Why is there no biosimilar of Erbitux®?

Douez, Emmanuel; D'Atri, Valentina; Guillarme, Davy; Antier, Daniel; Guerriaud, Mathieu; Debecker, Alain; Watier, Hervé; Foucault-Fruchard, Laura

How to cite

DOUEZ, Emmanuel et al. Why is there no biosimilar of Erbitux®? In: Journal of pharmaceutical and biomedical analysis, 2023, vol. 234, p. 115544. doi: 10.1016/j.jpba.2023.115544

This publication URL: https://archive-ouverte.unige.ch/unige:170658
Publication DOI: 10.1016/j.jpba.2023.115544

Journal of Pharmaceutical and Biomedical Analysis 234 (2023) 115544

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.journals.elsevier.com/journal-of-pharmaceutical-and-biomedical-analysis

Why is there no biosimilar of Erbitux®?

Emmanuel Douez [a,b,*,1], Valentina D'Atri [c,1], Davy Guillarme [c,d], Daniel Antier [a,e], Mathieu Guerriaud [f], Alain Beck [g], Hervé Watier [h,i], Laura Foucault-Fruchard [a,e]

[a] Pharmacy Department, Tours University Hospital, Tours, France
[b] EA6295, Nanomédicaments et Nanosondes, Université de Tours, Tours, France
[c] School of Pharmaceutical Sciences, University of Geneva, CMU - Rue Michel Servet 1, 1211 Geneva 4, Switzerland
[d] Institute of Pharmaceutical Sciences of Western Switzerland, University of Geneva, CMU - Rue Michel Servet 1, 1211 Geneva 4, Switzerland
[e] UMR 1253, iBrain, Université de Tours, Inserm, Tours, France
[f] CREDIMI Laboratory EA 7532 and Laboratory of Excellence LipSTIC ANR-11-LABX-0021, Faculty of Health Sciences (Pharmacy), University of Burgundy, Dijon, France
[g] IRPF - Centre D'Immunologie Pierre-Fabre (CIPF), 5 Avenue Napoléon III, BP 60497 Saint-Julien-en-Genevois, France
[h] Immunology Laboratory, Tours University Hospital, Tours, France
[i] UMR 1100, CEPR, Université de Tours, Inserm, Tours, France

ARTICLE INFO

Keywords:
Cetuximab
Biosimilar
Glycosylation
Sialic acids
Analytical strategy
Anaphylactic reactions

ABSTRACT

Monoclonal antibody (mAb)-based therapies have been a major advance in oncology patient care, even though they represent a significant healthcare cost. Biosimilars, launched in Europe in 2004 are an economically attractive alternative to expensive originator biological drugs. They also increase the competitiveness of pharmaceutical development. This article focuses on the case of Erbitux® (cetuximab). This anti-EGFR (Epidermal Growth Factor Receptor) monoclonal antibody is indicated for metastatic colorectal cancer (2004) and squamous cell carcinoma of the head and neck (2006). However, despite the expiration of the patent in Europe in 2014 and estimated annual sales of 1.681 million US dollars in 2022, Erbitux® has not yet faced any approved biosimilar challenges in the United States or in Europe. Here, we outline the unique structural complexity of this antibody highlighted by advanced orthogonal analytical characterization strategies resulting in risks to demonstrate biosimilarity, which may explain the lack of Erbitux® biosimilars in the European and US markets to date. The development of Erbitux® biobetters are also discussed as alternative strategies to biosimilars. These biologics offer expected additional safety and potency benefits over the reference product but require a full pharmaceutical and clinical development as for New Molecular Entities.

1. Introduction

The success of monoclonal antibody (mAb)-based therapies in oncology is inevitably accompanied with a significant increase in healthcare costs, which are always difficult to contain. Indeed, there is still a strong development momentum and a need for next generation antibody-based products [1]. Due to their lower development costs [2,3], biosimilars are an interesting alternative to increase patient access to treatment, with a proven, significant, and positive impact on public health [4]. Biologically similar medicines, commonly referred to as biosimilars, were defined by European law in 2004 (Directive 2004/27/EC amending directive 2001/83/EC), and by US law in 2009 (Biologics price competition and innovation act, amending the PHS Act in 2010). As stated by the consolidated Directive 2001/83/EC (article 10.4) concerning the EU community code relating to medicinal products for human use, if biological medicinal products do not meet the conditions in the definition of generic medicinal products, due to differences in raw materials or in manufacturing processes, the results of appropriate pre-clinical tests or clinical trials relating to these conditions must be provided. The European Medicines Agency (EMA) ensures that biosimilars are assessed according to the same standards of pharmaceutical quality, safety and efficacy that apply to all biological medicines approved in the EU. However, the marketing authorization for biosimilars is shortened: it does not require all the clinical trials to be repeated. This offers the hope of lower costs for healthcare payers, which is particularly attractive given the very high price of biotherapies.

* Corresponding author at: Pharmacy Department, Tours University Hospital, Tours, France.
E-mail address: emmanuel.douez@univ-tours.fr (E. Douez).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.jpba.2023.115544
Received 28 April 2023; Received in revised form 12 June 2023; Accepted 20 June 2023
Available online 22 June 2023

They also increase competition in pharmaceutical development [5].

The first antibody biosimilar (infliximab) was approved by EMA in 2013 [6], proactive in the field [7] and by the Food and Drug Administration (FDA) in 2016. In oncology, the first mAbs generation (i.e., rituximab, trastuzumab and bevacizumab) now have biosimilars in both Europe and in the United States. Patents and data protection period for many other mAbs will expire in coming years, and the number of mAb biosimilars entering the market is expected to consequently increase (Fig. 1).

Among the mAbs with an oncology indication, cetuximab, marketed as Erbitux®, was the first monoclonal antibody (IgG1) targeting the epidermal growth factor receptor (EGFR) for the treatment of wild-type K-Ras metastatic colorectal cancer (CRC) approved in 2004 [8]. It also plays an important role in head and neck cancer, where it has no antibody competitior. To date, no biosimilar of Erbitux® has been approved in either the US [9] or Europe [10], even though its EU patent expired in 2014 [11]. Interestingly, a WHO committee expert published by the end of 2022 a report on a collaborative study for the 1st International Standard to assess the in vitro biological activities of cetuximab (inhibition of proliferation activity, EGFR binding activity using cell-based and non-cell based assays, antibody dependent cell mediated cytotoxic activity (ADCC), FcγR binding assays and C1q binding assay) [12].

Hence, the purpose of this review paper is to examine and discuss the reasons that could explain the lack of Erbitux® biosimilars in the European and US markets, including the unique complexity of the antibody structure resulting in higher risk of failure of biosimilarity exercise as for other IgGs.

2. Structural complexity of cetuximab – need for advanced analytical strategies

2.1. Structural complexity of cetuximab and main CQAs

The process of biosimilarity assessment involves amino acid sequence confirmation [13] and comparison of the critical quality attributes (CQAs) in reference to the originator molecule [14]. Cetuximab is a chimeric mouse/human recombinant IgG1 monoclonal antibody produced in mouse myeloma SP2/0 cells. An important number of proteoforms and isoforms have been identified, showing an higher structural heterogeneity than other IgGs [15]. Beyond the canonical post-translational modifications (PTMs) that characterize mAbs (deamidations, oxidations, C-terminal lysines, etc.), the main reason for cetuximab structural heterogeneity is related to a second N-glycosylation consensus site located within the framework 3 of the variable domain of the heavy chain (Asn^{88}, Fab portion) in addition to the CH2 domain (Asn^{299}, Fc portion). In the case of cetuximab, the variable domain from the mouse parent antibody 225 was kept for the clinical chimeric candidate (C225). In the case of trastuzumab, the second N-glycosylation site in the Fab of the parent 4D5 mouse antibody site was removed during the humanization step resulting in a much more homogeneous clinical candidate [16]. In addition in mouse SP2/0 derived cell lines, N-glycolyl neuraminic acid (NGNA), the hydroxylated form of the N-acetyl neuraminic acid (NANA) expressed in humans, is preferentially expressed, together with a fraction of bisecting glycans, not present with Chinese Hamster Ovary (CHO) cell produced antibodies [17,18]. As shown in Fig. 2, the glycan composition of the Fc region mainly consists of typical core fucosylated biantennary glycans bearing variable galactosylation (mostly 0, 1 or 2 β-galactose residues). On the contrary, the Fab region is characterized by murine type glycans, such as α1–6 fucosylated structures terminating with alpha 1 – 3 galactose motifs, and a high content of sialic acids mainly due to the presence of NGNA.

