is assumed to increase over time, with the number of products and the severity (high-risk) of the changes [117-119]. Since biosimilars and reference products are considered to be standalone products after approval, consistency between the products is important, especially when with consideration of interchangeability or switching between biosimilars and originators [120-123]. One may argue that, in clinical practice, patients are often switched (for (non) medical reasons) between different reference products, such as insulin and epoetin products, without any negative impact on safety and efficacy, despite differences in quality in these products, including cell lines, glycosylation, sialyation and formulation [124]. However, the risk of product divergence between biosimilars and reference products and between biosimilars are assumed to increase over time, with the increase in the

number of products, and with the severity (high-risk) of changes [117-119]. Therefore, there is a need to develop and promote reference standards for biopharmaceuticals, with an emphasis on the CQAs, as these relate to patient care. In recent years, the Expert Committee on Biological Standardization has established the first World Health Organization reference standards for several mAbs [125-129]. Together with comparability exercises, reference standards standardize and harmonize potency estimates and clinical monitoring, which will be useful to HCPs for informed decision making and treatment strategies in clinical practice.

Post-approval quality-related RAs are important tools to minimize the potential risk of quality defects in patient care. The potential clinical consequences of quality defects may not be fully predictable but can be prevented with the actions that are required from the HCPs. The type of HCP actions depends on the nature of the quality defects, which means that there is "no size fits all" solution. For example, the actions required on the patient level to counter manufacturing issues are likely to be "restrict, monitor, and switch" whereas actions required on the product level to contain specification issues would be "check, handle and recall." Our study found variations in the content of quality-related RAs, which can be partially attributed to the different quality defects. However, this finding emphasizes the need to improve the clarity of the content and greater alignment between regulators regarding the type of HCP actions to minimize the potential risk of quality defects. The nature of the underlying quality defects in some quality-related RAs were not clear, such as in the case of manufacturing issues where the exact underlying issue was not explicitly defined in the RAs. This could hamper a complete understanding of the nature of the underlying quality defect, which is important for HCPs to make informed decisions and deal with (future) quality problems. Furthermore, a slight variation between EU and US regulators was observed regarding the type of HCP actions that they recommend for addressing the same quality defects detected in the same product in the two regions. In these cases, the US regulators focused more on HCP actions related to patient care compared to the EU regulators. Although the study intention was not to compare the regulatory behavior in the two regions, this slight variation in the recommendations could cause confusion for HCPs. Therefore, regulators should strive to increase the clarity and extent of the information on the nature of the underlying quality defects, potential clinical consequences and types of HCP actions, which are important elements that can help the HCPs to understand both the quality defects and the actions required to protect the patient.

## Thesis strengths

This thesis provides regulatory learnings of the quality of biopharmaceuticals during both the approval and post-approval phases. The thesis placed more emphasis on CQAs, as these are considered important for clinical outcomes and provide the clinical implications of minor differences for patient care. The use of EPARs as data source to reflect the current regulatory practice is an important strength. The thesis provides insights into post-approval quality changes in TNF-α-i products, especially after the introduction of biosimilars to the market, which complements the current evidence related to post-approval quality changes for biopharmaceuticals and identifies a new challenge to the regulatory system to ensure consistency between reference products and their biosimilars after approval. The thesis also reviewed the first study that explored different types of post-approval quality-related regulatory actions for biopharmaceuticals approved in the EU and the US, with up to 25 years of follow up, which provides a greater overview of quality-related RAs, a longer follow-up period and a larger sample size to provide longitudinal and helicopter views of quality-related RAs of more than 500 biopharmaceuticals in the EU and US markets. We developed a classification of the nature of the underlying quality defects and the type of HCP actions required to reflect current practice in the clinics and pharmacies, which can be used as a framework by HCPs to mitigate the potential risk for patient care and address (future) quality defects.



## Overall conclusion

To conclude, in this thesis, the quality of biopharmaceuticals, with a focus on biosimilars, has been studied through different lenses, namely comparability of CQAs between batches from the same manufacturer and between candidate biosimilars and originator products, and post-approval quality changes and defects that could pose a risk to patient care. All these lenses have different regulatory correlations and actions. Biopharmaceutical developers have to comply with a myriad of regulatory requirements. Some of these are merely technical, for example, specifications or standards. Some are more conceptual, for example, biosimilar pathways for biopharmaceuticals that are highly similar in, for instance, quality and non-clinical and clinical grounds, with the reference product that has already a license and is used in the clinic.

This thesis shows that the comparison of biopharmaceutical products, whether it concerns pre- and post-change batches from the same manufacturer or the comparison of candidate biosimilars and originator products, relies significantly on the comparability of QAs. This thesis has articulated that criticality of the QAs is key in the comparability exercises. The CQAs should be sensitive and specific enough to flag and characterize any

relevant differences between batches or products. However, it is difficult to establish what is relevant and how to determine a difference, both qualitatively and quantitively, and to establish what the found differences will mean for clinical outcomes and patient care. We have shown that all these factors are works in progress. Product developers and regulators across the world are constantly in conversation to balance the scientific and methodological possibilities for proof of likeness and meaningfulness from a benefit-risk perspective for the patient. The various studies in this thesis have also show the need for a measure of criticality of the attributes and that the CQAs included in the comparability are determined by the complexity of the molecules (e.g., growth factors versus mAbs) and the regulatory requirements.

Throughout the work presented in this thesis, we have witnessed the surge in innovation (from both product developer and regulators) to compare QAs of biopharmaceutical products. The introduction of biosimilars has definitely acted as a catalyst, and we have shown that many of the developments have found their way into scientific literature and public assessments reports from regulators. Although this is most likely unintended, the rapid development of biosimilars over recent decades and the eagerness to communicate and engage in conversations on the basis of the comparability exercises have contributed to what we could coin as an "open science exercise." Biosimilar developers, regulators, and, in their slipstream, academic groups have all shown a keen interest in trust building and liaising with the clinics to ensure that biosimilars can be considered reliable products. To this end, this thesis also highlights (again) the notion that modernizing and improving the quality and manufacturing of biopharmaceuticals is not a done deal after approval. The thesis has also shown that the quality of biopharmaceuticals should go hand in hand with safety and efficacy. Post-approval follow-up, surveillance and regulatory action as required remain essential building blocks of a trusted biopharmaceutical system.

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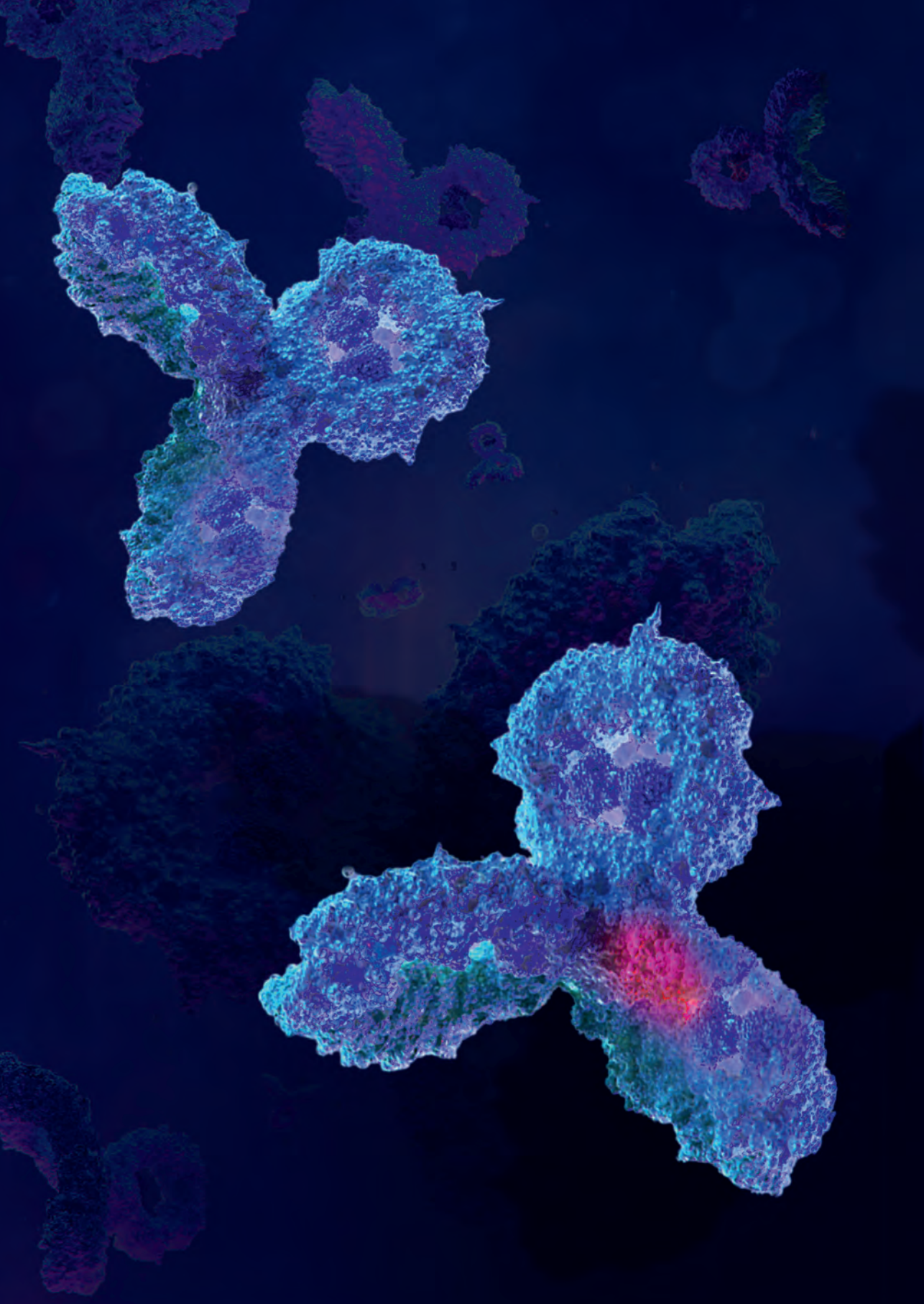
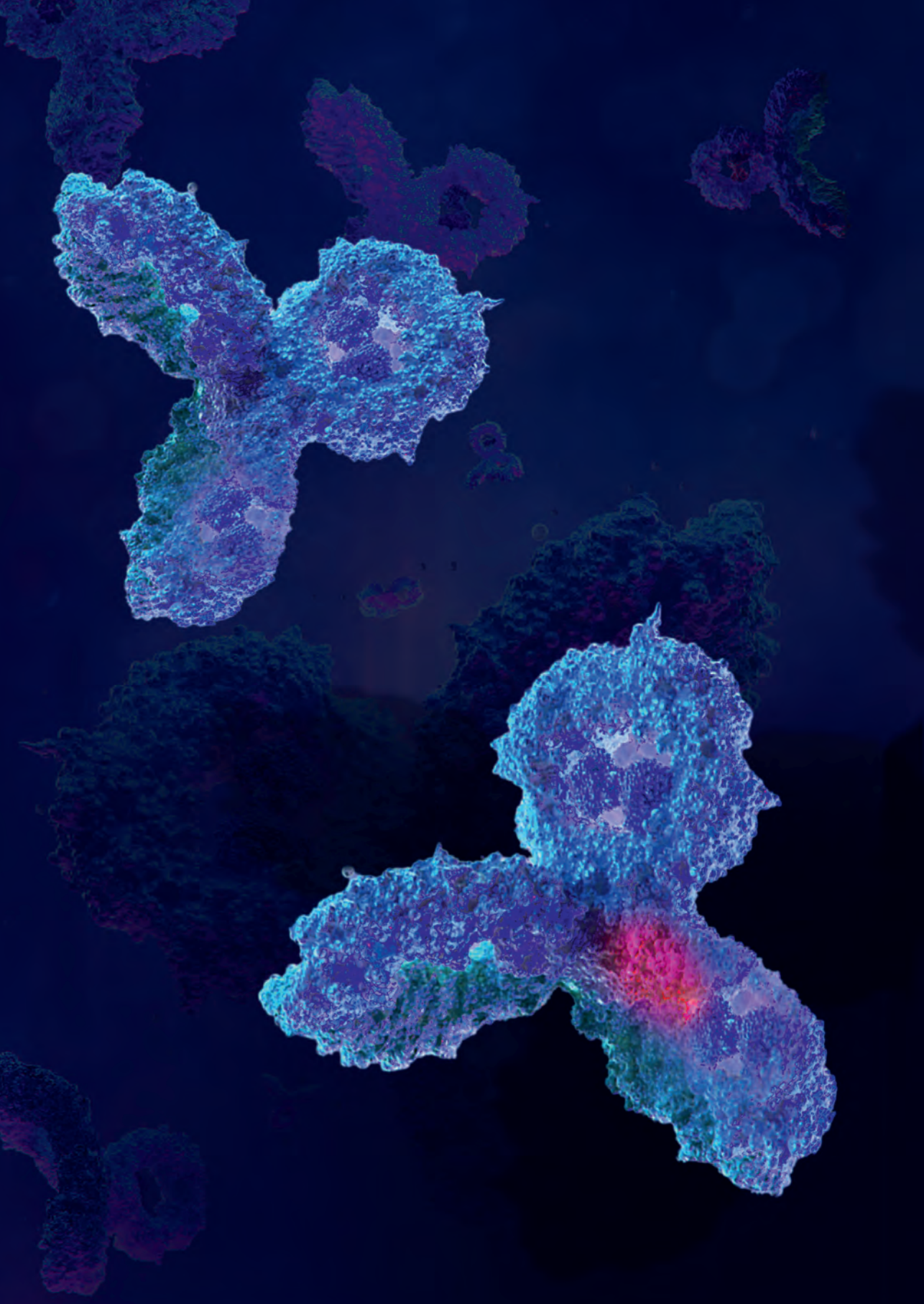


# Chapter 5

## Summaries



# Chapter 5.1

## English summary

In Chapter 1, we introduced the subject of the thesis, which is the quality of biopharmaceuticals. Biopharmaceuticals are a class of biological medicines that are produced in living cells with a biotechnology method, for example, recombinant deoxyribonucleic acid (DNA) technology, which has revolutionized treatments for several acute and chronic diseases, such as cancer, auto-immune diseases, and diabetes. Before the discovery of recombinant DNA technology, most biological medicines were extracted from biological materials from humans, animals, plants, yeasts, and viruses. The first biopharmaceutical produced by recombinant DNA technology was human insulin for diabetes, which was approved in the early 1980s and reduced potency variations and immunological complications associated with the use of animal-derived insulin. Since then, hundreds of biopharmaceuticals, ranging from simple polypeptides such as hormones and growth factors to more complex monoclonal antibodies, were developed and have emerged into clinical practice. Biopharmaceuticals are often expensive, which puts pressure on healthcare budgets and may impede patient access to important medicines. The expiration of patents and exclusivity rights of biopharmaceuticals pave the way for the introduction of biosimilars, which are follow-on biopharmaceuticals that contain highly similar active biological substances to an already approved originator, also known as the reference product.

Compared to small molecule pharmaceuticals, biopharmaceuticals, whether originators or biosimilars, are large and complex molecules produced, in a complex manufacturing process. This complexity results in a biopharmaceutical with various quality attributes (QAs), namely physical, chemical, biological, and microbiological properties. A subset of these QAs is known as critical QAs (CQAs), and these are vulnerable to changes in the process, storage, transportation, and handling of biopharmaceuticals and could potentially influence clinical outcomes and patient care. Because of the molecule and product process complexity, there is inherent variability, and minor differences are often observed in the (C)QAs of biopharmaceuticals, even between batches from the same manufacturer.



The regulatory requirements for the approval of biopharmaceuticals are different for originators and biosimilars. The regulatory approval for originators mainly relies on evidence from clinical trials that prove that the benefits outweigh the risks, whereas biosimilar approval relies significantly on a comparability exercise that demonstrates the biosimilarity of the biosimilar to the originator. The comparability exercise is required for biosimilar approval because the well-established regulatory requirements for small molecule generic drugs are not fit for the purpose of demonstrating the comparability of two versions of biopharmaceuticals from different manufactures.

The European Medicine Agency (EMA) established the first regulatory pathway and developed various guidelines for biosimilars in 2004, which were adopted to a lesser or greater extent by other regulatory and health authorities. This regulatory effort resulted

in the approval of more than 100 biosimilars for 17 reference products approved in the EU and US market. Despite this large number of biosimilars, the acceptance of and confidence in using biosimilars in clinical practice is still impeded by, among other factors, a lack of understanding of the biosimilar pathway.