Considering the presence of 4 glycosylation sites in total (2 for each heavy chain), it is easy to understand the great variability that the N-glycosylation profile of cetuximab can have. More generally, the glycan heterogeneities of mAbs might have an impact on safety/immunogenicity, biologic activity/efficacy and clearance (PK/PD).

Specifically, Reusch and Tejada [19] reported a comprehensive study

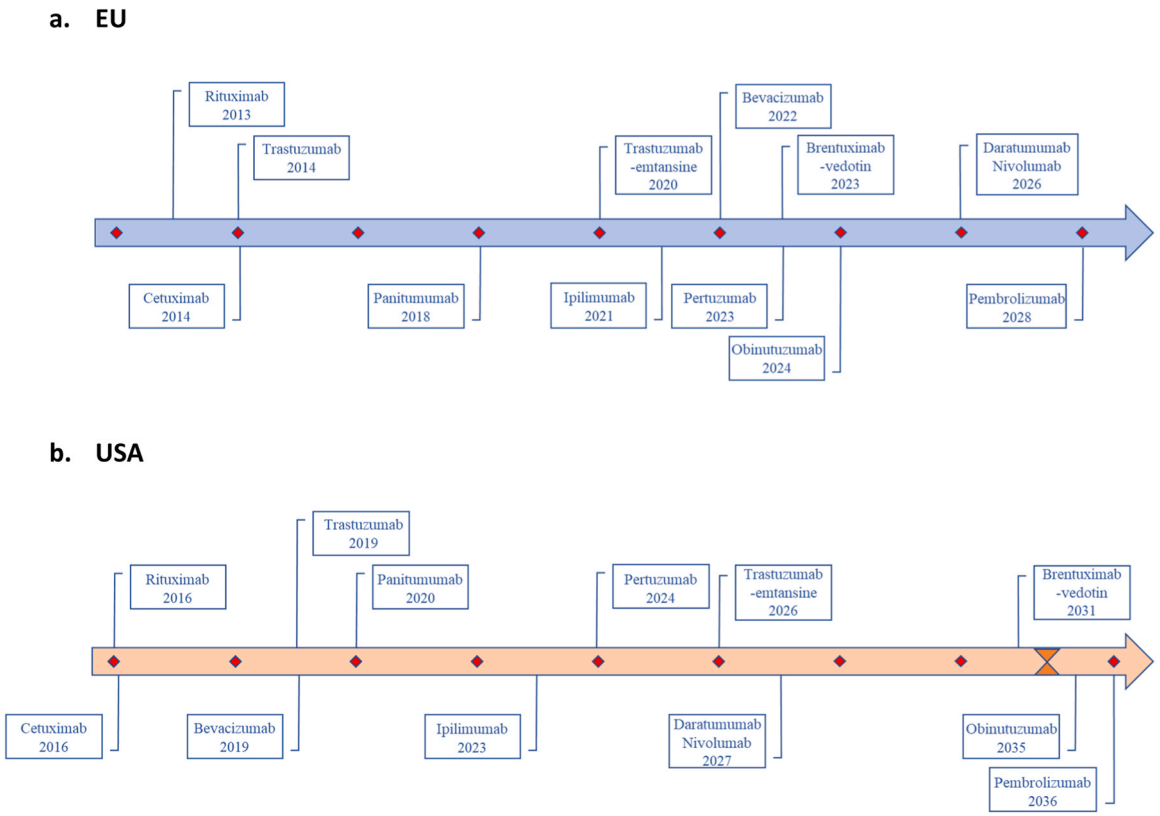

Fig. 1. Dates of patent expiry of the main mAbs used in oncology in European Union (a) and in the United States (b).

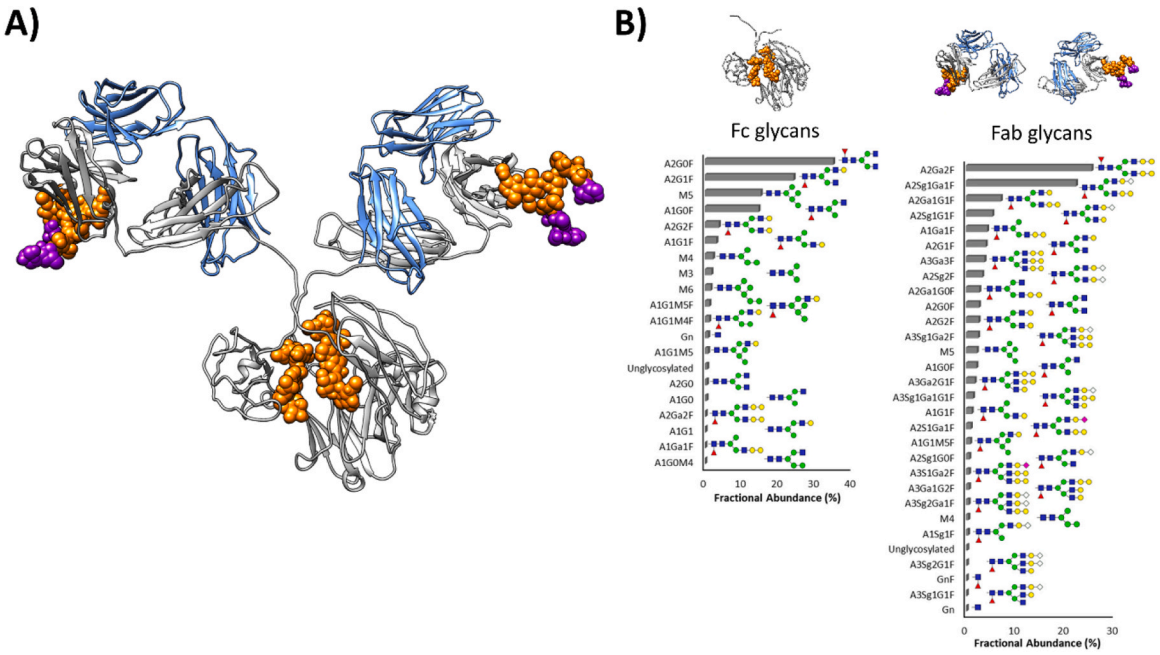

Fig. 2. Three-dimensional model of an IgG1 molecule (A) showing an N-glycosylation site at Asn88 (Fab portion) in addition to Asn299 (Fc portion). The kappa light and gamma heavy chains are in blue and grey, respectively. Fc- and Fab-associated biantennary N-glycans located on Asn299 and Asn88 are shown in orange. Terminal α3Gal residues of the Fab-associated N-glycans have been highlighted in purple. Image created with Chimera software from PDB 1IGY (IgG1) and 1YY9 (cetuximab Fab). Detailed glycosylation profile of cetuximab (B). List of cetuximab Fc and Fab N-glycans shown in order of decreasing fractional abundance from top to bottom. Comparative elucidation of cetuximab heterogeneity on the Intact protein level by cation exchange chromatography and capillary electrophoresis coupled to mass spectrometry, Anal. Chem. 2020, 92, 5431–5438 [15].
Reprinted with permission from Füssl, Trappe, Carillo, Jakes, Bones. Copyright 2020 American Chemical Society.

on this aspect highlighting how specific glycan species might have negative impacts, e.g. N-glycans terminated with α1,3-Galactose or NGNA might affect safety/immunogenicity, high mannose species the clearance, and NANA/NGNA the biologic activity/efficacy [19].