The biosimilar pathway starts with the development of knowledge of the (C)QAs of the reference product by analyzing multiple batches and then designing own manufacturing process to produce the candidate biosimilar. This step is followed by three comparability exercises to establish the biosimilarity to the reference product. The first comparability exercise is a comparison of the (C)QAs to demonstrate high similarity and detect potential differences between the candidate biosimilar and the reference product. The outcome of the comparison of the (C)QAs determines the extent and type of comparative nonclinical and clinical studies required to rule out the impact of minor differences, if any, and confirm the biosimilarity. Information on (C)QAs can emanate from the regulatory community through public assessment reports and the scientific community through scientific publications in peer-reviewed journals. Previous studies have provided insight into variabilities of certain (C)QAs between batches and products of less complex biopharmaceuticals, such as filgrastim and epoetin, obtained from different markets, but lack information on the (C)QAs that were assessed to support biosimilar approval, especially for more complex biopharmaceuticals such as monoclonal antibodies (mAbs).

The quality, safety, and efficacy of biopharmaceuticals, both originators and biosimilars, are ensured at the time of approval and are continually monitored throughout the life cycle through post-approval surveillance and pharmacovigilance. The post-approval surveillance system comprises various tools in addition to routine and proactive activities. The quality of biopharmaceuticals is mainly monitored through routine regulatory inspections to ensure compliance with good manufacturing practice and lot-release testing, which is mandatory for every single batch of biopharmaceuticals. However, two quality issues can occur after the approval of biopharmaceuticals, namely post-approval changes in the quality and manufacturing of the drug substance (DS)

and the drug product (DP) that require regulatory approval or notifications, and post-approval quality defects that require regulatory actions with instructions for healthcare professionals (HCPs) to minimize the potential risk for clinical outcomes and patient care. Depending on the seriousness of the issue, the regulatory actions range from direct letters or communications to HCPs to product or batch recalls and market withdrawal. Previous studies have investigated the number and risk levels of post-approval quality changes in a group of reference products of mAbs, but little is known about the type of changes, especially after the introduction of biosimilars. Furthermore, studies that investigated post-approval regulatory actions for biopharmaceuticals were either focused on safety and efficacy concerns or investigated the quality issues that led to recalls. Most

importantly, information on how HCPs should address the quality defects to minimize any potential risk to clinical outcomes and patient care remain uncharted.

Therefore, this thesis aims to provide insight into (1) the comparability of QAs with an emphasis on the CQAs (Chapter 2), and (2) post-approval quality-related surveillance and regulatory actions of biopharmaceuticals (Chapter 3). The key findings of Chapters 2 and 3 were discussed in a broader perspective, and combined with the potential implications for biopharmaceutical regulations and patient care that are reflected in Chapter 4.

In Chapter 2.1, we systematically reviewed the reporting of the QAs in the comparability assessments for (intended) biosimilars in peer-reviewed scientific publications. Since there is no consensus classification of the QA types that should be considered in the comparability exercise to support biosimilar approval, we developed a classification of QA types, based on the regulatory guidelines and discussions with experts on the quality assessment of biosimilars at the Medicines Evaluation Board in the Netherlands. We found an increase in the dynamics of publications that present comparability assessments of QAs over time, which suggests that there is a positive attitude toward increasing the available knowledge and also knowledge sharing, which can contribute to improving the understanding of the role of the comparability of (C)QAs in the biosimilar pathway. We also found the (as of 2019) comparability assessments of (C)QAs of only 60% of approved biosimilars reported in scientific publications, which suggests that the comparability exercises of (some) approved biosimilars are missing in scientific publications. Furthermore, the reporting of QA types in the comparability assessment has increased over time, which could be influenced by the development and publication of regulatory guidelines for biosimilars. We also show that the reporting frequencies varied between the QA types, which could be partially explained by the relevance of the QA to the molecule of interest, and what the authors deemed to be interesting enough to publish. In addition, the most frequently reported QA types were related to the function biological activity (94%); followed by structural attributes such as physicochemical properties (81%); post-translation modifications (79%); primary structure (77%); and purity and impurities (73%). The high reporting frequency of these QA types is probably related to the fact that these can provide first impressions and final insights into the (dis)similarity of the molecule. The study concluded that the availability of a complete, publicly accessible (open access) and unbiased comparability assessment of QAs, as part of a trusted and transparent regulatory process, will contribute to increased confidence in and acceptance of biosimilars in clinical practice.



In Chapter 2.2, we compared the consistency and complementarity in the descriptions of the type and extent of information on QAs in the comparability assessments in the European Public Assessment Reports (EPAR) from the regulatory community and in peer-reviewed scientific publications from scientific communities. The type of QAs defined as structural or functional attributes and extent of information were defined

by whether information on test results and biosimilarity interpretation were reported in each source. We used adalimumab biosimilars as a case study because adalimumab has the largest number of biosimilars containing the same active substance that have been approved by the EMA, as of the designing of this study in 2020. To facilitate the comparison, we developed a classification of 77 QAs related to the structure and functions of adalimumab, based on publicly available information. Adalimumab is a fully humanized monoclonal antibody (mAb) that targets tumor necrosis factor-α (TNF-α) and has broad spectrum therapeutic indications for inflammatory diseases among TNF-α inhibitors, such as infliximab and etanercept. We found that the number of reported QAs varied between the EPARs and scientific publications (range = 47 [61%] – 60 [78%]), which could be explained by the different objectives and motivations of the two sources. While the EPARs reflect regulatory assessments, the scientific publications reflect what the author(s) deem to be interesting enough to share. We also found that the functional QAs (mean = 21 QAs [88%]; range = 19–23) were more consistently described in both sources compared to the structural QAs (mean = 33 QAs [62%]; range = 27–34). This finding could be related to the fact that functional QAs can provide useful information to assess the impact of minor differences in structural QAs, reflect the mechanism of the action of adalimumab, and predict the (dis)similarity of clinical outcomes. Moreover, the assessment of functional QAs provide the basis for the assessment of the extrapolation of indications without the need for repeat clinical trials for each indication. Moreover, the EPARs focused on presenting the biosimilarity interpretation, whereas the scientific publications strongly focused on the test results and the biosimilarity interpretation. This finding shows that the sources provide information on QAs in a complementary manner, which means that both sources should be consulted for an improved understanding of the role of structural and functional QAs in establishing the comparability and the mechanism of action of biological substances in general.

In Chapter 2.3, we explored how EU regulators reflect their assessments of the comparability of the QAs for the adalimumab biosimilars approved by the EMA in 2020. In this study, we applied the same methodology described in Chapter 2.2 but we specifically focused on CQAs. We observed a variation in the number of QAs in EPARs of adalimumab biosimilars (35–75%), which could be explained by the fact that EPARs were prepared by different rapporteurs from different member states. This finding emphasizes the need to call for a uniform reporting approach to improve consistency in the reporting of QAs in EPARs, because EPARs are a valuable source of information for several stakeholders involved in biosimilar development (industry), regulation (regulators/policy makers), education, and prescription and use (HCPs) in clinical practice to support their decision-making processes. We proposed the adoption of review templates or to initiate a collaboration research project to improve the content of EPARs related to the comparability assessment of QAs for biosimilars. Furthermore, we found that regulators

diligently focused on the CQAs, which are all considered to be relevant to the functions and clinical outcomes. Most importantly, regulators often observed minor differences in CQAs, such as in glycosylation and charge variants and biological activities, which seem to be quantitively and clinically irrelevant differences that do not preclude the overall comparability of the (C)QAs between the biosimilars and the reference product of adalimumab. However, the omission of the test results in the EPARs could hamper the understanding of the users of EPARs of the regulatory interpretation and identification of the extent of the minor differences in these (C)QAs. Therefore, there should be greater consistency in the reporting of QAs in EPARs, with more emphasis on CQAs to improve the understanding of the relationship between the (C)QAs and the clinical outcomes, which may contribute positively to the adoption of biosimilars in clinical practice.

In Chapter 3.1, we assessed the nature (type and risk level) and timing of post-approval changes that were implemented in a group of biopharmaceuticals, including both originators and biosimilars of TNF-α inhibitors available on the European market up to May 2021. To facilitate the assessment of post-approval changes, we developed a classification of the type and risk level of the changes related to the quality of the DS and the DP of biopharmaceuticals. We found approximately 800 post-approval changes, with the majority of these related to manufacturing and quality control, which reflects that modernization and improvements in the quality and manufacturing of biopharmaceuticals is never finished. The implementation of these changes is likely stimulated by regulatory compliance, technical advances, upscaling, and innovation. The type of post-approval changes implemented to biosimilars were not related to those already introduced to originators, which reflects that biosimilars are standalone products with their own lifecycle after approval. Similar to the findings of Vezér et al., the majority of post-approval changes were rated as low- and medium-risk, which suggests that it is unlikely that the clinically relevant QAs of the DS and DP is influenced by post-approval changes. The low frequency of high-risk changes introduced to originators and biosimilars could be attributed to the possibility that these can potentially impact CQAs, which will, in turn, influence clinical outcomes and patient care. However, no safety or efficacy concerns were identified for the studied products by the post-approval surveillance and pharmacovigilance system, despite the implementation of many post-approval changes. In contrast to the findings in the study by Vezér et al., we found a slightly higher incidence rate of post-approval changes, which might suggest that the majority of these changes were implemented after the publication of Vezér et al.'s study in 2014. Thus, our data shows there is no reason to assume that post-approval quality changes will lead to differences between originators and biosimilars of TNF-α-i products in clinical practice.

In Chapter 3.2, we conducted a retrospective analysis to determine the type, content, and frequency of quality-related regulatory actions for biopharmaceuticals approved in the EU and US between January 1995 and December 2019, from their market autho-

rization date until August 2021. We found that the proportion of biopharmaceuticals (12.5%) that was subject to at least one quality-related regulatory action was lower than what was reported by Giezen et. al. (23.6%) on biopharmaceuticals that required safety-related regulatory actions. The difference could be attributed to the increase in knowledge on safety after the approval of the products, with the increase in experience and use of the products in clinical practice, whereas quality always has to be controlled to ensure consistency and prevent biopharmaceuticals with quality defects from affecting patients. The lower probability of biopharmaceuticals to be subject to quality-related regulatory actions could be attributed to the implementation of post-approval surveillance and pharmacovigilance, manufacturing control activities such as inspection and lot-release testing of biopharmaceuticals, the development of regulatory strategies such as quality-by-design, the advance in analytical technologies, and the high cost of the manufacturing of biopharmaceuticals. We also found that the type of regulatory actions were more often letters to HCPs and less frequently recalls, which shows that a focus on recalls may underestimate the number of quality-related regulatory actions for biopharmaceuticals. The letters often resulted from manufacturing issues that could potentially result in a shortage, which required HCP actions such as restrict, monitor, switch, and inform at the patient level. Recalls were often issued because of specification issues that could potentially result in lower/higher potency or, at least theoretically, trigger immunogenicity, which requires HCP actions such as check, handle, and recall at the product level. This finding shows that the type of HCP actions recommended by regulators depend on the underlying quality defects. We also found a variation in the content of quality-related regulatory actions, which can be partially explained by the differences in the underlying quality defects. Despite the highly advanced regulatory systems in the EU and the US, quality-related regulatory actions were issued for one out of eight biopharmaceuticals. Manufacturers and regulators should continue to strive to reduce the occurrence of any quality defects that may impact patient care. Further studies are required to assess the effectiveness and impact of the recommended HCP actions on clinical practice and patient care.

In Chapter 4, the key findings from the individual chapters were discussed in a broader perspective, with two themes, namely comparability of (C)QAs and post-approval quality surveillance of biopharmaceuticals. This discussion was followed by a reflection on the implications of the key findings of the thesis for biopharmaceutical regulation and patient care.

In the first discussion theme in Chapter 4, we elaborated on the way comparability exercises are applied in two different scenarios, namely "post-approval quality changes" and "biosimilar pathway." We discussed why the comparability of (C)QAs is important in the regulation of biopharmaceuticals and why knowledge of the (C)QAs of a molecule is key to establishing the specification and comparability of biopharmaceuticals. We

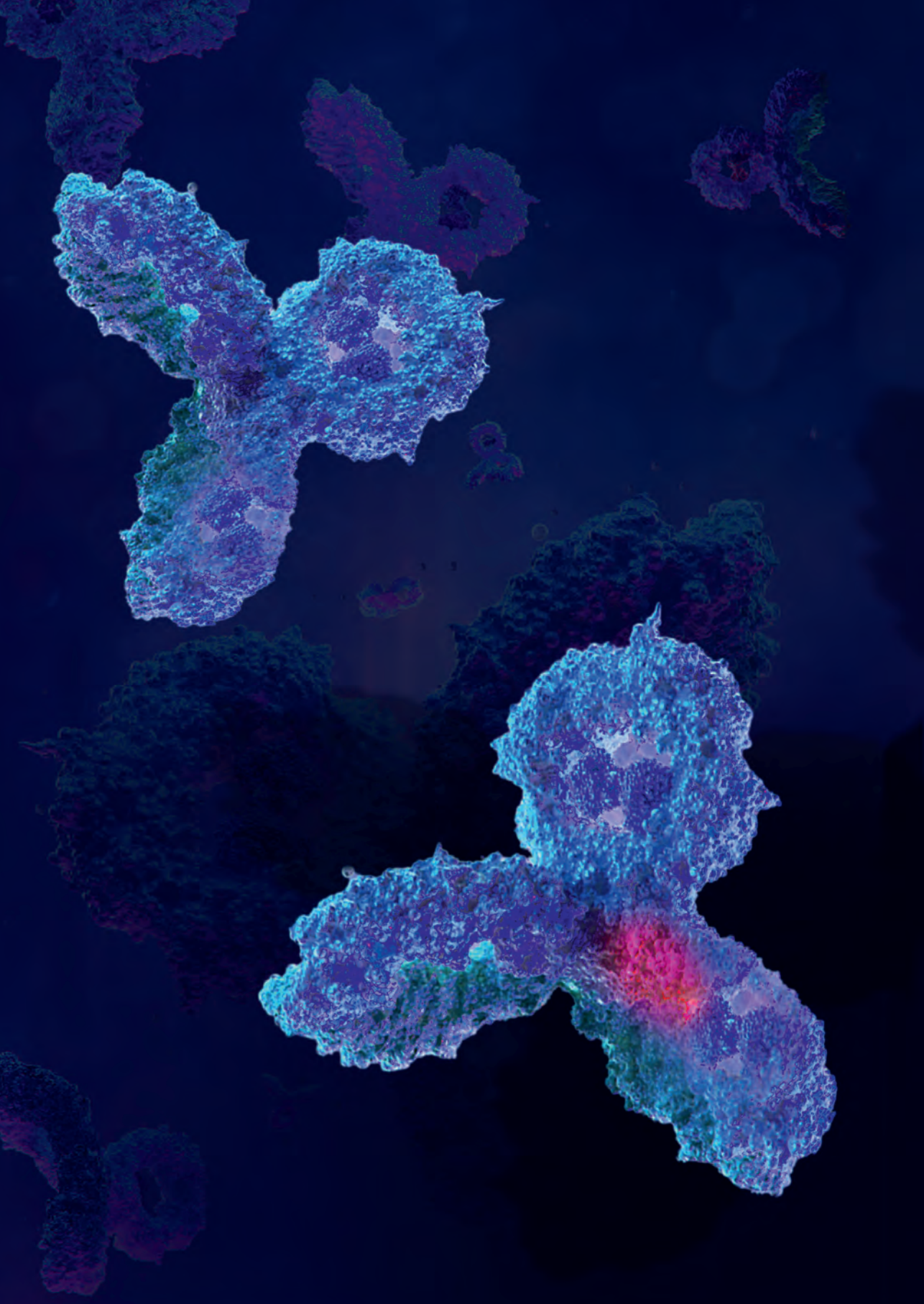
discussed why information on the comparability of (C)QAs for biosimilars should be available in the public domain. We explained what renders a QA a CQA, based on the risk assessment tools endorsed by regulators, and why CQAs are relevant. We showed that regulators often observe minor differences in CQAs during the regulatory assessment of biosimilars. However, it is important that the minor differences in (C)QAs do not impact the safety and efficacy of the biosimilar. The rapid advances in analytical technology has enabled the precise detection of minor differences, but the challenge is to understand whether the minor differences will impact clinical outcomes and patient care. We reflected on the potential implications of the availability of information on the comparability of (C)QAs in biopharmaceutical regulation, especially the reshaping of regulatory requirements for biosimilars. Biosimilar regulation is evolving, and regulators continue to emphasize the comparability of (C)QAs, which could result in a reduction in unnecessary comparative clinical trials in the near future. The current assessment is based on assessing the product with different comparability exercises, such a quantitative approach for acceptable minor differences that can lead to early prediction of the comparability and transition to tailored scientific-based requirements. The availability of comprehensive knowledge on the comparability of (C)QAs for biosimilars can assist HCPs to understand the science behind biosimilar approval and the clinical effects of minor differences in CQAs on patient care. The availability of this knowledge could contribute to increasing the acceptance of biosimilars in clinical practice, so that patients and healthcare systems can achieve the benefits of the biosimilars.



In the second discussion theme in Chapter 4, we discussed the importance of post-approval surveillance to ensure that the quality, safety and efficacy of (bio)pharmaceuticals are consistent and fit for the purpose of patient use throughout their life cycle. We elaborated on two quality aspects, including post-approval quality changes and quality-related regulatory actions. We discussed why quality changes were more frequently related to manufacturing and quality control for both originators and biosimilars of TNF-α inhibitors and what factors may trigger the implementation of quality changes after approval. We discussed why the proportion of biopharmaceuticals that were subject to quality-related regulatory actions was lower than those that received safety-related regulatory actions and the factors that influence this low probability. We explained why quality-related regulatory actions were more frequently in the form of letters to HCPs than recalls and why the content of quality-related regulatory actions vary. This variation was attributed to the different underlying quality defects that require different types of HCP actions to minimize the potential risk to clinical outcomes and patient care. We reflected on potential implications for the regulation of biopharmaceuticals if post-approval quality surveillance and post-approval benefits-and-risk are two separate silos that originated from the pre-approval system. However, we learned that there is a greater link between quality, safety and efficacy for biopharmaceuticals

than for small molecule pharmaceuticals. This means that biopharmaceutical regulation has to advance toward a combined approach, where quality can be integrated with benefit-and-risk through expanding the scope of the current post-approval regulatory tools. The biopharmaceutical regulation also needs to develop risk-based classification for post-approval quality changes, as the current classification is based on regulatory procedures of post-approval variation applications (i.e., type IA, IB and II). We also reflected on the potential implications of quality changes and quality-related regulatory actions for patient care. Some quality changes may influence the consistency of CQAs of a biopharmaceutical, which, in turn, could affect clinical outcomes and patient care. The current regulatory practice ensures the consistency of CQAs in batches from the same manufacturer. Since biosimilars are not compared to the originator after approval, there is a need to develop and promote the use of reference standards to ensure the consistency of CQAs between batches and products. The implementation of a reference standard could, at least theoretically, address the potential impact of product divergence and ensure that patients receive products with harmonized doses and comparable effects. In addition, quality-related regulatory actions are issued to minimize the potential risk of quality defects to patient care. Regulators should strive to increase the clarity and completeness of the content of quality-related regulatory actions, which is important because it can assist HCPs to make informed decisions and prepare for (future) quality problems.

The general discussion in Chapter 4 was wrapped up with an overall conclusion. The thesis investigated the quality of biopharmaceuticals throughout the lifecycle, pre- and post-approval, and studied both manufacturing changes and quality-related regulatory actions of marketed biopharmaceuticals and specifically focused on the pre-approval quality assessment of biosimilars. The studies reviewed for this thesis have shown that the comparison of biopharmaceuticals relies significantly on the comparability of (C)QAs and articulated that the criticality of QAs is key in the comparability exercise and flagging of minor differences. We have shown that work on questions regarding the potential effects of minor differences in clinical outcomes and patient care is in progress. Finally, this thesis also highlights (again) the notion that improving the quality and manufacturing of biopharmaceuticals is not finished after approval. Post-approval follow-up, surveillance, and regulatory action when needed remain essential building blocks of a trusted biopharmaceutical system.



# Chapter 5.2

## Nederlandse samenvatting

In hoofdstuk 1 is het onderwerp van het proefschrift, de kwaliteit van biologische geneesmiddelen, geïntroduceerd. Binnen de biologische geneesmiddelen is een specifieke groep geneesmiddelen welke in levende cellen wordt geproduceerd met een biotechnologische methode, bijvoorbeeld recombinant deoxyribonucleïnezuur (DNA)-technologie, welke een revolutie teweeg heeft gebracht in de behandeling van verschillende acute en chronische ziekten, zoals kanker, auto-immuun ziekten en diabetes. Voor de ontdekking van recombinant DNA-technologie werden de meeste biologische geneesmiddelen geëxtraheerd uit biologisch materiaal van mensen, dieren, planten, gisten en virussen. Het eerste biologische geneesmiddel geproduceerd met recombinant DNA-technologie was humaan insuline voor de behandeling van diabetes, dat begin jaren tachtig is goedgekeurd voor gebruik door patiënten. Insuline geproduceerd met recombinant DNA-technologie verminderde, in vergelijking met het tot dan toe in gebruik zijnde insuline geëxtraheerd vanuit een dierlijke bron, variaties in potentie tussen batches en immunologische complicaties. Sindsdien zijn honderden biologische geneesmiddelen ontwikkeld, variërend van eenvoudige polypeptiden, zoals hormonen en groeifactoren, tot complexere monoklonale antilichamen, welke in de klinische praktijk worden gebruikt. Biologische geneesmiddelen zijn vaak duur, wat druk legt op de budgetten van de gezondheidszorg en de toegang van patiënten tot belangrijke geneesmiddelen kan belemmeren. Het verstrijken van octrooien en exclusiviteitsrechten van biologische geneesmiddelen maken de weg vrij voor de introductie van biosimilars. Biosimilars zijn in hoge mate gelijkwaardig aan een bestaand biologisch geneesmiddel dat al in de EU in de handel is gebracht, ook wel bekend als het referentiegeneesmiddel.

In vergelijking met chemisch gesynthetiseerde geneesmiddelen zijn biologische geneesmiddelen, of het nu gaat om referentiegeneesmiddelen of biosimilars, grote en complexe moleculen die worden geproduceerd middels een complex productieproces. Deze complexiteit resulteert in een biologisch geneesmiddel met een groot aantal kwaliteitskenmerken, ook wel quality attributes (QA's) genoemd, namelijk fysische, chemische, biologische en microbiologische eigenschappen. Een subset van deze QA's staat bekend als kritische kwaliteitskenmerken, ook wel critical quality attributes (CQA's) genoemd, welke kwetsbaar zijn voor veranderingen in het traject van productie tot toedieningen en welke mogelijk invloed hebben op de effectiviteit en veiligheid en daarmee op de patiëntenzorg. Vanwege de complexiteit van het molecuul en het productieproces is er inherente variabiliteit, en kleine verschillen worden regelmatig waargenomen in de (C)QA's van biologische geneesmiddelen, zelfs tussen batches van dezelfde fabrikant.

De wettelijke eisen voor de regulatoire goedkeuring van biologische geneesmiddelen verschillen tussen het referentiegeneesmiddel en de biosimilar. De wettelijke goedkeuring voor het referentiegeneesmiddel berust voornamelijk op bewijs uit klinische onderzoeken waarin wordt aangetoond dat de klinische voordelen opwegen tegen de

risico's. Daarnaast dient het referentiegeneesmiddel aan te tonen dat er een robust en stabiel productieproces is waardoor de potentiële verschillen tussen de kwaliteitskenmerken geen invloed hebben op de klinische effectiviteit en veiligheid Goedkeuring van een biosimilar is in belangrijke mate afhankelijk van uitgebreide onderzoeken waarin de gelijkwaardigheid van de biosimilar ten aanzien van het referentiegeneesmiddel wordt aangetoond. Het uitgebreide aantal vergelijkende onderzoeken is vereist voor de goedkeuring van een biosimilar omdat de wettelijke vereisten voor registratie van chemisch gesynthetiseerde generieke geneesmiddelen niet geschikt zijn om de gelijkwaardigheid aan te tonen tussen twee biologische geneesmiddelen geproduceerd door verschillende farmaceuten.

Het Europees Geneesmiddelenbureau (EMA) introduceerde in 2004 een speciaal registratietraject voor biosimilars en ontwikkelde in de jaren na 2004 verschillende aanvullende richtlijnen voor de ontwikkeling en registratie van biosimilars. Deze richtlijnen zijn in meer of mindere mate overgenomen door andere registratieautoriteiten. Inmiddels zijn er meer dan 100 biosimilars voor 17 referentiegeneesmiddelen geregistreerd in de EU en de VS. Ondanks dit grote aantal biosimilars wordt de acceptatie en het vertrouwen in het gebruik van biosimilars in de klinische praktijk nog steeds belemmerd door, onder andere, een gebrek aan kennis over het registratietraject en de eigenschappen van biosimilars.

De ontwikkeling van een biosimilar begint met het verzamelen van kennis van de (C)QA's van het referentiegeneesmiddel door meerdere verschillende batches van het referentiegeneesmiddel tot in detail te analyseren en hierop het eigen productieproces voor de potentiële biosimilar te optimaliseren. Deze stap wordt gevolgd door drie stappen waarmee gelijkwaardigheid met het referentiegeneesmiddel wordt vastgesteld. De eerste stap is een vergelijking van de (C)QA's waarmee gelijkwaardigheid en mogelijk (kleine) verschillen tussen de biosimilar en het referentiegeneesmiddel worden onderzocht. Het resultaat van de vergelijking van de (C)QA's bepaalt de omvang en het type onderzoek welke nodig is om de impact van eventuele kleine verschillen uit te sluiten en de gelijkwaardigheid te bevestigen. Dit onderzoek vindt plaats middels niet-klinische (of preklinische) en klinische onderzoeken. Informatie over (C)QA's kan inzichtelijk worden gemaakt via de regelgevende instanties, zoals de EMA, middels openbare beoordelingsrapporten en via de wetenschappelijke gemeenschap middels wetenschappelijke publicaties in peer-reviewed tijdschriften. Eerdere studies hebben inzicht gegeven in de variabiliteit van bepaalde (C)QA's tussen batches van dezelfde farmaceut en tussen producten van verschillende farmaceuten van minder complexe biologische geneesmiddelen, zoals filgrastim en epoëtine, verkregen van verschillende landen. Deze studies hebben echter geen onderzoek gedaan naar beschikbare informatie over (C)QA's die werden beoordeeld om de goedkeuring van biosimilars te ondersteunen. Met name

voor meer complexe biologische geneesmiddelen zoals monoklonale antilichamen, is deze informatie beperkt beschikbaar.

De kwaliteit, veiligheid en werkzaamheid van biologische geneesmiddelen, zowel referentiegeneesmiddelen als biosimilars, zijn gewaarborgd op het moment van goedkeuring en worden voortdurend gemonitord gedurende de levensduur door middel van toezicht na goedkeuring en geneesmiddelenbewaking. Het bewakingssysteem na goedkeuring omvat naast routinematige en proactieve activiteiten verschillende instrumenten. De kwaliteit van biologische geneesmiddelen wordt voornamelijk gecontroleerd door middel van routinematige inspecties om ervoor te zorgen dat de geldende regels voor de productie van biologische geneesmiddelen worden nageleefd en middels testen welke worden uitgevoerd op elke afzonderlijke batch en waarmee wordt aangetoond dat verschillende batches van hetzelfde biologische geneesmiddel gelijkwaardig zijn. Na registratie kunnen er bedoelde en onbedoelde wijzigingen in de (C)QA's van biologische geneesmiddelen optreden. Voor bedoelde wijzigingen in het productieproces met als gevolg veranderingen in de kwaliteit en productie van het actieve bestanddeel (Drug substance (DS)) dan wel in het uiteindelijke product, actieve bestanddeel inclusief o.a. hulpstoffen (Drug Product (DP)) is goedkeuring of tenminste kennisgeving aan de regulatoire autoriteiten vereist. Een tweede groep betreft onbedoelde kwaliteitsgebreken welke worden ontdekt na regulatoire goedkeuring en waarvoor maatregelen nodig zijn met instructies voor beroepsbeoefenaren in de gezondheidszorg om het potentiële risico op klinische effecten en de patiëntenzorg te minimaliseren. Afhankelijk van de ernst van het probleem variëren de maatregelen van brieven of mededelingen aan beroepsbeoefenaren in de gezondheidszorg tot terugroepacties van producten of specifieke batches en terugtrekking uit de markt. Eerdere studies hebben het aantal en potentiële invloed van kwaliteitsveranderingen op de effectiviteit en veiligheid na goedkeuring in een groep referentiegeneesmiddelen van monoklonale antilichamen onderzocht, maar er is weinig bekend over de frequentie en het type veranderingen na de introductie van biosimilars. Bovendien waren studies die maatregelen na goedkeuring voor biologische geneesmiddelen onderzochten ofwel gericht op problemen met betrekking tot de veiligheid en/ of werkzaamheid of er werd specifiek gekeken naar de kwaliteitsproblemen die tot terugroepacties leidden. Het belangrijkste is dat informatie over de wijze waarop beroepsbeoefenaren in de gezondheidszorg de kwaliteitsgebreken moeten behandelen om een potentieel risico voor de klinische praktijk en patiëntenzorg te minimaliseren niet uitgebreid bestudeerd is in eerdere studies.

Daarom beoogt dit proefschrift inzicht te geven in (1) de gelijkwaardigheid van QA's met de nadruk op de CQA's (hoofdstuk 2), en (2) controle op kwaliteitskenmerken na registratie en acties van regulatoire autoriteiten ten aanzien van de kwaliteit van biologische geneesmiddelen (hoofdstuk 3). De belangrijkste bevindingen van de hoofdstukken 2

en 3 zijn in een breder perspectief besproken, en gecombineerd met de mogelijke implicaties voor regelgeving en patiëntenzorg in hoofdstuk 4.

In hoofdstuk 2.1 hebben we systematisch de rapportage van de QA's beoordeeld welke is uitgevoerd om gelijkwaardigheid aan te tonen tussen de (beoogde) biosimilar en het referentiegeneesmiddel en is gepubliceerd in peer-reviewed wetenschappelijke publicaties. Aangezien er geen consensus bestond over de classificatie van QA's welke gebruikt wordt voor het aantonen van gelijkwaardigheid tussen biosimilar en referentiegeneesmiddel , hebben we een classificatiesysteem van QA's ontwikkeld. Hierbij zijn we uitgegaan van de richtlijnen van de regulatoire autoriteiten en discussies met experts van het College ter Beoordeling van Geneesmiddelen in Nederland welke betrokken zijn bij de kwaliteitsbeoordeling van biosimilars. We vonden in de loop van de tijd een toename in het aantal publicaties waarin het vergelijkende onderzoek van QA's tussen biosimilar en referentiegeneesmiddel werd vastgelegd. Dit suggereert dat er een positieve houding is ten opzichte van het vergroten van de beschikbare kennis en ook het delen van kennis, wat kan bijdragen aan het verbeteren van het begrip van de rol van de gelijkwaardigheid van (C)QA's in het registratietraject van biosimilars. We vonden ook dat de vergelijkende onderzoeken van (C)QA's voor slechts 60% van de goedgekeurde biosimilars gerapporteerd is in wetenschappelijke publicaties, wat impliceert dat de vergelijkende onderzoeken van (sommige) goedgekeurde biosimilars ontbreekt in wetenschappelijke literatuur. Bovendien is de rapportage van de verschillende QA's in de vergelijkende onderzoeken in de loop van de tijd toegenomen, wat zou kunnen zijn beïnvloed door de ontwikkeling en publicatie van richtlijnen voor biosimilars door de regulatoire autoriteiten. We lieten ook zien dat de frequentie waarmee verschillende QA's worden gerapporteerd varieert, wat gedeeltelijk kon worden verklaard door het type QA welke in meer of mindere mate van belang is voor het molecuul, en wat de auteurs interessant genoeg vonden om te publiceren. Bovendien waren de meest gerapporteerde QA's gerelateerd aan de werking van het molecuul en daarmee aan de biologische activiteit (94%); gevolgd door kenmerken gerelateerd aan de structuur zoals fysisch-chemische eigenschappen (81%); post-translationele modificaties (79%); primaire structuur (77%); en zuiverheid en onzuiverheden (73%). De hoge rapportagefrequentie van deze QA's houdt waarschijnlijk verband met het feit dat deze een goed beeld geven ten aanzien van de gelijkwaardigheid van de biosimilar ten opzichte van het referentiegeneesmiddel . De studie concludeerde dat de beschikbaarheid van een volledige, publiek toegankelijke (open access) beoordeling van de gelijkwaardigheid van QA's, als onderdeel van een vertrouwenswaardig en transparant ontwikkelings- en registratieproces, zal bijdragen tot een groter vertrouwen in en acceptatie van biosimilars in de klinische praktijk.