As concerns cetuximab, about 30% of the N-glycans of the Fab domain, terminated with one or two α1,3-Galactose, have been recognized as epitopes associated with severe, sometimes fatal, hypersensitivity reactions in some patients with various reported incidences typically within minutes after first exposure and compatible with IgE-mediated anaphylaxis resulting in a FDA Black Box Warning [20]. However, changing the expression cell lines to prefer the use of mammalian cells, such as CHO of human cell line, would have a remarkable impact on the N-glycosylation profile as the expression of NGNA would be replaced by the human-like sialic acids (NANA) together with lower levels of galactose-α-1,3-galactose motifs expression. But the resulting mAb would not be any more a highly similar version of the originator because of the very different glyco-profile, although it can be expected to show the same efficacy and a better safety profile [17,21].

Given the critical importance of the glycosylation profile, on top of other PTMs, several analytical strategies have been reported in the literature to ensure accurate monitoring of the N-glycan profile together with the possibility of using valid analytical tools in case of comparability assessment [13,15,17,22–25]. Of note, during the manufacture of a mAb product, lot-to-lot variability is also observed in the isoform content. Most often, isolated isoforms indicate that this variability is caused by sialic acid content, as well as truncation of C-terminal lysine of the individual isoforms, although the set specifications for lot release are generally met [26].

2.2. Innovative strategies to assess glycosylation of cetuximab

The N-glycosylation profile is usually characterized by performing an enzymatic release of the glycans from the protein by using the PNGase F enzyme, followed by chemical derivatization of the glycans with 2-aminobenzamide (2-AB) label, to add a chromophore to the carbohydrate structure. The labelled glycans can then be analyzed by hydrophilic interaction chromatography (HILIC) with either fluorescence detection (FLD) or mass spectrometry (MS) [27,28]. However, for biopharmaceuticals with a highly heterogeneous glycosylation profile, such as cetuximab, alternative methods may be useful. In this section, we have described several advanced/innovative analytical strategies that allow a detailed characterization of cetuximab glycoforms.

2.2.1. New labelling agents for released glycans analysis

First of all, it is important to mention that the reference 2-AB labelling has a number of drawbacks, such as *i)* long and laborious labelling procedure, *ii)* poor ionization efficiency in ESI-MS, *iii)* possible desialylation due to the acidic reductive amination reaction (the characterization of sialic acids is particularly critical for cetuximab) [29]. Fortunately, it is now possible to use new labelling agents, such as Waters RapiFluor-MS (RFMS) or Agilent InstantPC which have been developed to avoid the abovementioned problems. For example, in a 5-minute reaction, enzymatically released N-glycans are labelled with RFMS, which contains a quinoline fluorophore, and a tertiary amine to enhance ESI-MS detection. A rapid PNGase F deglycosylation procedure was also incorporated to achieve the complete deglycosylation in only 10 min. This approach has already been applied to cetuximab, allowing an accurate and rapid identification and confirmation of a wide range of glycans [30,31].

2.2.2. HILIC analysis at the subunits level of analysis

As an alternative strategy, it is also possible to characterize cetuximab glycoforms at the protein subunit level, using HILIC-FLD or HILIC-MS. Protein subunits can be obtained after enzymatic digestion with a specific protease (IdeS), and chemical reduction with DTT or TCEP. The whole procedure is fast, easily automated and takes less than 1 h. At the chromatographic level, it is essential to use a HILIC column packed with

sub-2 μm particles and large pore sizes (ideally 300 Å) to analyse mAb subunits of around 25 kDa [32]. Such an approach has already been used for the characterization of cetuximab and a biosimilar candidate [23]. Fig. 3 shows the comparison of RPLC and HILIC-MS for the two samples at the subunit level. As highlighted, the RPLC separation does not provide any selectivity between the different glycoforms, whereas HILIC can achieve a partial separation of the glycoforms, allowing a more direct visualization of the glycol-heterogeneity and an easier deconvolution of the MS signals. In the HILIC chromatogram, the LC subunit was eluted as the first peak, followed by the Fc/2 and its glycoforms shortly thereafter, and Fd' together with its various glycoforms. Not surprisingly, the elution order of the different Fc/2 and Fd′glycoforms seemed to be related to the number of glycan residues involved in the formation of the biantennary/triantennary complex oligosaccharide structures. This elution order was confirmed by MS. Interestingly, the Erbitux® Fd′fragment was found to be populated with larger, hyper-galactosylated glycans and structures containing NGNA. The presence of bisecting N-acetylglucosamines forming triantennary complex oligosaccharides was also detected. The biosimilar candidate product was identical in terms of amino acid sequence for the LC and HC but showed a different glycosylation pattern due to its expression from a CHO cell line instead of SP2/0 murine myeloma cells. In fact, the Fc/2 region was more abundant in fucosylated glycans, whereas the Fd' region was more heterogeneously populated with fucosylated glycans and few sialylated glycans containing NANA. Besides glycans, it is important to mention that the middle-up LC-MS approach described above can simultaneously assess the presence of additional PTMs, such as oxidation, C-terminal lysine truncation and others.

2.2.3. *Multidimensional LC for in-depth characterization*

To further improve the characterization of biopharmaceuticals having complex glycosylation patterns and numerous PTMs, it is possible to combine multiple chromatographic dimensions to achieve unique selectivity and high peak capacity. In recent years, there have been numerous demonstrations of two-dimensional LC (2D-LC) for the detailed characterization of therapeutic monoclonal antibodies, using either ion exchange (IEX), reversed phase (RP), hydrophobic interaction (HIC), hydrophilic interaction (HILIC) or size exclusion (SEC) chromatography in the two dimensions, in combination with MS [33,34]. In addition, depending on the application, such 2D-LC separations have been demonstrated using a heart cutting, multiple heart cutting, selective comprehensive or full comprehensive mode of operation [35,36]. Finally, both intact and subunit samples have been analyzed under such conditions.

Interestingly, 2D-LC-MS has already been used to characterize cetuximab and its biosimilar candidate [33,37]. A fully comprehensive 2D-LC-MS strategy using either CEX or HILIC in the first dimension, followed by RPLC in the second dimension, was developed. As shown in Fig. 4, for the CEX x RPLC setup, CEX was found to be particularly powerful in resolving charge variants (i.e. the variants containing sialic acid as part of their glycan composition generally eluted before those that did not contain such moieties, due to their negative charges), while RPLC provided separation primarily by hydrophobicity (elution of Fc/2, followed by LC and Fd). This pattern in the 2D chromatograms can be very helpful in identifying unknown modifications of mAb fragments. On the other hand, the combination of HILIC with RPLC proved to be more challenging, as the high proportion of acetonitrile in the HILIC mobile phase causes severe broadening and peak distortion in the second-dimension separation. This challenge was overcome by