In hoofdstuk 2.2 vergeleken we de consistentie en complementariteit van de beschreven informatie over het type QA en de omvang hiervan in de vergelijkende

onderzoeken in de openbare beoordelingsrapporten van de EMA en in peer-reviewed wetenschappelijke publicaties van de wetenschappelijke gemeenschap. Het type QA gedefinieerd als zijnde structurele (gerelateerd aan de structuur) of functionele (gerelateerd aan de biologische/ farmacologische activiteit) kenmerken en de omvang van de informatie werden bepaald door de vraag of informatie over testresultaten en interpretatie van gelijkwaardigheid in elke bron werd gerapporteerd. We gebruikten biosimilars van adalimumab omdat, op het moment dat deze studie werd uitgevoerd, adalimumab het grootste aantal biosimilars had welke zijn goedgekeurd door de EMA. Om de vergelijking te kunnen uitvoeren, hebben we een classificatiesysteem ontwikkeld van 77 QA's gerelateerd aan de structuur en functies van adalimumab, gebaseerd op openbaar beschikbare informatie. Adalimumab is een volledig gehumaniseerd monoklonaal antilichaam dat zich richt op tumornecrosefactor-α (TNF-α) en is binnen de groep van de TNF-α-remmers, waar ook infliximab en etanercept onder vallen, geregistreerd voor de meeste indicaties. We ontdekten dat het aantal gerapporteerde QA's varieerde tussen de openbare beoordelingsrapporten en wetenschappelijke publicaties (bereik = 47 [61%] - 60 [78%]), wat kon worden verklaard door de verschillende doelstellingen van de twee bronnen. Hoewel de openbare beoordelingsrapporten de beoordelingen door de regulatoire autoriteiten rapporteren, rapporteren de wetenschappelijke publicaties wat de auteur(s) interessant genoeg achten om te delen. We ontdekten eveneens dat de QA's gerelateerd aan functionele kenmerken (gemiddelde = 21 QA's [88%]; bereik = 19-23) consistenter werden beschreven in beide bronnen in vergelijking met de QA's gerelateerd aan structurele kenmerken (gemiddelde = 33 QA'S [62%]; bereik = 27-34). Deze bevinding zou verband kunnen houden met het feit dat functionele QA's nuttige informatie kunnen bieden om de impact van kleine verschillen in structurele QA's te beoordelen. Functionele QA's zijn gerelateerd aan het werkingsmechanisme van adalimumab, voorspellen daarmee de gelijkwaardigheid van klinische uitkomsten en vormen de basis voor de beoordeling van de extrapolatie van indicaties zonder dat voor elke indicatie herhaalde klinische onderzoeken nodig zijn. Bovendien concentreerden de openbare beoordelingsrapporten zich op het presenteren van de interpretatie van de gevonden resultaten, terwijl de wetenschappelijke publicaties sterk gericht waren op de testresultaten en de interpretatie van de gevonden resultaten. Deze bevinding toont aan dat de bronnen complementaire informatie over QA's verstrekken, wat betekent dat beide bronnen moeten worden geraadpleegd voor een beter begrip over de rol van structurele en functionele QA's bij het vaststellen van de gelijkwaardigheid en het werkingsmechanisme van biologische geneesmiddelen.

In hoofdstuk 2.3 hebben we onderzocht op welke manier regulatoire autoriteiten in de EU hun beoordelingen van de gelijkwaardigheid van de QA's voor de biosimilars van adalimumab rapporteren. Hiervoor hebben we de adalimumab biosimilars meegenomen welke tot en met 2020 door de EMA zijn goedgekeurd. In deze studie hebben

we dezelfde methodologie toegepast welke is beschreven in hoofdstuk 2.2, maar we hebben ons specifiek gericht op CQA's. We constateerden een variatie in het aantal gerapporteerde CQA's in openbare beoordelingsrapporten van biosimilars van adalimumab (35-75%), wat kan worden verklaard door het feit dat openbare beoordelingsrapporten zijn opgesteld door verschillende rapporteurs uit verschillende lidstaten. Deze bevinding benadrukt de noodzaak om te komen tot een uniforme rapportage zodat QA's in openbare beoordelingsrapporten consistenter worden gerapporteerd. Openbare beoordelingsrapporten zijn een waardevolle informatiebron voor verschillende belanghebbenden die betrokken zijn bij de ontwikkeling van biosimilars (industrie), regelgeving (beleidsmakers), onderwijs, en voorschrijvers en gebruikers in de klinische praktijk. We stelden voor om standaard sjablonen te ontwikkelen voor de rapportage van QA's in openbare beoordelingsrapporten of om een onderzoeksproject te starten met als doel de inhoud van openbare beoordelingsrapporten met betrekking tot de gelijkwaardigheid van QA's voor biosimilars te verbeteren. Bovendien ontdekten we dat regulatoire autoriteiten met name CQA's rapporteerden, welke als relevant worden beschouwd voor het werkingsmechanisme en de klinische resultaten. Het belangrijkste is dat toezichthouders vaak kleine verschillen in CQA's observeerden, zoals in glycosylering en biologische activiteit, welke vervolgens zijn beoordeeld als zijnde klinisch niet relevant en gelijkwaardigheid van de (C)QA's tussen de biosimilar en het referentiegeneesmiddel niet uitsluiten. Aangezien testresultaten niet worden gerapporteerd in de bestudeerde openbare beoordelingsrapporten kan de interpretatie en de vaststelling van de omvang van deze kleine verschillen in (C)QA's het begrip bij de zorgverleners en patiënten kunnen belemmeren. Om die reden is het van belang dat er meer consistentie komt in de rapportage van QA's in openbare beoordelingsrapporten, met meer nadruk op CQA's om het begrip van de relatie tussen de (C)QA's en de klinische resultaten te verbeteren, wat een positieve bijdrage kan leveren aan de acceptatie van biosimilars in de klinische praktijk.

In hoofdstuk 3.1 beoordeelden we de aard (type en risiconiveau) en het moment van wijzigingen in de kwaliteitskenmerken welke werden geïmplementeerd na goedkeuring van een groep biologische geneesmiddelen, waaronder zowel referentiegeneesmiddelen als biosimilars van TNF-α-remmers die beschikbaar zijn op de Europese markt tot mei 2021. Om de beoordeling van wijzigingen na goedkeuring te vergemakkelijken, hebben we een classificatiesysteem ontwikkeld van het type en het risiconiveau van de wijzigingen met betrekking tot de kwaliteit van de DS en de DP van biologische geneesmiddelen. We vonden ongeveer 800 wijzigingen na goedkeuring, waarvan de meeste betrekking hadden op productie en kwaliteitscontrole, wat impliceert dat modernisering en verbeteringen in de kwaliteit en productie van biologische geneesmiddelen continu plaatsvindt. De implementatie van deze veranderingen wordt waarschijnlijk gedreven door naleving van de regelgeving, technische vooruitgang, opschaling en innovatie. Het

type wijzigingen na goedkeuring dat voor biosimilars werd doorgevoerd, hield geen verband met de wijzigingen die al bij de referentiegeneesmiddelen waren aangebracht, wat bevestigt dat biosimilars op zichzelf staande producten zijn met een eigen levenscyclus na goedkeuring. Net als bij de bevindingen van Vezér et al., werden de meeste wijzigingen na goedkeuring beoordeeld als zijnde een laag en gemiddeld risico, wat suggereert dat het onwaarschijnlijk is dat de wijziging van de kwaliteitskenmerken van de DS en DP na regulatoire goedkeuring invloed heeft op de effectiviteit en veiligheid. De lage frequentie waarmee veranderingen met een hoog risico bij referentiegeneesmiddelen en biosimilars werden geïntroduceerd, kan mogelijk worden toegeschreven aan de invloed welke deze kunnen hebben op CQA's. Dit kan op hun beurt de klinische resultaten van het biologische geneesmiddel en daarmee de patiëntenzorg beïnvloeden. Er werden echter geen problemen met de veiligheid en/ of de effectiviteit vastgesteld voor de bestudeerde producten binnen het systeem van geneesmiddelenbewaking, ondanks de implementatie van de vele wijzigingen. In tegenstelling tot de bevindingen in de studie van Vezér et al., vonden we een iets hogere incidentie van wijzigingen, wat zou kunnen suggereren dat de meerderheid van de wijzigingen werden doorgevoerd na de publicatie van de studie van Vezér et al. in 2014. De reden voor deze bevinding vereist verder onderzoek. Onze gegevens tonen aan dat er geen reden is om aan te nemen dat wijzigingen in kwaliteitskenmerken na goedkeuring zullen leiden tot verschillen tussen referentiegeneesmiddelen en biosimilars van TNF-α-remmers in de klinische praktijk.

In hoofdstuk 3.2 hebben we een retrospectieve analyse uitgevoerd welke zich richt op het ingrijpen door regulatoire autoriteiten in verband met kwaliteitsproblemen van biologische geneesmiddelen. Hierbij is gekeken naar het type, de inhoud en de frequentie van ingrijpen voor biologische geneesmiddelen welke tussen januari 1995 en december 2019 in de EU en de VS zijn goedgekeurd. We ontdekten dat het aandeel biologische geneesmiddelen (12,5%) waarbij ten minste éénmaal moest worden ingegrepen door de regulatoire autoriteiten lager lag dan wat werd gemeld door Giezen et. al. (23,6%). Giezen et al. heeft zich echter specifiek gericht op regulatoire maatregelen in verband met de veiligheid van biologische geneesmiddelen. Het verschil kan worden toegeschreven aan de toename van kennis over veiligheid na de goedkeuring van de producten, met een toename van de ervaring en het gebruik van de producten in de klinische praktijk, terwijl de kwaliteit altijd moet worden gecontroleerd om consistentie te garanderen en te voorkomen dat biologische geneesmiddelen met kwaliteitsgebreken patiënten treft. De lagere kans dat biologische geneesmiddelen worden onderworpen aan maatregelen door de regulatoire autoriteiten in verband met kwaliteitsproblemen kan mogelijk worden toegeschreven aan het systeem van toezicht en geneesmiddelenbewaking na goedkeuring, controles binnen het productieproces zoals inspectie en lot-release testen, vooruitgang in analytische technologieën en de hoge kosten van de productie van biologische geneesmiddelen. We ontdekten ook dat de kwaliteitsproblemen vaker middels

brieven aan zorgverleners werden gecommuniceerd en minder vaak terugroepacties betrof, wat aantoont dat een focus op enkel terugroepacties het aantal maatregelen in verband met kwaliteitsproblemen door de toezichthoudende instanties voor biologische geneesmiddelen kan onderschatten. De brieven waren vaak het gevolg van productieproblemen die mogelijk zouden kunnen resulteren in een tekort, waarvoor acties van zorgverleners nodig waren, zoals beperken, bewaken, wisselen van therapie en informeren van patiënten. Terugroepacties werden vaak geïnitieerd vanwege problemen met specificaties die mogelijk zouden kunnen resulteren in een lagere dan wel hogere potentie of, althans theoretisch, immunogeniciteit zouden kunnen veroorzaken, waarvoor acties zoals controle, aanpassingen in bijvoorbeeld de toediening en een terugroepactie op productniveau vereist waren. Deze bevinding toont aan dat het type acties dat door regulatoire autoriteiten wordt aanbevolen, afhankelijk is van de onderliggende kwaliteitsgebreken. We vonden ook een variatie in de inhoud van maatregelen door de regulatoire autoriteiten als gevolg van kwaliteitsproblemen, wat gedeeltelijk kan worden verklaard door de verschillen in de onderliggende kwaliteitsgebreken. Op basis van dit onderzoek concludeerden we dat ondanks de zeer geavanceerde en stringente regelgeving in de EU en de VS maatregelen als gevolg van kwaliteitsproblemen door de regulatoire autoriteiten zijn uitgevaardigd voor één op de acht biologische geneesmiddelen. Fabrikanten en regulatoire autoriteiten moeten blijven streven naar het verminderen van het optreden van kwaliteitsproblemen. Verdere studies zijn nodig om de effectiviteit en impact van de aanbevolen acties door zorgverleners op de klinische praktijk en patiëntenzorg te beoordelen.

In hoofdstuk 4 zijn de belangrijkste bevindingen uit de afzonderlijke hoofdstukken in een breder perspectief besproken, waarbij uitgegaan wordt van twee thema's: gelijkwaardigheid van (C)QA's en bewaking van de kwaliteit na goedkeuring van biologische geneesmiddelen. Deze discussie is gevolgd door een reflectie op de implicaties van de belangrijkste bevindingen van het proefschrift voor de regulatie van biologische geneesmiddelen en voor de patiëntenzorg.

In het eerste thema van de discussie in hoofdstuk 4 zijn we dieper ingegaan op de manier waarop onderzoeken naar de gelijkwaardigheid worden toegepast in twee verschillende scenario's voor biologische geneesmiddelen; (1) veranderingen in kwaliteitskenmerken na regulatoire goedkeuring en (2) voor de ontwikkeling en registratie van biosimilars. We bespraken waarom de vergelijkbaarheid van (C)QA's belangrijk is bij de ontwikkeling en registratie van biologische geneesmiddelen en waarom kennis van de (C)QA's van een molecuul essentieel is voor het vaststellen van de specificaties en de noodzakelijke mate van gelijkwaardigheid van biologische geneesmiddelen. We bespraken waarom informatie over de gelijkwaardigheid van (C)QA's voor biosimilars beschikbaar zou moeten zijn in het publieke domein. We hebben uitgelegd wat een QA tot een CQA maakt, op basis van de beoordelingsinstrumenten welke zijn goedgekeurd

door regulatoire autoriteiten, en waarom CQA's relevant zijn. We toonden aan dat regulatoire autoriteiten vaak kleine verschillen in CQA's waarnemen tijdens de beoordeling van biosimilars als onderdeel van het registratietraject. Het is echter belangrijk dat de kleine verschillen in (C)QA's geen invloed hebben op de veiligheid en werkzaamheid van de biosimilar. De grote vooruitgang in analytische technologieën heeft de nauwkeurige detectie van kleine verschillen mogelijk gemaakt, maar de uitdaging is om te begrijpen of de kleine verschillen van invloed zijn op klinische effecten en daarmee op de patiëntenzorg. We dachten na over de mogelijke implicaties van de beschikbaarheid van informatie ten aanzien van de gelijkwaardigheid van (C)QA's binnen de regulatie van biologische geneesmiddelen. De regulatoire eisen voor biosimilars evolueren en toezichthouders blijven het aantonen van de gelijkwaardigheid van (C)QA's benadrukken, wat zou kunnen resulteren in een vermindering van onnodige vergelijkende klinische onderzoeken in de nabije toekomst. De huidige beoordeling is gebaseerd op een beoordeling van het product aan de hand van verschillende vergelijkende onderzoeken, zoals een kwantitatieve benadering voor aanvaardbare verschillen op het niveau van (C)QA's welke kan leiden tot een vroege voorspelling van de gelijkwaardigheid en de overgang naar gespecificeerde wetenschappelijk onderbouwde vereisten voor de vervolgstappen binnen het ontwikkeltraject van een biosimilar. De beschikbaarheid van uitgebreide kennis ten aanzien van de gelijkwaardigheid van (C)QA's voor biosimilars kan zorgverleners helpen de wetenschappelijke achtergrond rondom de registratie van biosimilars en de effecten van kleine verschillen in CQA's op de patiëntenzorg te begrijpen. De beschikbaarheid van deze kennis zou kunnen bijdragen aan het vergroten van de acceptatie van biosimilars in de klinische praktijk, zodat patiënten en de gezondheidszorg de volledige voordelen van de biosimilars kunnen benutten.

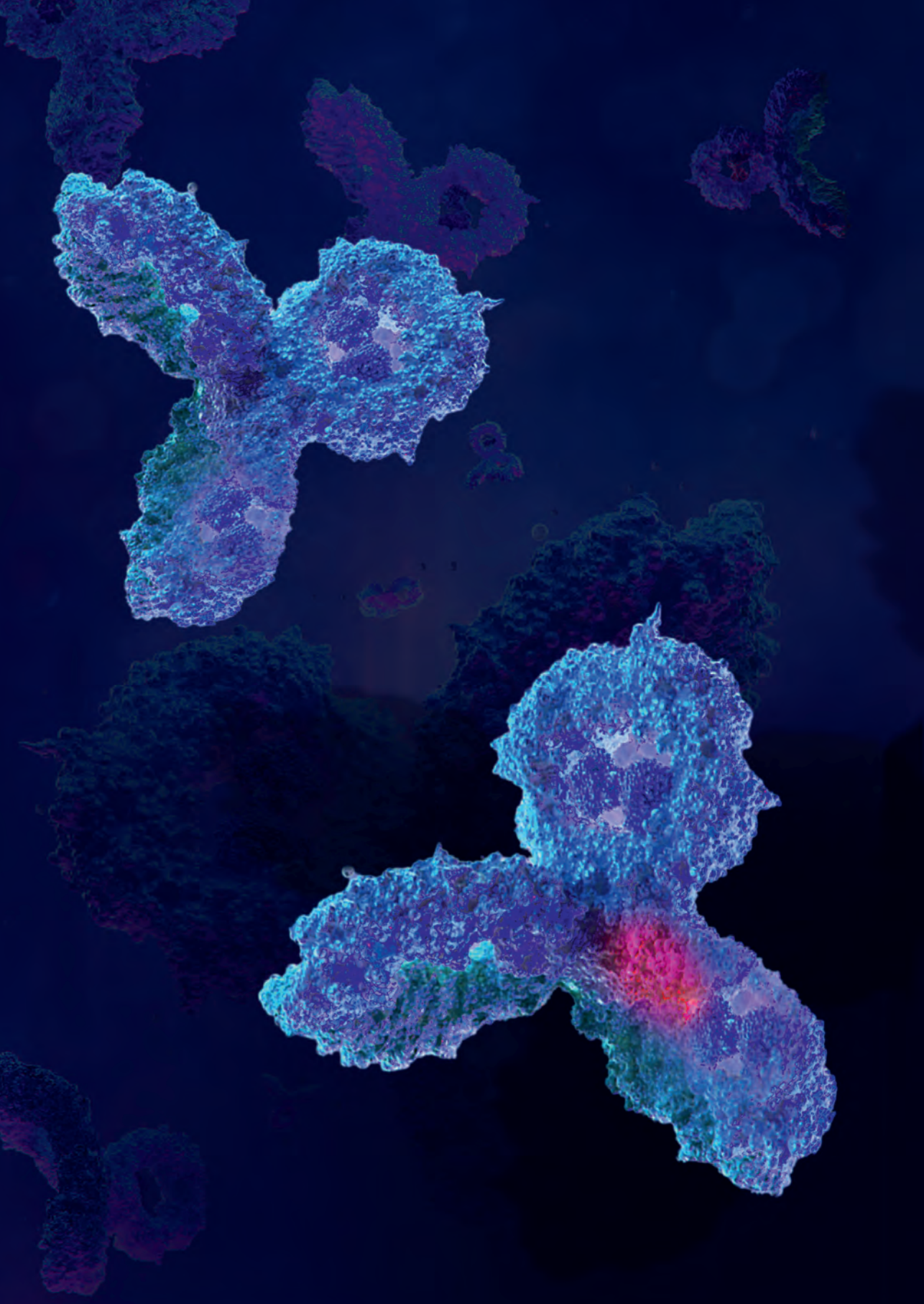


In het tweede thema van de discussie bespraken we het belang van continu toezicht na regulatoire goedkeuring om ervoor te zorgen dat de kwaliteit, veiligheid en werkzaamheid van (biologische) geneesmiddelen consistent zijn en geschikt zijn voor gebruik door patiënten gedurende hun levenscyclus. We hebben twee aspecten op dit gebied uitgewerkt; (1) wijzigingen in de kwaliteit na goedkeuring en (2) genomen maatregelen door regulatoire autoriteiten vanwege geconstateerde kwaliteitsproblemen. We bespraken waarom veranderingen in de kwaliteit vaker verband hielden met productie en kwaliteitscontrole, voor zowel referentiegeneesmiddelen als biosimilars van TNF-α-remmers, en welke factoren de implementatie van veranderingen in kwaliteit na goedkeuring kunnen verklaren. We bespraken waarom het aandeel biologische geneesmiddelen dat onderworpen was aan regulatoire maatregelen door problemen met de kwaliteit lager was dan het aandeel regulatoire maatregelen in verband met veiligheidsproblemen en de factoren die deze lagere waarschijnlijkheid mogelijk beïnvloeden. We legden uit waarom regulatoire maatregelen door kwaliteitsproblemen vaker brieven aan zorgverleners betroffen met een bepaalde waarschuwing dan terugroepacties en

waarom de inhoud van regulatoire maatregelen door kwaliteitsproblemen varieert. Deze variatie werd toegeschreven aan de verschillende onderliggende kwaliteitsdefecten die verschillende soorten acties van zorgverleners vereisen om het potentiële risico voor klinische werkzaamheid en effectiviteit en daarmee voor de patiëntenzorg te beperken. We hebben nagedacht over mogelijke implicaties voor de regulering van biologische geneesmiddelen indien surveillance op het gebied van kwaliteit na registratie en de afweging van baten en risico's na registratie twee afzonderlijke silo's zijn gerelateerd aan het systeem van initiële registratie. We hebben echter geleerd dat er een groter verband bestaat tussen kwaliteit, veiligheid en werkzaamheid voor biologische geneesmiddelen dan voor chemisch gesynthetiseerde geneesmiddelen. Dit betekent dat regelgeving van biologische geneesmiddelen moet evolueren naar een gecombineerde aanpak, waarbij kwaliteitskenmerken kunnen worden gerelateerd aan klinische voordelen en risico's. Binnen de regulatie van biologische geneesmiddelen is het van belang een classificatiesysteem te ontwikkelen waarbij op basis van verwachte risico's van wijzigingen in de kwaliteit kan worden beoordeeld wat de klinische effecten zullen zijn. We hebben ook nagedacht over de mogelijke implicaties van veranderingen in de kwaliteit en regulatoire maatregelen en de impact voor de patiëntenzorg. Sommige veranderingen in de kwaliteit kunnen de consistentie van CQA's van een biologisch geneesmiddel beïnvloeden, wat op zijn beurt de klinische resultaten en daarmee de patiëntenzorg kan beïnvloeden. De huidige regulatoire praktijk zorgt voor consistentie van CQA's tussen batches van hetzelfde biologische geneesmiddel van dezelfde fabrikant. Aangezien biosimilars na goedkeuring niet meer met het referentiegeneesmiddel worden vergeleken, moet de ontwikkeling en het gebruik van referentiestandaarden worden bevorderd om de consistentie van CQA's tussen batches geproduceerd door dezelfde farmaceut en tussen producten geproduceerd door verschillende farmaceuten te waarborgen. De implementatie van een referentiestandaard zou, althans theoretisch, de mogelijke impact van verschillen binnen en tussen producten kunnen aanpakken en er voor kunnen zorgen dat patiënten producten krijgen met geharmoniseerde doses en daarmee samenhangend vergelijkbare effecten. Daarnaast worden regulatoire maatregelen door kwaliteitsproblemen uitgevaardigd om het potentiële risico op gebreken in de kwaliteit voor de patiëntenzorg te minimaliseren. Regulatoire autoriteiten moeten ernaar streven de inhoud van de regulatoire maatregelen door kwaliteitsproblemen duidelijker en volledige te communiceren, wat belangrijk is omdat het zorgverleners kan helpen weloverwogen beslissingen te nemen en zich voor te bereiden op (toekomstige) kwaliteitsproblemen.

De algemene discussie in hoofdstuk 4 werd afgesloten met een algemene conclusie. Het proefschrift onderzocht de kwaliteit van biologische geneesmiddelen vanuit verschillende gezichtspunten waarbij kwaliteitskenmerken werden bestuurd voor en na regulatoire goedkeuring. Daarnaast zijn wijzigingen in de kwaliteitskenmerken na

regulatoire goedkeuring bestudeerd en is er specifiek aandacht besteed aan de regulatoire beoordeling en de rapportage van de gelijkwaardigheid van biosimilars. De studies in dit proefschrift, hebben aangetoond dat het aantonen van gelijkwaardigheid van biologische geneesmiddelen in grote mate afhankelijk is van de gelijkwaardigheid van (C)QA's waarbij een beoordeling van de CQA's de sleutel is bij het aantonen van gelijkwaardigheid en het signaleren van kleine verschillen. We hebben laten zien dat er wordt gewerkt aan antwoorden ten aanzien van de mogelijke effecten van kleine verschillen in (C)QA's in relatie tot de klinische resultaten en de patiëntenzorg. Ten slotte benadrukt dit proefschrift ook (opnieuw) het idee dat het verbeteren van de kwaliteit en productie van biologische geneesmiddelen niet is voltooid na regulatoire goedkeuring. Follow-up na goedkeuring, surveillance en regulatoire maatregelen, indien nodig, blijven essentiële bouwstenen van het vertrouwen in biologische geneesmiddelen.



# Chapter 5.3

## Arabic summary

Flip the book

الأوروبية للأدوية وإدارة الغذاء والدواء الأمريكية، إلا أن خلل الجودة قد يحدث ولو بشكل نادر مما يتطلب الاستمرار في تحسين أدوات مراقبة الجودة من قبل الجهات الرقابية والشركات لسرعة الكشف عن خلل الجودة ومنع وصولها إلى المريض.

في الفصل الرابع، استعرضنا مناقشة لأهم النتائج من منظورين: المنظور الأول كان متعلقاً بدراسات المقارنة للمواصفات (الحرجة) لجودة للأدوية الحيوية. حيث تمت مناقشة أهمية تقييم المواصفات الحرجة للجودة في دراسات المقارنة للأدوية الحيوية، واكتساب المعرفة بالتأثير المحتمل لأي اختلافات في مواصفات الجودة وإن كانت طفيفة على فاعلية ومأمونية الدواء الحيوي. حيث فد تسهم تلك المعرفة في التنبؤ المبكر لتكافؤ الأدوية الحيوية، مما قد تساعد الجهات الرقابية في التخلص من بعض المتطلبات غير الضرورية لتسجيل وتقييم الأدوية الحيوية الكفيئة، مما يساهم في استدامة تطوير وصناعة هذه الأدوية. الأمر الذي قد يعود بالنفع على أنظمة الرعاية الصحية والمريض تحديداً من خلال توفر بدائل علاجية بتكلفة منخفضة. حيث من المتوقع أن تلعب نتائج دراسات المقارنة للمواصفات الحرجة للجودة دوراً جوهرياً في تحديد أوجه التشابه والاختلاف وتسجيل الأدوية الحيوية الكفيئة في المستقبل القريب. لكن الأهم هو معرفة التأثير المحتمل لاي اختلافات في مواصفات الجودة علي فاعلية الدواء وسلامة المريض. ونظراً لأن أنظمة الجهات الرقابية الحالية لا تتطلب إعادة مقارنة الأدوية الحيوية الكفيئة بعد تسجيلها، الأمر الذي قد يتطلب تطويراً ودعماً لاستخدام معايير للمواصفات الحرجة للجودة تساعد الجهات الرقابية والشركات في ضمان تكافؤ الأدوية الحيوية. المنظور الثاني كان متعلقاً بمتابعة جودة الأدوية الحيوية بعد التسجيل خلال استخدام المرضى لهذه الأدوية وفقاً للممارسات الطبية. أوضحت الدراسات في هذه الرسالة أن هناك علاقة محتملة مباشرة أو غير مباشرة بين مواصفات الجودة وفاعلية ومأمونية الأدوية الحيوية بالمقارنة مع الأدوية الكيميائية. لكن أنظمة الجهات الرقابية الحالية تقوم بمراقبة وضبط الجودة بشكل منفصل عن متابعة فاعلية ومأمونية الأدوية الحيوية. الأمر الذي قد يتطلب تطوير أدوات جديدة أو دمجاً لبعض الأدوات التنظيمية الحالية لتتم مراقبة ومتابعة جودة وفاعلية ومأمونية الأدوية الحيوية.

فيزير وآخرون (٢٠١٤م)، مما يعني أن تأثير تعديلات التصنيع على جودة عينة الأدوية الحيوية متدنٍ أو غير وارد. لاحظنا كذلك أن نسبة التعديلات عالية الخطورة منخفضة، وقد يكون ذلك بسبب تأثيرها المحتمل على مواصفات الحرجة للجودة. على الرغم من إجراء العديد من التعديلات، إلا أنه لم يتم اكتشاف أي تأثير لهذه التعديلات على فاعلية ومأمونية لعينة الأدوية الحيوية المدروسة في أنظمة الجهات الرقابية لمراقبة الجودة والتيقظ الدوائي. الاستنتاج هو أنه لا يوجد أي سبب لافتراض أن التعديلات في التصنيع قد تؤدي إلى اختلاف بين الأدوية الحيوية الكفيئة ونظيرتها المبتكرة المرجعية في الاستخدام العلاجي وفقاً للممارسات الطبية.

الدراسة الخامسة (الفصل ٣.٢) تهدف إلى التعرف على القرارات الصادرة من الجهات الرقابية بسبب خلل في جودة الأدوية الحيوية المسجلة في الوكالة الأوروبية للأدوية وهيئة الغذاء والدواء الأمريكية في الفترة بين ١٩٩٥م إلى ٢٠١٩م. وجدنا ١٢.٥٪ من الأدوية الحيوية قد حصلت على قرار أو أكثر من الجهات الرقابية بسبب خلل في الجودة. حيث كانت هذه النسبة أقل مما تم نشره مسبقاً بواسطة خيزين وآخرون (٢٠٠٨م) الذين وجدو ٢٣.٦٪ من الأدوية الحيوية حصلت على قرار أو أكثر من الجهات الرقابية بسبب مخاطر على فاعلية ومأمونية الأدوية الحيوية مثل الاعراض جانبية. ويعزى هذا الاختلاف إلى ان المعرفة بالمخاطر المتعلقة بفاعليته ومأمونيته يزداد بزيادة عدد المرضى المستخدمين للأدوية الحيوية بعد التسجيل وفقاً للممارسات الطبية. على النقيض تهدف أنظمة الجهات الرقابية وأدوات ضبط الجودة في المصانع إلى إحكام السيطرة علي جودة الأدوية الحيوية ومنع وصول أي دواء تعرض إلى خلل في الجودة على المريض. وقد يكون التطور في تقنيات وأدوات مراقبة الجودة والتفتيش على المصانع واختبارات الفسح و أنشطة التيقظ الدوائي من الأسباب التي أدت إلى انخفاض احتمالية صدور قرار من الجهات الرقابية بسبب خلل في جودة الأدوية الحيوية. غالبية قرارات الجهات الرقابية كانت عبارة عن منشورات تحتوي على توصيات للممارسين الصحيين للتعامل مع خلل الجودة لحماية المريض من أي مخاطر محتملة. وجدنا أيضا أن التوصيات للممارسين الصحيين تختلف باختلاف نوع خلل الجودة. على سبيل المثال، إذا كان خلل الجودة في أحد خطوات التصنيع مما قد يؤدي إلى إيقاف التصنيع وعدم توفر الدواء في السوق فإن التوصيات غالبا ما تكون متعلقة بمتابعة ومراقبة المريض والبحث عن بدائل علاجية. أما إذا كان خلل الجودة في مواصفات الجودة فإن التوصيات تكون متعلقة بفحص وإعادة الدواء المتضرر إلى الشركة. الاستنتاج هو على الرغم من صرامة الأنظمة في الوكالة

الدراسة الثالثة (الفصل ٢.٣) تهدف إلى مراجعة التقييم العلمي لدراسات المقارنة لمواصفات الجودة في تقارير الوكالة الأوروبية للأدوية لنفس عينة الأدوية الحيوية التي تم مقارنتها في الدراسة الثانية، مع التركيز على تقييم المواصفات الحرجة لجودة الأدوية الحيوية. لاحظنا تباينًا في عدد مواصفات الجودة (٣٥٪ – ٧٥٪) بين تقارير الوكالة الأوربية للأدوية الحيوية الكفيئة، وقد يكون ذلك التباين بسبب أن التقارير يتم أعدادها بواسطة جهات رقابية في دول مختلفة ضمن الاتحاد الأوروبي. لذلك قد تكون هناك حاجة إلى آلية موحدة لإعداد التقارير تسهم في تحسين اكتمال البيانات لأن هذه التقارير تعتبر مصدراً أساسياً لصناع القرار في العديد من الشركات والجهات الرقابية والصحية والبحثية في مجال الأدوية الحيوية الكفيئة. حيث قدمنا مقترحين لتحسين محتوى اكتمال بيانات دراسات المقارنة للمواصفات الحرجة لجودة الأدوية الحيوية الكفيئة في تقارير الجهات الرقابية. لاحظنا اهتمام الجهة الرقابية في استعراض التقييم العلمي للمواصفات الحرجة للجودة في دراسات المقارنة، وقد يُعزى ذلك الاهتمام إلى وجود رابط بين المواصفات الحرجة للجودة وفاعلية ومأمونية الدواء الحيوي. وجدنا أيضا أن الجهات الرقابية غالبا ما يلاحظون اختلافات كمية طفيفة في بعض المواصفات الحرجة للجودة بين الأدوية الحيوية الكفيئة حينما يتم مقارنتها مع الدواء المبتكر، إلا أن هذه الاختلافات لم تؤثر على الوظيفة والفاعلية و المأمونية ولم تكن عائقا لتسجيل الأدوية الحيوية الكفيئة.