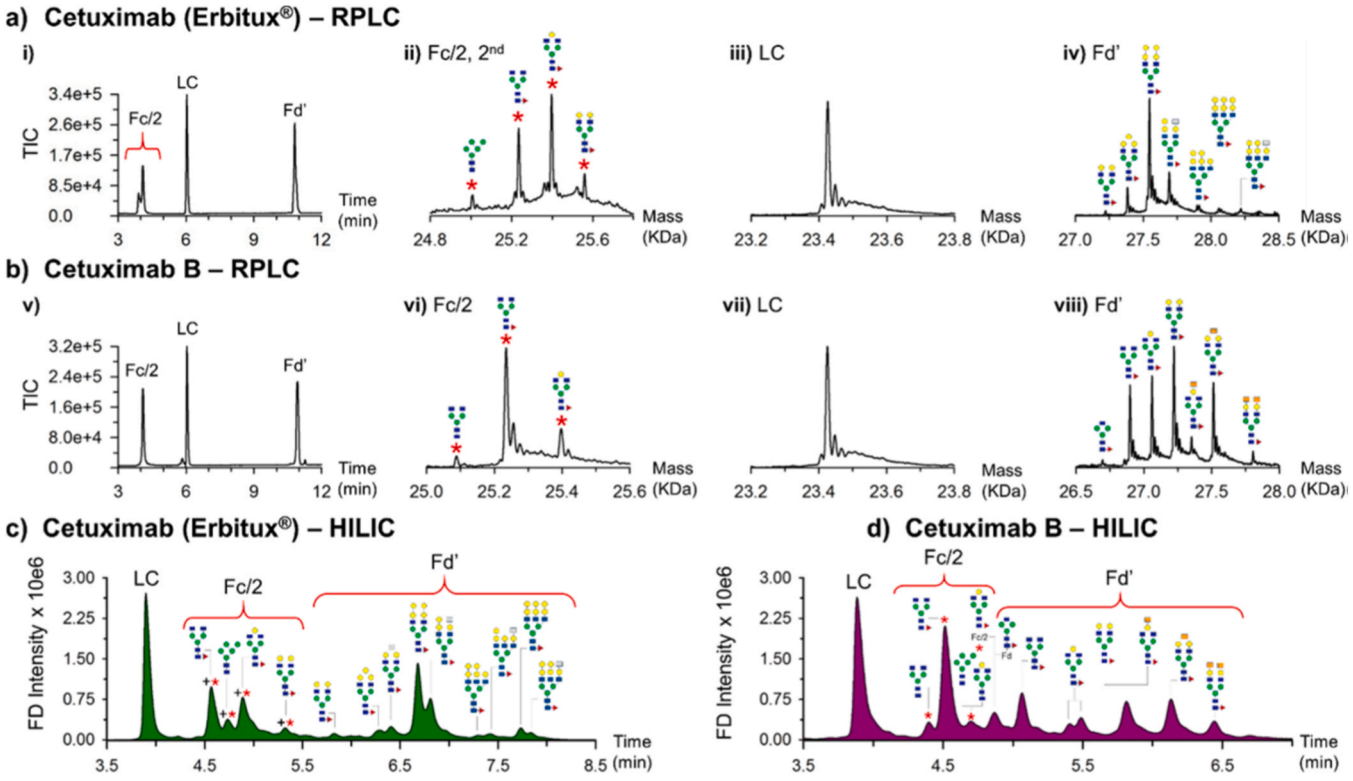

Fig. 3. Middle-up RPLC-MS analysis of (a) cetuximab (Erbitux) and (b) cetuximab biosimilar candidate. Total ion chromatograms (i, v) and associated deconvoluted mass spectra of Fc/2 (ii, vi), LC (iii, vii), and Fd′ (iv, viii) fragments. Middle-up HILIC-MS analysis of Erbitux (c) and cetuximab biosimilar candidate (d). For experimental conditions and peak annotations, the reader is referred to the original article. Hydrophilic Interaction Chromatography Hyphenated with Mass Spectrometry: A Powerful Analytical Tool for the Comparison of Originator and Biosimilar Therapeutic Monoclonal Antibodies at the Middle-up Level of Analysis. Anal. Chem. 2017, 89, 2086–2092 [23].
Reprinted with permission from D'Atri, Fekete, Beck, Lauber, Guillarme. Copyright 2017 American Chemical Society.

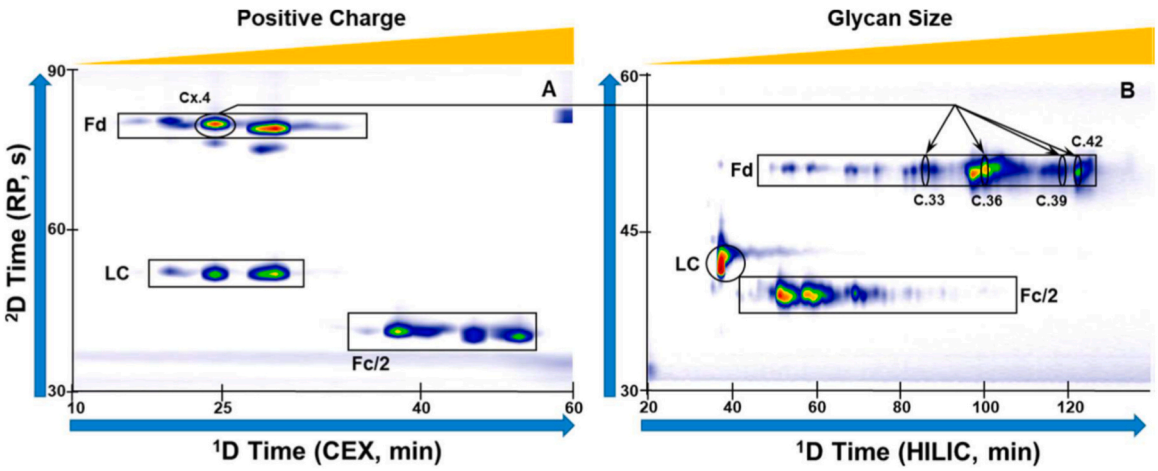

Fig. 4. Comparison of glycan analysis of cetuximab subunits using (A) CEX × RP and (B) HILIC × RP. It was demonstrated that using HILIC in the first dimension, instead of 1D-CEX, could resolve several co-eluting glycoforms (Cx.4) on the heavy chain portion of the antigen binding fraction (Fd) of cetuximab. Chromatograms are based on UV absorbance at 280 nm, peak identification was based on TOF-MS detection. For experimental conditions and peak annotations, the reader is referred to the original article. Development of Comprehensive Online Two-Dimensional Liquid Chromatography/Mass Spectrometry Using Hydrophilic Interaction and Reversed-Phase Separations for Rapid and Deep Profiling of Therapeutic Antibodies. Anal. Chem. 2018, 90, 5923–5929 [33].
Reprinted with permission from Stoll, Harmes, Staples, Potter, Dammann, Guillarme, Beck. Copyright 2018 American Chemical Society.

implementing a valve-based approach, called active solvent modulation (ASM) to dilute the weak solvent fractions of the ^{1}D effluent. As shown in Fig. 4, the improvement in resolution offered by the ^{1}D in the case of HILIC x RPLC over CEX x RPLC was quite striking. Many peaks that coeluted in the Cx.4 region using the CEX dimension are now clearly resolved with the HILIC separation.

Finally, a significant number of fragments were identified using the approach described above, provided that the 2D-LC separation was sufficiently optimized. This confirms that conventional MS instrumentation (such as Time of Flight (ToF)-MS) can be used for in-depth characterization of cetuximab, without the need for much more expensive high-resolution MS instrumentation.

2.2.4. *Capillary zone electrophoresis coupled with mass spectrometry*

In addition to chromatography, capillary electrophoresis (CE) can also be successfully coupled to MS detection. A systematic comparison has recently been carried out for Erbitux® at the protein level, using pH gradient CEX-MS and CZE-MS (using the ZipChip microfluidic CE-MS technology) [15]. These two separation modes are able to characterize the charge variants of complex products such as cetuximab. The high content of sialic acid (mostly NGNA) present on the Fab region of cetuximab is responsible for acidic forms, whereas the basic variants are due to the incomplete enzymatic lysine truncation.

As shown in Fig. 5, eight well-resolved charge variant peaks of varying abundance were identified in both cases, each giving access to native mass spectra without any sign of protein denaturation, allowing the annotation of the proteoforms corresponding to each peak.