الدراسة الرابعة (الفصل ٣.١) تهدف إلى التعرف على أنواع ودرجة خطورة تعديلات التصنيع التي تمت الموافقة عليها من الوكالة الأوروبية للأدوية لعينة من الأدوية الحيوية (ادالبيوماب، انفليكسيماب، ايتانيرسيبت) وهي عبارة عن أجسام مضادة تستخدم في علاج الالتهابات. لنتمكن من تحليل التعديلات المصنعية، قمنا بتطوير تصنيف لأنواع التعديلات ودرجة الخطورة المحتملة على جودة الأدوية الحيوية (منخفض أو متوسط أو عالي الخطورة). خلال فترة الدراسة، وافقت الوكالة الأوروبية للأدوية على عدد ٨٠٠ تعديل للأدوية الحيوية التي تم دراستها وكانت غالبية هذه التعديلات تتعلق بطرق التصنيع واختبارات ضبط الجودة. حيث يمكن تفسير هذه النتيجة على أن إجراء التعديلات بغرض تحسين جودة الأدوية الحيوية هي عملية مستمرة لانهاية لها. من العوامل المحفزة للشركات لأجراء هذه التعديلات قد تكون الالتزام بمتطلبات الجهات الرقابية، وتطور تقنيات التصنيع والتحليل والرغبة في الابتكار وإعادة الابتكار. لم نجد في هذه الدراسة أي ارتباط في أنواع التعديلات بين الأدوية الحيوية الكفيئة والمبتكرة، مما يعني أن الأدوية الحيوية الكفيئة لها دورة حياة مستقلة بعد التسجيل. لاحظنا أن درجة الخطورة لغالبية التعديلات كانت منخفضة أو متوسطة وهذه النتيجة متوافقة مع ما توصل له

الجودة التي تم تقييمها في دراسات المقارنة تليها مواصفات الجودة المتعلقة بشكل الدواء الحيوي (٨١٪). قد يكون ذلك إشارة إلى أهمية مواصفات الجودة المتعلقة بوظيفة الدواء الحيوي بسبب ان نتائج تحليل هذه المواصفات يعطي لمحة أولية عن أوجه التشابه والاختلاف بين الأدوية الحيوية الكفيئة والمبتكر (المرجعي). الاستنتاج من هذه الدراسة أن توفر دراسات المقارنة لجميع أنواع مواصفات الجودة المتعلقة بالدواء الحيوي قد تسهم في زيادة المعرفة بدور دراسات المقارنة في تسجيل الأدوية الكفيئة حيوياً، مما يعزز من قبول الأدوية الحيوية الكفيئة كبدائل علاجية وفقاً للممارسات الطبية.

الدراسة الثانية (الفصل ٢.٢) تهدف إلى مقارنة للبيانات المنشورة في تقارير الوكالة الأوروبية للأدوية والدراسات العلمية في الدوريات العلمية المحكمة عن مواصفات الجودة التي تم تقييمها في دراسات المقارنة لعينة من لأدوية الحيوية الكفيئة. العينة لنوع واحد من الأدوية الحيوية (ادااليموماب) وهو جسم مضاد يستخدم لعلاج الالتهابات ومنها التهاب المفاصل ولهذا الدواء أكبر عدد من الأدوية الحيوية الكفيئة المسجلة في أوروبا. لاحظنا أن هناك اختلاف في عدد مواصفات الجودة المدونة لنفس الدواء بين تقارير الجهات الرقابية الدراسات العلمية، وقد يكون ذلك بسبب أن المصدرين يختلفان بالدوافع والاهداف. حيث تمثل تقارير الجهات الرقابية انعكاساً وتلخيصاً للتقييم العلمي الذي قامت به الوكالة الأوروبية للأدوية، بينما الدراسات العلمية انعكاس لما يراه المؤلفون مثيرًا للاهتمام. مواصفات الجودة المتعلقة بوظيفة الدواء الحيوي غالبا ما تحظى باهتمام في تقارير الجهات الرقابية والدراسات العلمية، مما يعكس أهمية هذا النوع من المواصفات في تقييم دراسات المقارنة. حيث إن المواصفات الوظيفية تعطي معلومات عن تأثير أي اختلاف في مواصفات الجودة ولو كان طفيفا على وظيفة وآلية عمل الدواء في جسم الإنسان. بالإضافة إلى أن تقييم المواصفات الوظيفية قد يساعد في التنبؤ المبكر في تكافؤ الدواء حيوياً. وكذلك يعتبر تقييم المواصفات الوظيفية هو حجر الأساس في تقييم تعميم الادعاءات الطبية دون إعادة إجراء الدراسات السريرية لكل ادعاء طبي، إذا ما توافرت الشروط اللازمة. وجدنا أن تقارير الجهات الرقابية غالباً ما تركز على تدوين تفسير المقيم للنتائج في حين أن الدراسات العلمية تقوم بتدوين نتائج التحليل الكمي والكيفي مع استعراض لتفسير الباحث لهذه النتائج. لذلك ينبغي مراجعة كلا المصدرين للحصول على تصور أكبر عن مواصفات الجودة التي تم تقييمها في دراسات المقارنة الداعمة لتسجيل الأدوية الحيوية الكفيئة، مما يساعد في معرفة دور تقييم المواصفات الوظيفية في دراسات المقارنة وآلية عمل الأدوية الحيوية بشكل عام.

الجهات الرقابية بمراقبة وضبط جودة الأدوية الحيوية بشكل أساسي من خلال عمليات تفتيش للمصانع للتحقق من الالتزام بأسس التصنيع الجيد وأيضا الاختبار الإلزامي لفسح الأدوية الحيوية عن طريق مختبرات الجهات الرقابية. من الأمور التي قد تحدث لجودة الأدوية الحيوية بعد التسجيل هما أجراء تعديلات على التصنيع الذي يتطلب موافقة الجهات الرقابية أو حدوث خلل في الجودة والذي يتطلب صدور قرار من الجهات الرقابية الذي قد تتراوح القرارات بين منشور مع توصيات للممارسين الصحيين إلى سحب العينات المتضررة أو إلغاء تسجيل الدواء لحماية المرضى من أي مخاطر محتملة.

لذلك فالهدف من رسالة الدكتوراه هو دراسة جودة الأدوية الحيوية من جانبين من خلال إجراء دراسات بحثية: الجانب الأول هو التعرف على دور تقييم دراسات المقارنة للمواصفات (الحرجة) للجودة في تنظيم الأدوية الحيوية (الفصل الثاني). الجانب الثاني هو متابعة جودة الأدوية الحيوية بعد التسجيل من خلال دراسة موافقات وقرارات الجهات الرقابية (الفصل الثالث). وقد أتبعنا ذلك بفصل المناقشة العامة لأهم نتائج الدراسات الرئيسية للفصلين الثاني والثالث من منظور شامل، بالإضافة إلى استعراض أهمية نتائج الرسالة لتنظيم الأدوية الحيوية ورعاية المرضى وفقا للممارسات الطبية.

الدراسة الأولى (الفصل ٢.١) تهدف إلى إجراء مراجعة شاملة للدراسات المنشورة في الدوريات العلمية المحكمة للتعرف على مواصفات الجودة التي تم تقييمها في دراسات المقارنة للأدوية الحيوية الكفيئة. نظرًا لعدم وجود تصنيف متفق عليه لمواصفات الجودة للأدوية الحيوية، قام فريق البحث بتطوير تصنيف لمواصفات الجودة للأدوية الحيوية التي يتطلب تقييمها في دراسات المقارنة، بناءً على ماورد في الأدلة الإرشادية التنظيمية. حيث تمت مناقشة التصنيف مع خبراء في تقييم جودة الأدوية الحيوية من الجهة الرقابية الهولندية. لاحظنا أن هناك ازدياداً متسارعاً في نشر دراسات المقارنة لمواصفات الجودة في الدوريات العلمية، وهو ما قد يسهم في تحسين المعرفة بدور دراسات المقارنة في تسجيل الأدوية الحيوية الكفيئة. لاحظنا عدم توفر دراسات المقارنة ل ٤٠٪ من الأدوية الحيوية الكفيئة المسجلة في الجهات الرقابية مما قد يتطلب البحث في مصادر أخرى مثل تقارير الجهات الرقابية. وجدت الدراسة ازدياد ملحوظ في انواع مواصفات الجودة التي يتم تقييمها في دراسات المقارنة، من المحتمل ان يكون ذلك بسبب التحديث التي قامت به الجهات الرقابية للأدلة الارشادية الخاصة بدراسات المقارنة لمواصفات الجودة في عام ٢٠١٢م وعام ٢٠١٤م. وجدنا أيضا اختلاف في أنواع مواصفات الجودة بين الدراسات وقد يكون علاقة نوع مواصفة الجودة بالدواء الحيوي الذي تم دراسته وقد يكون اختيار الباحثين لما يرونه مثيرا للاهتمام دورا بذلك الاختلاف. مواصفات الجودة المتعلقة بوظيفة الدواء الحيوي (٩٤٪) كانت من أكثر أنواع مواصفات

لجودة الدواء الحيوي المبتكر. بعد ذلك، يقوم المطور بتطوير طريقة التصنيع ليتمكن من إنتاج عينات أولية للدواء الحيوي الكفيْ الذي سوف يتم مقارنته مع الدواء الحيوي المبتكر (أو المرجعي). يقوم المطور بأجراء عدة دراسات مقارنة. أول هذه الدراسات هي مقارنة المواصفات (الحرجة) للجودة لتحديد أوجه التشابه والاختلاف بين الدواءين ودراسة تأثير أي اختلاف ولو كان طفيفاً على فاعلية ومأمونية الدواء. تكمن أهمية دراسات المقارنة للمواصفات الحرجة للجودة في إعطاء التصور المبدئي عن تكافؤ الأدوية الحيوية. بناء على نتائج دراسات المقارنة للمواصفات (الحرجة) للجودة، يتم تحديد نوع دراسات المقارنة السريرية على عينة من المتبرعين والمرضى لإثبات عدم تأثير أي اختلافات في مواصفات الجودة على فاعلية ومأمونية الدواء الحيوي.

الرحلة الثانية هي تنظيم وتسجيل الدواء ومتابعة جودته وفاعليته ومأمونيته بعد التسجيل خلال الممارسات الطبية. وتعتبر الجودة والفاعلية والمأمونية أركاناً أساسية في تنظيم وتسجيل الأدوية. فتسجيل الأدوية مبني على التقييم العلمي للأدلة والبراهين المقدمة من الشركة. حيث تتضمن الأدلة والبراهين أنواعاً متعددة من الدراسات التحليلية والسريرية التي تهدف لإثبات جودة وفاعلية ومأمونية الدواء. للأدوية الحيوية مسارين للتسجيل مع اختلاف في أنواع الدراسات الداعمة للأدلة والبراهين. مسار تسجيل الأدوية الحيوية المبتكرة يعتمد على نتائج الدراسات السريرية بكل مراحلها والتي تهدف إلى إثبات أن المنافع للدواء تفوق المخاطر، بينما مسار تسجيل الأدوية الحيوية الكفيئة يعتمد على نتائج دراسات المقارنة والتي تهدف إلى إثبات تكافؤ الجودة والفاعلية والمأمونية بين الأدوية الحيوية الكفيئة والمبتكرة. ففي عام ٢٠٠٤م، تم استحداث مسار تسجيل الأدوية الحيوية الكفيئة، والذي تم تبنيه من قبل العديد من الجهات الرقابية حول العالم. حيث تم تسجيل ما يقارب عدد ١٠٠ من الأدوية الحيوية الكفيئة كبدائل لعدد ١٧ دواءً حيوياً مبتكراً في السوق الأوروبي والأمريكي حتى منتصف سنة ٢٠٢٢م. على الرغم من توافر هذا العدد من الأدوية الحيوية الكفيئة، إلا أنها تواجه تحدي قبول استخدامها كبدائل علاجية في الممارسات الطبية. حيث يُعزى ذلك لأسباب متعددة من أهمها: عدم الإلمام بماهية الأدلة والبراهين المبنية على تسجيل الأدوية الحيوية الكفيئة بناء على نتائج دراسات المقارنة. ومن أهم مصادر المعرفة لنتائج دراسات المقارنة هي التقارير المنشورة من الجهات الرقابية أو المقالات العلمية المنشورة في الدوريات العلمية المحكمة.

لا تنتهي رحلة الدواء عند تسجيله، بل تقوم الجهات الرقابية بمراقبة ومتابعة الدواء بعد التسجيل؛ لذلك طورت الجهات الرقابية عدة أنظمة لمراقبة وضبط الجودة وأنشطة التيقظ الدوائي لضمان جودة وفاعلية ومأمونية الأدوية للمرضى. وتقوم

## جودة الأدوية الحيوية من منظور دراسات المقارنة ومراقبة الجودة بعد التسويق

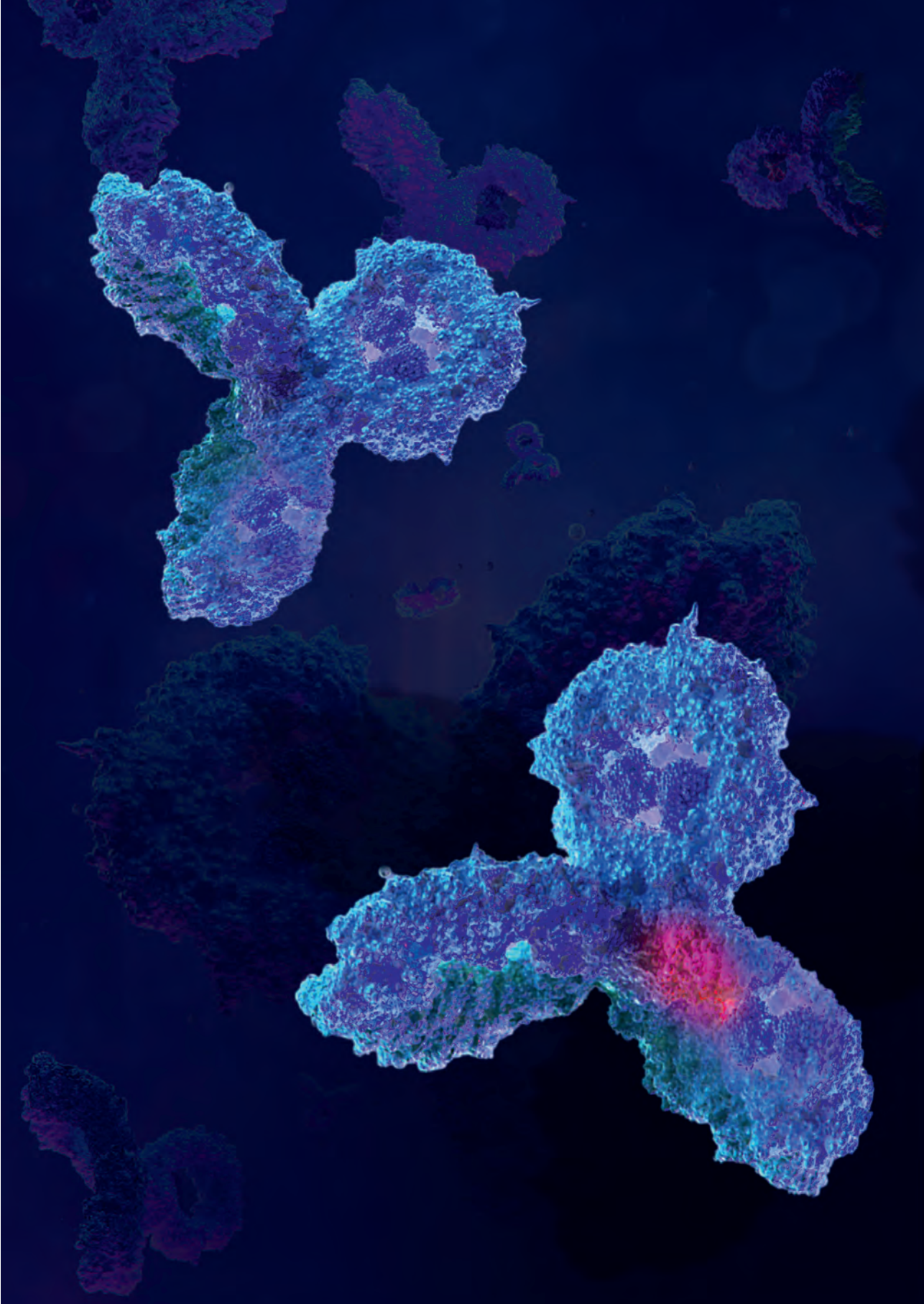
الفصل الأول يحتوي على مقدمة لجودة الأدوية الحيوية، وهي أدوية إما أن تكون مستخلصة من كائنات حية مثل الإنسان، والحيوان، والنباتات، والبكتيريا والفيروسات أو تكون مصنعة بطريقة التقنية الحيوية. ساهمت الأدوية الحيوية في توفر – بعد مشيئة الله - العلاج للعديد من الأمراض الحادة والمزمنة، منها السرطان والالتهابات والسكري، وكذلك السيطرة على الأوبئة مثل وباء كورونا ١٩. ركزت هذه الرسالة على الأدوية الحيوية المصنعة بالتقنية الحيوية؛ والصناعة بالتقنية الحيوية تعمل على مبدأ حقن شفرة وراثية في خلية أو بكتيريا، وبدورها تقوم بترجمة هذه الشفرة لتصنيع الدواء الحيوي. ففي عام ١٩٨٢م، تمت الموافقة على تسجيل أول دواء حيوي مصنع بالتقنية الحيوية وهو الأنسولين بوصفه علاجاً لمرض السكري. حيث أسهمت التقنية الحيوية في سد فجوة التفاوت في فاعلية وتقليل الأعراض الجانبية التي كانت متلازمة لاستخدام الأنسولين المستخلص من الحيوانات. منذ ذلك الحين، تم التطوير والموافقة على تسجيل المئات من الأدوية الحيوية المبتكرة مثل الهرمونات والأجسام المضادة التي تتفاوت درجة التعقيد للتركيبة الكيميائية. تختلف الأدوية الحيوية عن الأدوية الكيميائية في كون طريقة تصنيعها وتركيبتها الكيميائية معقدة. لذلك فإن الأدوية تمتلك العديد من مواصفات الجودة التي تصف الخواص الشكلية والوظيفية للدواء الحيوي. حيث إن بعض هذه الخواص تعرف بالمواصفات الحرجة لجودة الأدوية الحيوية بسبب حساسيتها لأي تغيير في طرق التصنيع أو ظروف التخزين والنقل. لذلك فأي تغير يطرأ على المواصفات الحرجة للجودة قد يؤثر على فاعلية ومأمونية الدواء الحيوي.

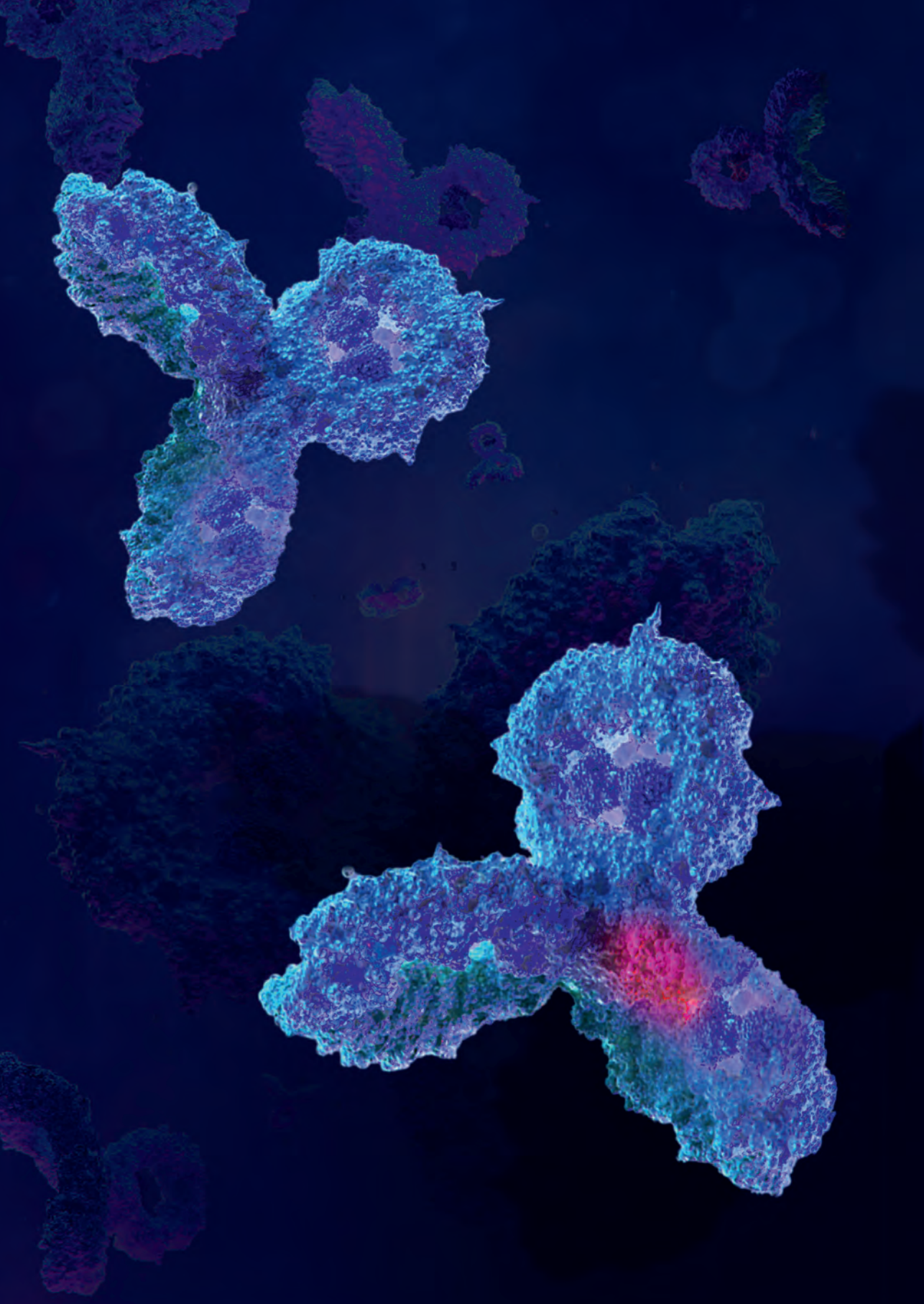
تعتبر الأدوية الحيوية في الوقت الحالي عنصراً مهماً في الممارسات الطبية. في الوقت نفسه تعتبر مكلفة مادياً مما قد يثقل كاهل الاقتصاد وقد يتسبب في عدم وصول هذه الأدوية المهمة لجميع المرضى. حيث أسهمت انتهاء فترة براءة الاختراع للأدوية الحيوية المبتكرة بالإضافة إلى نشوء تنظيمات جديدة في إتاحة الفرصة لتطوير أدوية كفيئة حيوياً كبدائل للدواء المبتكر كأحد الحلول لزيادة توفر هذه الأدوية للمرضى وخفض العبء الاقتصادي.

بشكل عام، للدواء رحلتان قبل أن يصل إلى المريض: الرحلة الأولى هي الابتكار والتطوير والتي قد تستغرق سنوات من البحث والتطوير لاستكشاف الدواء لعلاج مرض محدد وقد تصل تكلفة تطوير الدواء إلى ٢.٦ بليون دولار. في هذه الرسالة تم استعراض رحلة تطوير الأدوية الحيوية الكفيئة. حيث تبدأ هذه الرحلة بدراسة شاملة للدواء الحيوي المبتكر (المرجعي) من خلال تحليل عينات باستخدام العديد من طرق التحليل المخبرية ليتم بناء معرفة عن المواصفات (الحرجة)

# الفصل ٥.٣

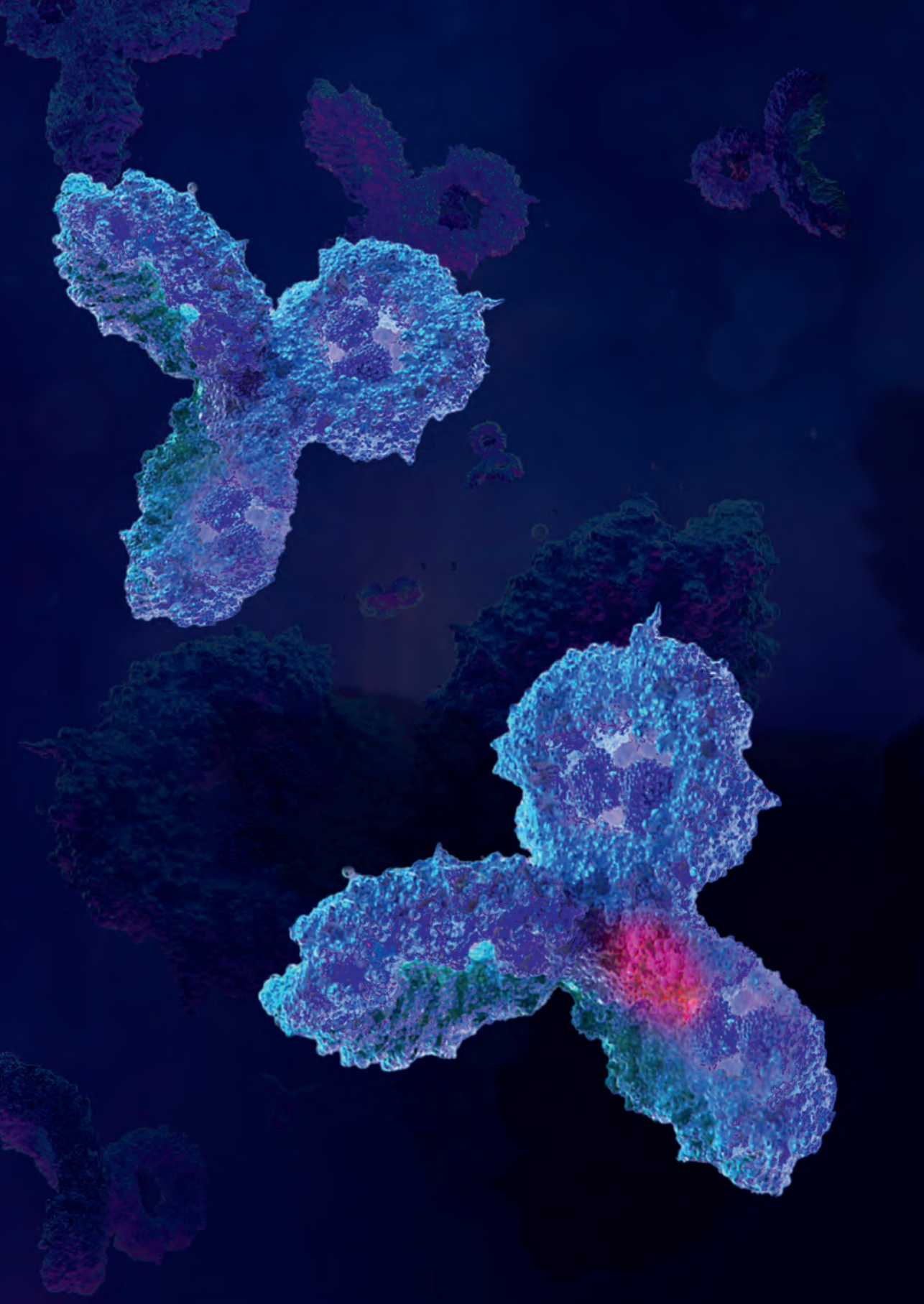
## الملخص باللغة العربية





# Chapter 6

## Appendices



# Chapter 6.1

## Acknowledgments

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**Farhan, Abdulrahaman Alshalani.** We had wonderful gatherings, which were rich of joy and full of fun. I hope that you achieve your goals, and we can stay connected after completing your journeys.

I will never forget those who visited my family and I in the Netherlands during the PhD journey. Thanks for **Ghaida** (together with her kid **Nasser**) for visiting her sister Lama in March 2018 and helping Lama with the decoration of our apartment at Utrecht, the Netherlands. Thanks for my parents (together with my sister **Razan**) for visiting us in December 2018, where we had a lot of fun and happiness time and traveled together to Spain during the new year of 2019. Thanks to my brother **Anas** (together with my cousin **Fahad** and a close friend **Faisal**) for visiting me in November 2019, where we had a lot of joy, fun and celebrity times in the Netherlands and traveled to Madrid, Spain. Thanks also for my sister **Shatha** (together her husband **Daifallah** and baby **Lina**) for visiting us in October 2021, which was one of the biggest surprises that I have ever experienced in my life. **Shatha** planned this surprise visit with my wife while I was thinking that **Shatha** was traveling to Qassim, Saudi Arabia. But in a sudden **Shatha** touched my shoulder near the library of Leidsche Rijn, the moment was unbelievable! The visit was full of surprises, joy, fun, and happiness. Thanks also to another close friend **Mansour** for visiting me in Utrecht, the Netherlands, where we had fun and reminisce our old days in Chicago, New Haven, Hamden, Miami and other places in the US.

My deepest emotions will not be sufficient to express my gratitude to my wife **Lama Alsannat**, my daughters **Lara and Haifa**, who stood beside me throughout my PhD journey and particularly at the time of discouragements and happiness, and all in between. **Lama,** we started our life journey years before the PhD journey, and I am praying for Allah (God) that we continue to stay together forever and enjoy seeing our kids growing up and achieving their own life goals. **Lama**, I am genuinely grateful for the unconditional love and care, unlimited support, and staying beside me all the time. I feel lucky to have you in my life. **Lara and Haifa,** you both lighten our life and I hope you have enjoyed growing up in the Netherlands. I am happy that you grow up in a community with diverse cultures and background. I admire that you respect the culture diversity while being proud of your own culture and principles.

To my brothers (**Osama, Anas, Hamad, and Obi**) and sisters (**Aljoharah, Eman, Shatha, and Razan**), thank you for your unlimited support and standing beside me throughout my life. Despite far distance, your communication never stops. Thanks for all your efforts including the arrangement of mailing a shipment form home, where unpacking the parcel was something like you are all visiting us. Thanks, **Eman** for homemade cookies, it should be a brand by now, go for it!

Finally, a heart-felt thank is to my parents **Mohammed Alsamil and Sharefa Alsamil** for their countless sacrifices to set a smooth road for me to reach this point. Proudly now, I can say your planting has been harvested and here I am as you always love to see me.

Special thanks to my father for the critical review and helpful suggestion of the Arabic summary of the thesis. My parents, May Allah (God) bless you and we (siblings) hope that we can repay you for all your love, care and unlimited support.

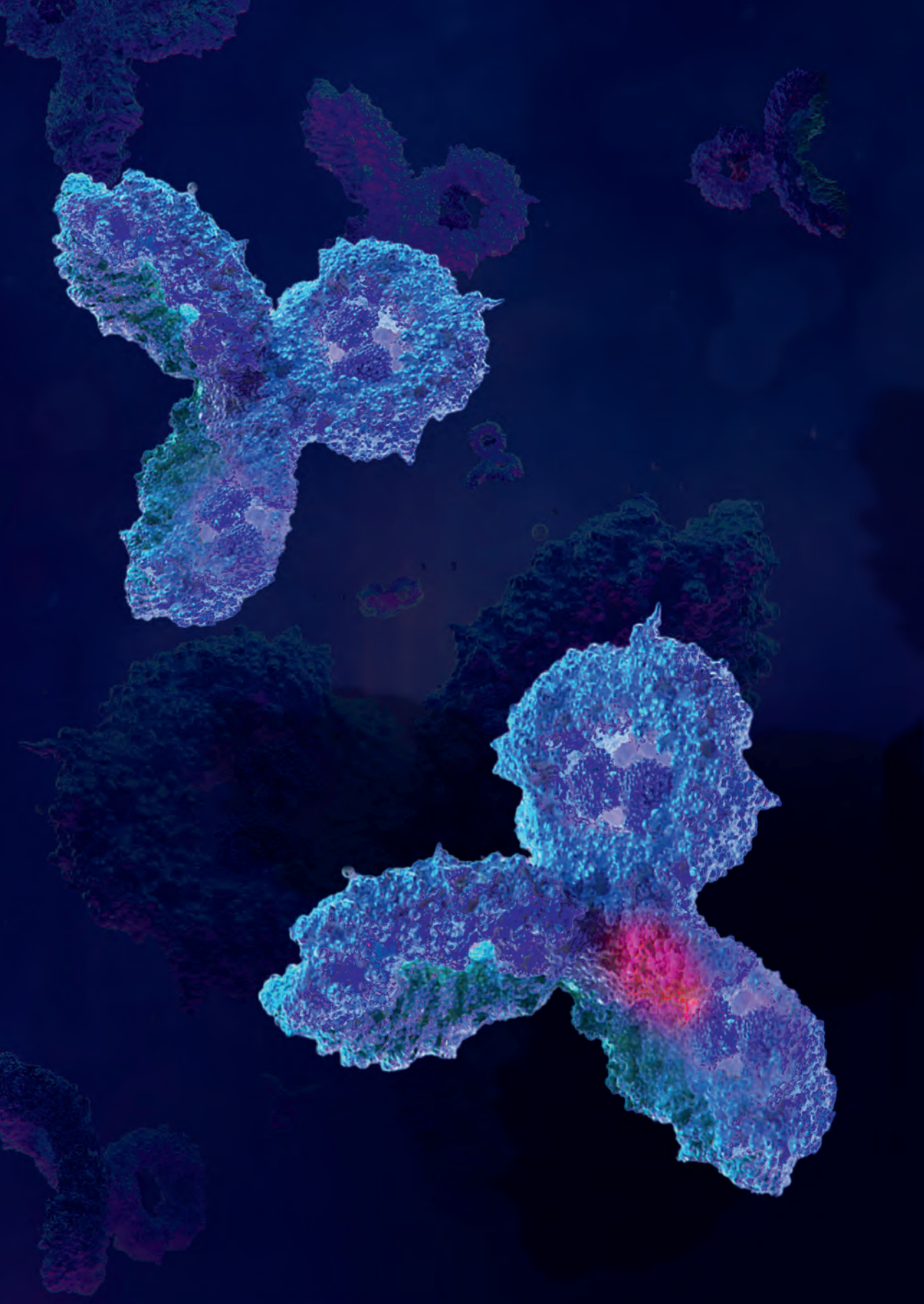
إلى زوجتي الغالية **لمى بنت ابراهيم الصنات** حفظها الله. وجودك في حياتي ساعدني كثيرا في التغلب على تحديات الدراسة والغربة، وخصوصا في فترة جائحة كورونا. لن أنسى صبرك وتفهمك انشغالي في كل مراحل رحلة الدكتوراة، وتحملك مشقة البعد عن الأهل والوطن. كُنتِ يا لمى، السند والمعين والداعم الأول لي في كل سنوات الدراسة. لن أنسى تحفيزك لي خلال أصعب المراحل. شكرا على السعادة والحب الذي قدمتيه من خلال رعايتك واهتمامك. اسأل الله أن يسخرني لدعمك في السعي إلى تحقيق امنياتك، ويقر أعيننا بصلاح بناتنا. الشكر والدعاء لوالدك إبراهيم بن محمد الصنات ووالدتك هند بنت عبدالله العليان على دعمهم ودعواتهم بالتيسير والتسخير وأن يجزيهم عنا خير الجزاء.