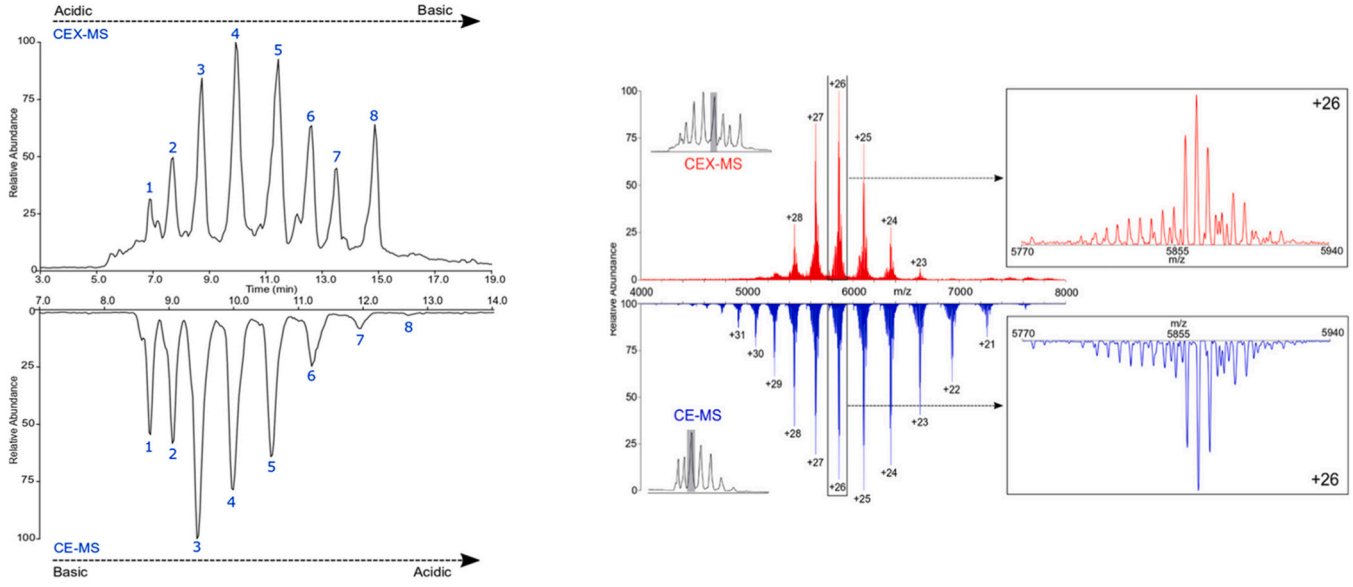

Fig. 5. Comparison of the separations obtained in CEX-MS and CZE-MS (left panel). Comparison of the charge envelopes obtained by the two orthogonal analytical approaches (right panel). For experimental conditions and peak annotations, the reader is referred to the original article. Comparative Elucidation of Cetuximab Heterogeneity on the Intact Protein Level by Cation Exchange Chromatography and Capillary Electrophoresis Coupled to Mass Spectrometry. Anal. Chem. 2020, 92, 5431–5438 [15].
Reprinted with permission from Fussl, Trappe, Carillo, Jakes and Bones. Copyright 2020 American Chemical Society.

However, the selectivity was found to be different between the two modes of separation, as were the charge states (mainly due to differences in the separation mechanism, but also to the presence of different additives in the mobile phase and background electrolyte). In addition to charge variant separation, CE-MS also allowed hydrodynamic radius-dependent separation of species based on different glycoform complexity within individual charge variant peaks. In this study, 109 and 218 isoforms were found by CEX-MS and CZE-MS, respectively. The main advantages of the technique were that CZE-MS was found to be fully generic (similar conditions can be used regardless of the mAb under investigation), sample consumption was very low (only 1 ng) and MS sensitivity was enhanced due to the operational flow rate in the low nL range.

Interestingly, the potential of CEX and CZE was also combined in a single workflow. Charge fractionation was first performed using pH gradient CEX conditions and 8 different fractions were collected. All these individual fractions were then re-analysed using CZE-MS. This strategy allowed the simultaneous characterization of the heterogeneity resulting from N-glycosylation bearing sialylation, and also additional modifications such as C-terminal lysine residues [38].

Finally, CZE-MS has also been successfully applied in another work, where cetuximab was successfully analysed at the subunit level of analysis (after IdeS digestion), highlighting the interest of CZE-MS for the analysis of mAb samples with a high degree of sialylation [25].

2.2.5. *Innovative MS-based approaches*

MS-based approaches are becoming increasingly popular for the characterization of mAb products. Cetuximab and a biosimilar candidate were thoroughly characterized at the intact level using denaturing MS, native MS and ion mobility MS (IM-MS) [24]. The experiments were performed using matrix assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI). As reported in Fig. 6, analysis at the intact level in denaturing MS or native MS allowed easy measurement of intact mAb masses and differentiation of batch-to-batch heterogeneity. However, it was not possible to assess the glycosylation heterogeneity. Combining ion mobility with native MS, additional information on disulphide bridge pairing heterogeneity of the mAb were obtained. As for the biosimilar candidate, the driftscope plot (Fig. 6) was found to be equivalent to the cetuximab originator. Based on these experiments, it appeared that the evaluation of the glycosylation pattern of cetuximab at the intact level of analysis was very limited when using either denaturing or native MS alone. Therefore, as highlighted in the previous sections, the combination of state-of-the-art MS methods with high resolution separation methods (LC, CZE) it is therefore recommended to clearly demonstrate the similarity between the reference product and the biosimilar candidate.

Interestingly, there are already some commercial biosimilar versions of etanercept [39] and epoetin alfa (EPO), although these two products are much more difficult to characterize than cetuximab in terms of glycosylation complexity. In the case of etanercept, the detailed characterization of the glycoforms was achieved by a combination of different and complementary enzymatic digestion procedures (i.e. glycosidase, sialidase, and protease). After the enzymatic treatment, two different strategies can be applied, namely an HILIC-MS analysis using a simple QTOF/MS instrument [40] or a native MS using a high resolution Orbitrap MS instrument (high spatial resolution at lower charge state) [41]. Using these enzymes, it was possible to support the MS spectra deconvolution and assess (i) the major PTMs (mainly C-terminal lysine clipping), (ii) the subunit-specific distribution of glycans, and (iii) the overall N/O-glycan composition and sialylation profiles of each subunit. For EPO, to the best of our knowledge, the most powerful analytical strategy for glycoform characterization combines anion exchange chromatography (AEX) and Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) [42]. In a single experiment, assessment of critical quality attributes, such as charge heterogeneity, sialic acid content and number of N-acetyllactosamine units could be assessed, while providing additional information on other modifications such as O-acetylation and deamidation. Based on these data, it is therefore clear that the analytical complexity of cetuximab is not the main reason why a biosimilar is not available yet.

3. Regulatory aspects

In 2009, the EMA “Guideline on Evaluation of Similar Biopharmaceutical Products” reported that additional studies such as receptor activity or preclinical studies (both *in vitro* and *in vivo*) are required when

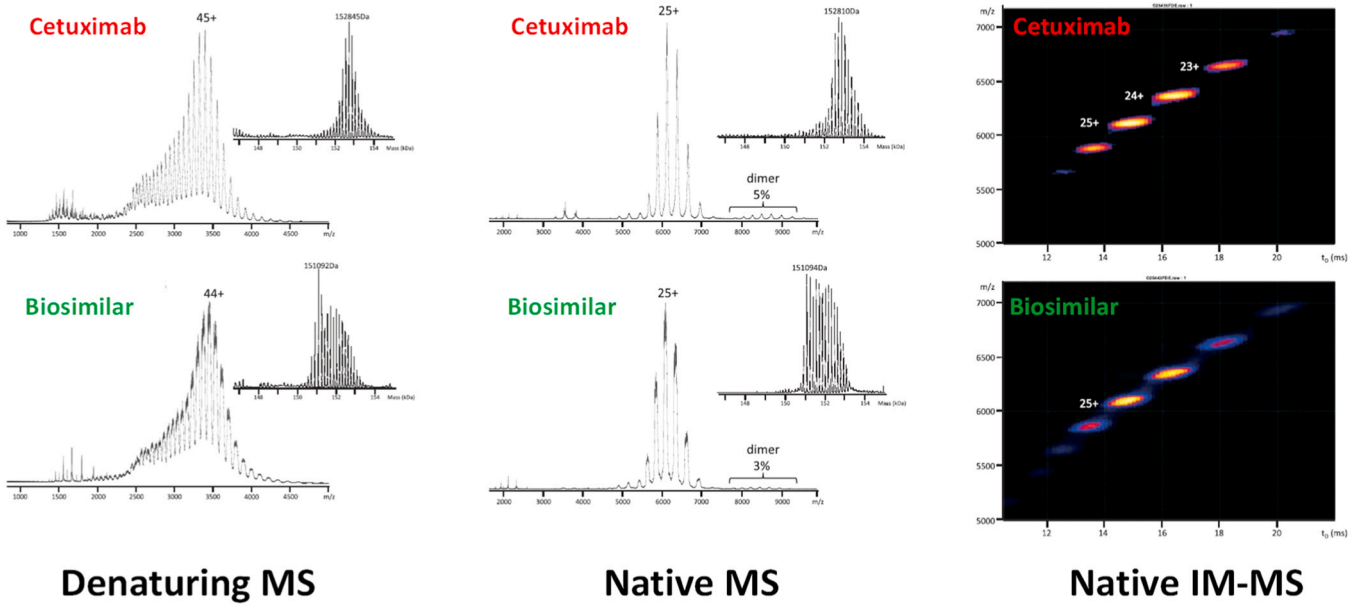

Fig. 6. Denaturing MS, native MS and native IM-MS of cetuximab and a biosimilar candidate. For experimental conditions and peak annotations, the reader is referred to the original article. Cutting-edge mass spectrometry characterization of originator, biosimilar and biobetter antibodies. J. Mass Spec. 2015, 50, 285–297 [24].
Reprinted with permission from beck, Debaene, Diemer, Wgner-Rousset, Colas, Van Dorsselaer and Cianferani.

there are changes in the glycosylation pattern [43]. Within the EU, guidelines for similar biological medicinal products do not exclude variations in structure. Thus, it is possible to obtain a biosimilar with glycosylation structures different from the reference, but this must be adequately justified [44]. For example, infliximab biosimilars have been approved by the EMA despite some analytical glycosylation differences related to CHO cell line expression. Furthermore, if intended changes to improve efficacy, such as glyco-optimisation, are not compatible with the biosimilarity approach, differences that may have a safety benefit in terms of reduced immunogenicity or allergenicity should be addressed but may not preclude biosimilarity [44]. For example, it is possible to imagine a modified cetuximab (designed to prevent adverse reactions) being approved as biosimilar.

The FDA guidelines (first published in 2009) appear to be more flexible than the European guidelines, being based on a "case-by-case totality-of-evidence" and a risk-based approach [45], even though this form of regulation may discourage companies from submitting biosimilar candidates, due to the uncertainty of the approval process. In addition, Van de Wiele et al. identified two barriers to biosimilar entry in the US: the high number of patents and the non-compliance with the complex legislative process created by the "Biologics Price Competition and Innovation Act" of 2010 [46]. Thanks to all innovative strategies described in previous paragraph, more chances of approval process could be expected for modified cetuximab showing a different glycan profile but purposely designed to prevent fatal adverse reaction linked to α3 Gal residues.

4. Side effects of cetuximab – towards a biobetter product?

The frequency (around 1.5%) and severity of the anaphylactic reactions reported with cetuximab may hamper the development of Erbitux® biosimilars. This was not the case during the development of Erbitux® more than a decade ago, as the drug was already approved by the time the problem was identified, but it will undoubtedly affect the development of biosimilars. To date, no other monoclonal antibody causes so much severe hypersensitivity reactions, which may be life threatening [47]. Developing copies of a molecule that is known to have flaws can pose ethical problems. It makes no sense from the point of view of pharmaceutical and medical ethics to market which we know the reference drug poses safety problems. European medicinal product regulations have only one aim: to ensure the quality, efficacy, and safety of the molecule to act in the interests of the patient. This seems ethically unacceptable given that European regulations allow slight modifications of the biosimilar structure in order to improve its safety profile. This possibility remains only theoretical, for this reason, instead of biosimilars, it might be more reassuring for a company to develop biobetter versions of Erbitux®.

Biobetter is a term introduced by biotech companies, probably Reddy's Laboratories in 2007 [48,49]. Biobetters are copies that have been structurally and/or functionally altered to improve the activity or risk-benefit ratio compared to the originator [12,50]. A biobetter product is very often associated with an improvement of mAb properties such as affinity and selectivity for the target epitope, Fc effector functions improvement and/or stability against degradation, but in this context the desired biobetter would be an antibody with increased safety. Biobetters are treated by regulatory authorities as original products and are evaluated as new drugs in a standard approval process. Of course, the risk of failure in clinical trials is higher than for biosimilars [51]. Eliminating α3 Gal residues and making cetuximab safer fits very well with the biobetter concept.

There are two main issues to consider regarding the existence of a serious adverse event regarding this medication: *i)* A biological test is available to detect anti-alpha Gal IgE and prevent anaphylactic shock, thereby reducing morbidity/mortality. Despite its availability, this test is rarely performed prior to cetuximab administration. *(ii)* There is no biosimilar or biobetter without alphaGal. One of the possible solutions to reduce this problem of anaphylactic reaction would be to change the recommendations and the SPC (summary of product characteristics) to make the measurement of anti-alpha IgE mandatory before the first administration. Unfortunately, this solution does not encourage the development of the biosimilar and therefore has a negative impact on the pharmacoeconomic of the biological product.

As reported in Table 1, at least four cetuximab-related molecules are still in preclinical development: ABP-494, CT-P15, MabioEGFR, and APZ001. So far, none of them has been sufficiently described to know whether they could be considered as true biosimilars by regulatory authorities in Europe and the US. Five other products have entered clinical development: HLX05 and HLX07, A140, CMAB009 (also known as STI-001) and tomozotuximab (see Table 1). Structural details are only available for the last two products. CMAB009 is produced in CHO cells, which do not add terminal α3 Gal residues [52]. An editorial in the journal Generics and Biosimilars Initiative journal (GaBi) suggests that CMAB009 could not be approved as a biosimilar to Erbitux® in Europe, unless rigorous preclinical and clinical comparability is performed in the Shi et al. study [53]. A decrease in hypersensitivity reactions was observed compared to the incidence reported with Erbitux® (2%) in phase III [53], but this is not a fair comparison. Interestingly, ASCO considers CMAB009 to be a biosimilar of Erbitux® [54], demonstrating that the medical community is not sufficiently familiar with the distinction between a biosimilar and a biobetter. Tomuzotuximab (CetuGEX, WHO RL 78 2017) is a glycoengineered second generation antibody of cetuximab produced in the human GlycoExpress expression system (Glycotope GmbH, Berlin, Germany). It is an IgG1 glycoengineered mAb of cetuximab with the same binding properties to epidermal growth factor receptor (EGFR) as cetuximab but with enhanced ADCC [55].

Produced in a glyco-engineered cell line derived from a human erythroleukemia (K562). Glycans are mostly biantennary complex glycans with 5% sialylated glycans, 10% bisecting N-acetylglucosamine bearing glycans and no N-glycolylneuraminic acid (WHO INN RL 78, 2017). It is also based on the same Erbitux® amino-acid sequence but combines two major changes in the glycosylation profile, the absence of the terminal α3 Gal residues mentioned above, and the absence of core fucosylation to trigger higher ADCC. A different INN (tomuzotuximab) has been attributed to this antibody showing clearly that it is not a biosimilar of cetuximab.