إلى بناتي **لارا وهيفاء**، كم أنا فخور بكم فأنتم من أجمل هدايا ربي. رحلة الدراسة كانت مليئة بالتحديات التي سرقت وقتكم الثمين. كلي حماس لمشاركة أسعد اللحظات والمغامرات معكم. أسأل الله أن يحفظكم وأن ييسر لكم تحقيق أمانيكم.

إلى إخواني (**أسامه، أنس، حامد، أبي**) وأخواتي (**الجوهرة، ايمان، شذا، رزان**)، شكراً لكم من القلب على دعمكم الدائم وتواصلكم المستمر. لن أنسى تحفيزكم ووقفاتكم ودعواتكم التي رافقتني خلال رحلة مرحلة الدكتوراة.

إلى والدي الغالي **محمد بن علي الصامل** حفظه الله، أنتَ معلمي وقدوتي التي أفخر بها. والدي لقد غرست بنا الكثير من مكارم الاخلاق والصفات الحميدة ومنها حب العلم والتعليم. اغدقت علينا والدي بالدعم والتحفيز الوفير منذ المراحل التعليمية الأولية. حيث لم تكتفِ يا والدي بالدعم، بل اتبعت ذلك بتشجيع مستمر للتميز والتفوق الدراسي. كان يا والدي حصولك على أعلى الدرجات العلمية وتكليفك في أعلى المناصب الأثر الإيجابي في ارتفاع سقف طموحاتنا، حتى باتت لا سقف لها. بفضل الله ثم دعمك المستمر تحققت العديد من الغايات والمنجزات ومنها حصولي على درجة الدكتوراة. أهديك هذا المنجز الذي هو ثمرة من ثمار غرسك. كلمات الشكر لن توفيك حقك، اكرمتني وزوجتي وبناتي باهتمامك وحرصك ومتابعتك المستمرة خلال كل مراحل رحلة الدكتوراة في مملكة هولندا. لن أنسى زيارتك مع والدتي وأختي رزان في بداية رحلة الدكتوراة، التي خففت من الم الغربة والبعد عن الوطن. أسأل الله العلي القدير أن يجزيك عنا خير الجزاء وأن يرزقك سعادة الدارين. وأن يطيل الله في عمرك وأنت في أتم صحة وعافية. ونسأل الله أن يرزقني وزوجتي وابنائي واخواني واخواتي وابنائهم أعلى درجات البر بك وبوالدتي حفظها الله.

إلى والدتي الغالية **شريفة بنت محمد الصامل** حفظها الله، يا من تعلمت منها الحكمة وطيبة القلب. أن كان لقرار السفر للدراسة في الخارج من ألم فهو البعد الجسدي عنك يا والدتي. تواصلك وسؤالك المستمر كان سبباً في تخفيف ألم الغربة. على الرغم من بعد المسافة الا أنك كنت قريبة جدا من خلال تواصلك وسؤالك شبه اليومي للاطمئنان علينا. وقد لامست أثر هذا التواصل في تعلق طفلتي لارا بك. أغدقتني بالحب والحنان والحرص والرعاية والدعم والتحفيز المستمر. كانت دعواتك سبباً بعد توفيق الله في تيسير رحلة الدراسة وتحقيق العديد من الغايات والمنجزات ومنها حصولي على درجة الدكتوراة. لم يكن ليتحقق هذا المنجز لولا متابعتك وصبرك واهتمامك منذ ان كنت طفلاً يحاول أن يحبو، ولا أزال طفلا لك حتى مماتي. أنتِ مصدر سعادتي ورضاك هو غايتي. أسأل الله العلي القدير أن يجزيك عني خير الجزاء وأن يرزقك سعادة الدارين ويمتعك بموفور الصحة والعافية. ونسأل الله أن يرزقني وزوجتي وابنائي واخواني واخواتي وابنائهم أعلى درجات البر بك وبوالدي حفظه الله.



# Chapter 6.2

## List of co-authors

Co-authors of works presented in this thesis, in alphabetical order

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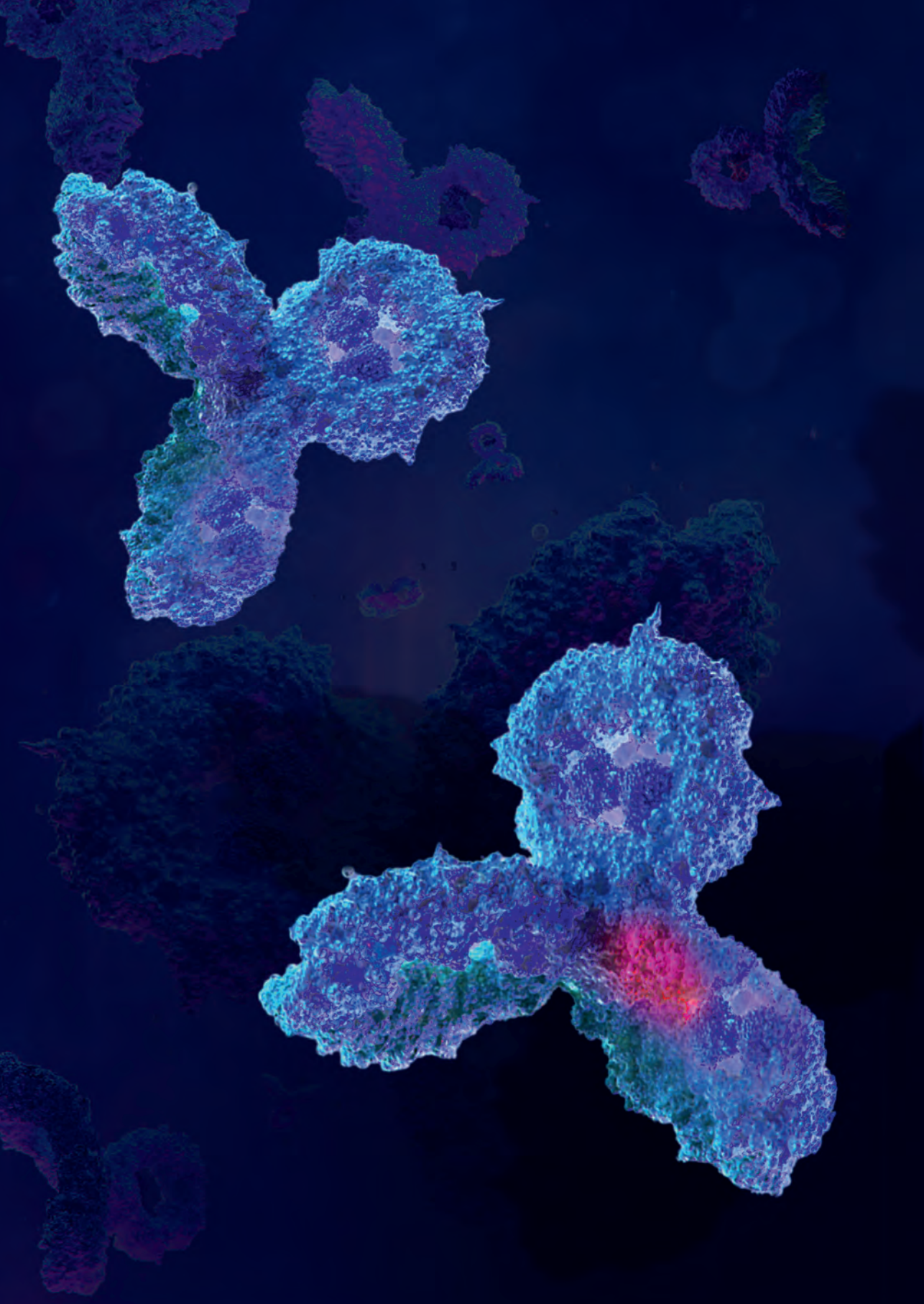


# Chapter 6.3

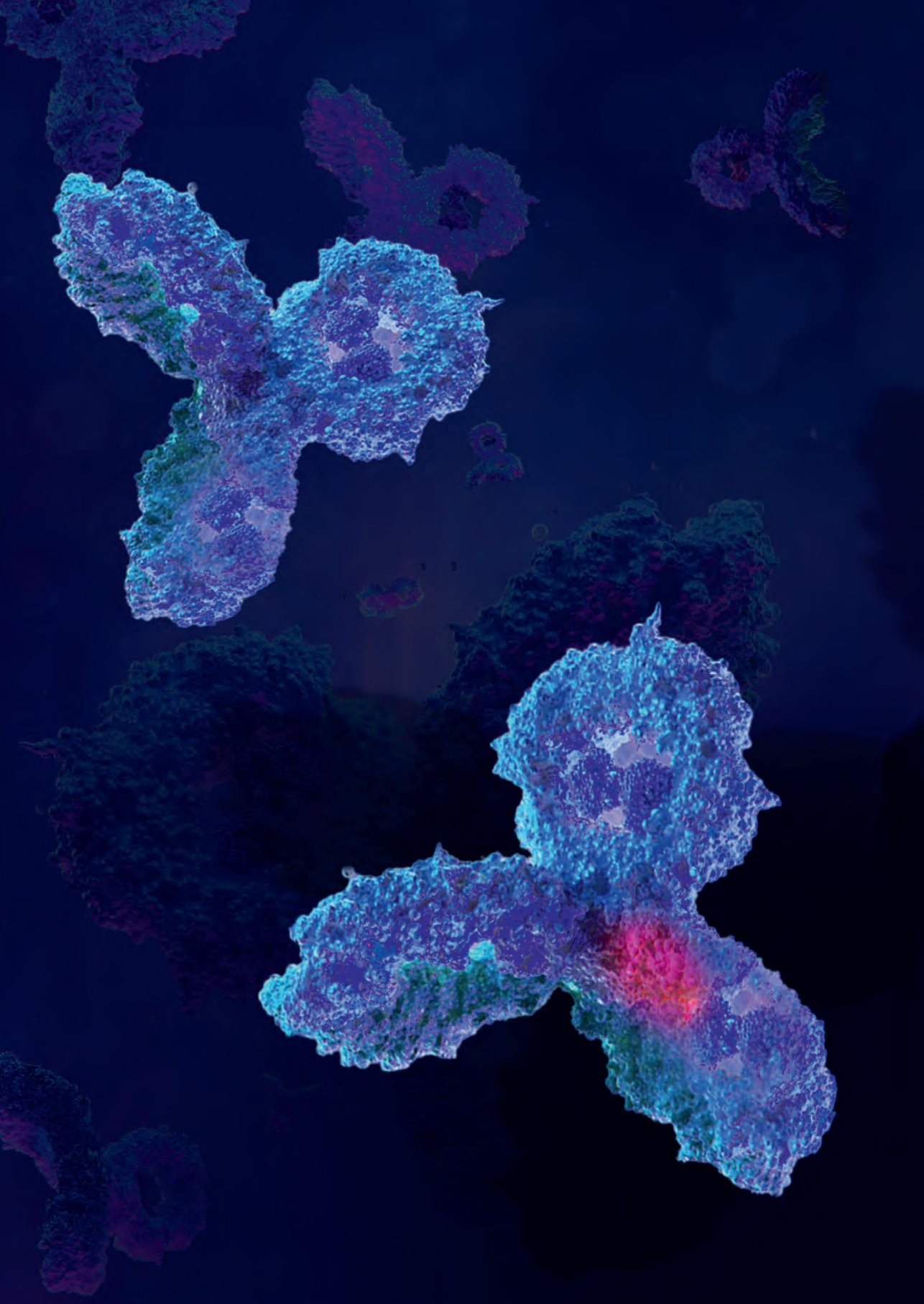
## List of publications

**Alsamil, A.M**., Giezen, T.J., Egberts, T.C., Leufkens, H.G., Vulto, A.G., van der Plas, M.R. and Gardarsdottir, H., Reporting of quality attributes in scientific publications presenting biosimilarity assessments of (intended) biosimilars: a systematic literature review. European Journal of Pharmaceutical Sciences, 2020, 154, p.105501.

**Alsamil, A.M.,** Giezen, T.J., Egberts, T.C., Leufkens, H.G. and Gardarsdottir, H., Comparison of consistency and complementarity of reporting biosimilar quality attributes between regulatory and scientific communities: An adalimumab case study. Biologicals, 2021, 69, pp.30-37.

**Alsamil, A.M.,** Giezen, T.J., Egberts, T.C., Leufkens, H.G. and Gardarsdottir, H., Type and extent of information on (Potentially critical) quality attributes described in european public assessment reports for adalimumab biosimilars. Pharmaceuticals (Basel), 2021,14(3), p.189.

**Alsamil, A.M.,** Giezen, T.J., Egberts, T.C., Doevendans, E., Leufkens, H.G. and Gardarsdottir, H., Nature and timing of post-approval manufacturing changes of tumour necrosis factor α inhibitor products: a 20-year follow-up study of originators and biosimilars. European Journal of Pharmaceutical Sciences, 2022, p.106227.



# Chapter 6.4

## About the author

Ali M. Alsamil was born in Riyadh, Saudi Arabia, in 1984. Ali is married to Lama I. Alsannat, who greatly stood beside him during his PhD journey. Ali and Lama have two beautiful daughters, Lara and Haifa.

In 2006, Ali completed a bachelor's degree in Biochemistry from the College of Science at King Saud University (KSU) in Riyadh. Thereafter, he was appointed as a graduate student in the Biochemistry Department at KSU. His main responsibility was conducting laboratory-based research that aims to extract and purify glucose-6-phosphate dehydrogenase from blood samples of camels with a variety of health conditions and to compare the enzyme activity of the samples.

A year later, Ali became a regulator in the drug sector at the Saudi Food and Drug Authority (SFDA). In 2009, he was awarded a scholarship from the SFDA for pursuing a master's degree in Biomedical Sciences from the College of Health Sciences at Quinnipiac University, Hamden, Connecticut, USA, where he earned the degree and completed a research project entitled "Benchmark of Blood Products and Establishment Regulations in Highly Regulated Markets; a Comparison to the Current Regulation in Saudi Arabia." He then returned to the SFDA and (co)established the evaluation department for the biological drugs along with his SFDA colleagues and worked as a scientific assessor of biological drugs. He was also appointed as a member of several local scientific committees and became a volunteer member and government liaison in the scientific committee for biologics monographs during the 2015–2020 cycle at the United States Pharmacopeia, Rockville, Maryland, USA.

To continue his education, in September 2017, Ali started a new journey as a PhD candidate in the Utrecht Institute for Pharmaceutical Sciences at Utrecht University, Utrecht, the Netherlands. His PhD research projects focused on the quality of biopharmaceuticals and explored comparability and post-approval surveillance.

After completing his PhD research project, Ali started a collaboration research project between Utrecht University, the Dutch Medicines Evaluation Board, and members from the Biologics, Biosimilars and Scientific Advice Working Parties of the European Medicines Agency. The research project aims to investigate glycosylation of monoclonal antibodies and to assess whether nonclinical in-vivo studies were requested during early dialogue between developers and regulators. From December 2022 onwards, he will start a new journey as a regulator and assessor in the quality evaluation department of biopharmaceuticals at the SFDA, Riyadh, Saudi Arabia.