5. Market size of cetuximab

A financial argument can also be used to explain the lack of an Erbitux® biosimilar in the therapeutic armamentarium. In 2014, Grabowski et al. argued that there are high market entry costs of biosimilars compared to generics [62]. Currently, there are seven mAbs/Fc fusion protein drugs with approved biosimilars in Europe. All are blockbusters (top ten) with significant sales. Despite reported sales of 1.681 million USD per year, Erbitux® profits are not included among the top ten biopharmaceuticals in the world [63,64]. Among biological drugs, Erbitux® ranks forty-second in terms of global sales in 2022 [65]. By contrast, the global sales of originators bevacizumab, rituximab and trastuzumab in the same year were 2.316 million, 2.265 million and 2.338 million USD, respectively, making their market more attractive. Knowing that these have a reduced turnover because biosimilars are in competition with each other. Also, etanercept which is a biological with more glycosylation (O-glycosylation…) complexities than cetuximab, has biosimilars since 2016 in Europe.

Of course, pharmaceutical companies think in terms of return on investment. Developing a cetuximab biosimilar is a major financial risk for manufacturers. In fact, there are many other antibody candidates that are more likely to reach the market in terms of biosimilars and sales. Panitumumab (Vectibix®) is the direct competitor to cetuximab in colorectal cancer. It has similar sales, although it is prescribed less frequently than cetuximab. Its patent has also just expired in Europe and

Table 1
Biosimilars/Biobetters of cetuximab in development. *N.A. for non-applicable.*

Molecules	Companies	Expression system	Dev. Phase	Last available data	Properties	Reference
ABP-494	Actavis/ Amgen	N.A	Preclinical	2019	?	Busse & Luftner[2]
CT-P15	Celltrion	N.A	Preclinical	2019	?	Busse & Luftner[2]
MabioEGFR	Mabion	N.A	Preclinical	2019	?	Busse & Luftner[2]
APZ001	N.A	N.A	Preclinical	2018	?	Wang et al.[56]
HLX05	Henlius biotech	N.A	Phase I	2016	?	Henlius[57]
HLX07	Henlius biotech	N.A	Phase I	2021	Humanized	Henlius[58,59]
Tomuzotuximab	Glycotope	Human GlycoExpress	Phase I Phase II (ResGEX)	2021	No terminal α3 Gal residues Afucosylated	Fiedler et al.[55] Klinghammer et al. [60]
A140	Sichuan Kelun Pharmaceutical Research Institute	N.A	Phase III	2021	?	NCT04835142
CMAB009 STI-001	Sorrento therapeutics	CHO	Phase III	2019	No terminal α3 Gal residues*	Shi et al.[48] Xiao-Hui et al.[61]

the US and there is no biosimilar on the market.

Furthermore, Moorkens *et al.* reported that the manufacturing process could be a financial barrier as well as numerous step protected by patents [66], which could discourage companies from developing complex biosimilars [64], to the point of abandoning an Erbitux® biosimilar market estimated to be worth more than one million dollars in 2019 [67].

It should also be noted that Erbitux has been granted orphan drug status for the treatment of squamous cell carcinoma of the (oro/hypo)-larynx only in the US since 2000. But so far like this designation had a limited encouragement impact for the development of a cetuximab biosimilar in the US.

6. Conclusion

To date, no biosimilar of Erbitux® has been approved in Europe or in the US, despite patent and data protection expiry. This situation has a pharmacoeconomic issue as it maintains high prices for a product that has already paid for itself, and it creates unfair competition that could favor a putative biobetter. In comparison to all other canonical EMA and FDA approved IgGs, one of main limitation of cetuximab is probably the unique structural complexity related to the presence of a second N-glycosylation in the Fab domain. This increases importantly the risk to not show analytical and functional biosimilarity which is the basis of the successful approval (totality-of-evidence).

More complex glycoproteins such as etanercept biosimilars have been successfully developed [68,69] but the market of the originator is higher (7.217 million USD in 2019). Many more have also failed [70]. Certolizumab pegol (Cimzia®) is another off-patent biomedicine without a biosimilar. Like for cetuximab, the structural complexity related to the PEG moiety maybe the reason of lack of biosimilar.

The new and upcoming antibody biosimilars that maybe approved in the next 5 years are Stelara®/ ustekinumab, Actemra®/ tocilizumab, Tysabri®/ natalizumab or Perjeta®/ pertuzumab to name a few [71]. All are canonical IgGs less complex than cetuximab and with a higher originator market.

On May 2023, Alkem Oncology has announced the launch of Cetuxa®, a biosimilar according to India regulations. It received Drugs Controller General of India (DCGI) approval on 16th January 2023 for head and neck cancer. This organization modified its recommendations in 2016 to align with European and American standards. Nevertheless, these probably do not yet correspond to EMA and FDA standards for approving a biosimilar [72,73]. According to the Indian Practitioner, the production of Cetuxa® on CHO cells (in contrast to SP2/0 with Erbitux®) could change all the N-glycosylation profile (removing alpha3Gal) and improve its pharmacodynamics/safety [27]. At the time of finalizing the manuscript, no data were available in the literature on this biobetter.

CRediT authorship contribution statement

Emmanuel Douez and Valentina D'Atri were the lead author and the writers. Laura Foucault-Fruchard, Hervé Watier and Alain Beck managed the manuscript. Hervé Watier has provided significant expertise in the field of immunological drugs. Valentina D'Atri and Davy Guillarme has provided significant expertise in bioanalysis and in analytical strategies. Mathieu Guerriaud made a major contribution to the field of ethics and patents. The eight authors carried out an important critical revision of the content of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

No data was used for the research described in the article.

Acknowledgments

This work was supported by the French Higher Education and Research Ministry under the program "Investissements d'Avenir'', grant agreement: LabEx MAbImprove ANR-10-LABX-53–01.

References

[1] H. Kaplon, S. Crescioli, A. Chenoweth, J. Visweswaraiah, J.M. Reichert, Antibodies to watch in 2023, mAbs 15 (2023), 2153410.
[2] A. Busse, D. Lüftner, What does the pipeline promise about upcoming biosimilar antibodies in oncology? Breast Care 14 (2019) 10–16.
[3] D.J. Kerr, A. Jani, S.M. Gray, Strategies for sustainable cancer care, Am. Soc. Clin. Oncol. (2016) 5.
[4] G.H. Lyman, R. Zon, R.D. Harvey, R.L. Schilsky, Rationale, opportunities, and reality of biosimilar medications, N. Engl. J. Med. (2018) 9.
[5] M.-I. Farfan-Portet, S. Gerkens, I. Lepage-Nefkens, I. Vinck, F. Hulstaert, Are biosimilars the next tool to guarantee cost-containment for pharmaceutical expenditures? Eur. J. Health Econ. 15 (2014) 223–228.
[6] A. Beck, J.M. Reichert, Approval of the first biosimilar antibodies in Europe, mAbs 5 (2013) 621–623.
[7] J.M. Reichert, A. Beck, H. Iyer, European medicines agency workshop on biosimilar monoclonal antibodies, mAbs 1 (2009) 394–416.
[8] P. Kirkpatrick, J. Graham, M. Muhsin, Cetuximab, Nat. Rev. Drug Discov. 3 (2004) 549–550.
[9] IQVIA Institute, Biosimilars in the United States 2023–2027.
[10] Troein, P., Newton, M., Stoddart, K., Arias, A., The Impact of Biosimilar Competition in Europe. *IQVIA Inc* n.d.
[11] E. Moorkens, A.G. Vulto, I. Huys, An overview of patents on therapeutic monoclonal antibodies in Europe: are they a hurdle to biosimilar market entry? mAbs 12 (2020), 1743517.
[12] Sandra Prior, Angela Holder, Bernard Fox, Eleanor Atkinson, Jason Hockley, Peter Rigsby, and Simon Hufton, and participants in the collaborative study, Report on a

Collaborative Study for Proposed Candidate 1st International Standard for the biological activities of Cetuximab. *WHO* 2022.
[13] D. Ayoub, W. Jabs, A. Resemann, W. Evers, C. Evans, L. Main, C. Baessmann, E. Wagner-Rousset, D. Suckau, A. Beck, Correct primary structure assessment and extensive glyco-profiling of cetuximab by a combination of intact, middle-up, middle-down and bottom-up ESI and MALDI mass spectrometry techniques, mAbs 5 (2013) 699–710.
[14] N. Nupur, S. Joshi, D. Gulliarme, A.S. Rathore, Analytical similarity assessment of biosimilars: global regulatory landscape, recent studies and major advancements in orthogonal platforms, Front. Bioeng. Biotechnol. (2022) 10.
[15] F. Füssl, A. Trappe, S. Carillo, C. Jakes, J. Bones, Comparative elucidation of cetuximab heterogeneity on the intact protein level by cation exchange chromatography and capillary electrophoresis coupled to mass spectrometry, Anal. Chem. 92 (2020) 5431–5438.
[16] P. Carter, L. Presta, C.M. Gorman, J.B. Ridgway, D. Henner, W.L. Wong, A. M. Rowland, C. Kotts, M.E. Carver, H.M. Shepard, Humanization of an anti-p185HER2 antibody for human cancer therapy, Proc. Natl. Acad. Sci. 89 (1992) 4285–4289.
[17] A. Trappe, F. Füssl, S. Millán-Martín, R. Ronan, I. Zaborowska, J. Bones, Correlative N-glycan and charge variant analysis of cetuximab expressed in murine, chinese hamster and human expression systems, J. Chromatogr. B 1194 (2022), 123186.
[18] F. Li, N. Vijayasankaran, A. Shen, (Yijuan), R. Kiss, A. Amanullah, Cell culture processes for monoclonal antibody production, mAbs 2 (2010) 466–479.
[19] D. Reusch, M.L. Tejada, Fc glycans of therapeutic antibodies as critical quality attributes, Glycobiology 25 (2015) 1325–1334.
[20] C.H. Chung, B. Mirakhur, E. Chan, Q.-T. Le, J. Berlin, M. Morse, B.A. Murphy, S. M. Satinover, J. Hosen, D. Mauro, R.J. Slebos, Q. Zhou, D. Gold, T. Hatley, D. J. Hicklin, T.A.E. Platts-Mills, Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3-galactose, N. Engl. J. Med. 358 (2008) 1109–1117.
[21] D.R. Goulet, S. Chatterjee, W.-P. Lee, A.B. Waight, Y. Zhu, A.N.-S. Mak, Engineering an enhanced EGFR engager: humanization of cetuximab for improved developability, Antibodies 11 (2022) 6.
[22] J. Qian, T. Liu, L. Yang, A. Daus, R. Crowley, Q. Zhou, Structural characterization of N-linked oligosaccharides on monoclonal antibody cetuximab by the combination of orthogonal matrix-assisted laser desorption/ionization hybrid quadrupole–quadrupole time-of-flight tandem mass spectrometry and sequential enzymatic digestion, Anal. Biochem. 364 (2007) 8–18.
[23] V. D'Atri, S. Fekete, A. Beck, M. Lauber, D. Guillarme, Hydrophilic interaction chromatography hyphenated with mass spectrometry: a powerful analytical tool for the comparison of originator and biosimilar therapeutic monoclonal antibodies at the middle-up level of analysis, Anal. Chem. 89 (2017) 2086–2092.
[24] A. Beck, F. Debaene, H. Diemer, E. Wagner-Rousset, O. Colas, A.V. Dorsselaer, S. Cianférani, Cutting-edge mass spectrometry characterization of originator, biosimilar and biobetter antibodies, J. Mass Spectrom. 50 (2015) 285–297.
[25] M. Biacchi, R. Gahoual, N. Said, A. Beck, E. Leize-Wagner, Y.-N. François, Glycoform separation and characterization of cetuximab variants by middle-up off-line capillary zone electrophoresis-UV/electrospray ionization-MS, Anal. Chem. 87 (2015) 6240–6250.
[26] S. Sundaram, A. Matathia, J. Qian, J. Zhang, M.-C. Hsieh, T. Liu, R. Crowley, B. Parekh, Q. Zhou, An innovative approach for the characterization of the isoforms of a monoclonal antibody product, mAbs 3 (2011) 505–512.
[27] B.L. Duivelshof, W. Jiskoot, A. Beck, J.-L. Veuthey, D. Guillarme, V. D'Atri, Glycosylation of biosimilars: recent advances in analytical characterization and clinical implications, Anal. Chim. Acta 1089 (2019) 1–18.
[28] L. Zhang, S. Luo, B. Zhang, Glycan analysis of therapeutic glycoproteins, mAbs 8 (2016) 205–215.
[29] B.L. Duivelshof, S. Denorme, K. Sandra, X. Liu, A. Beck, M.A. Lauber, D. Guillarme, V. D'Atri, Quantitative N-glycan profiling of therapeutic monoclonal antibodies performed by middle-up level HILIC-HRMS analysis, Pharmaceutics 13 (2021) 1744.
[30] M.A. Lauber, Y.-Q. Yu, D.W. Brousmiche, Z. Hua, S.M. Koza, P. Magnelli, E. Guthrie, C.H. Taron, K.J. Fountain, Rapid preparation of released N-Glycans for HILIC analysis using a labeling reagent that facilitates sensitive fluorescence and ESI-MS detection, Anal. Chem. 87 (2015) 5401–5409.
[31] C.E. Reed, J. Fournier, N. Vamvoukas, S.M. Koza, Automated preparation of MS-sensitive fluorescently labeled N-glycans with a commercial pipetting robot, SLAS Technol. Transl. Life Sci. Innov. 23 (2018) 550–559.
[32] A. Periat, S. Fekete, A. Cusumano, J.-L. Veuthey, A. Beck, M. Lauber, D. Guillarme, Potential of hydrophilic interaction chromatography for the analytical characterization of protein biopharmaceuticals, J. Chromatogr. A 1448 (2016) 81–92.
[33] D.R. Stoll, D.C. Harmes, G.O. Staples, O.G. Potter, C.T. Dammann, D. Guillarme, A. Beck, Development of comprehensive online two-dimensional liquid chromatography/mass spectrometry using hydrophilic interaction and reversed-phase separations for rapid and deep profiling of therapeutic antibodies, Anal. Chem. 90 (2018) 5923–5929.
[34] D.R. Stoll, D.C. Harmes, J. Danforth, E. Wagner, D. Guillarme, S. Fekete, A. Beck, Direct identification of rituximab main isoforms and subunit analysis by online selective comprehensive two-dimensional liquid chromatography–mass spectrometry, Anal. Chem. 87 (2015) 8307–8315.
[35] D.R. Stoll, P.W. Carr, Two-dimensional liquid chromatography: a state of the art tutorial, Anal. Chem. 89 (2017) 519–531.
[36] B.W.J. Pirok, A.F.G. Gargano, P.J. Schoenmakers, Optimizing separations in online comprehensive two-dimensional liquid chromatography, J. Sep. Sci. 41 (2018) 68–98.
[37] M. Sorensen, D.C. Harmes, D.R. Stoll, G.O. Staples, S. Fekete, D. Guillarme, A. Beck, Comparison of originator and biosimilar therapeutic monoclonal antibodies using comprehensive two-dimensional liquid chromatography coupled with time-of-flight mass spectrometry, mAbs 8 (2016) 1224–1234.
[38] C. Váradi, C. Jakes, J. Bones, Analysis of cetuximab N-Glycosylation using multiple fractionation methods and capillary electrophoresis mass spectrometry, J. Pharm. Biomed. Anal. 180 (2020), 113035.
[39] B.L. Duivelshof, A. Murisier, J. Camperi, S. Fekete, A. Beck, D. Guillarme, V. D'Atri, Therapeutic Fc-fusion proteins: Current analytical strategies, J. Sep. Sci. 44 (2021) 35–62.
[40] V. D'Atri, L. Nováková, S. Fekete, D. Stoll, M. Lauber, A. Beck, D. Guillarme, Orthogonal middle-up approaches for characterization of the glycan heterogeneity of etanercept by hydrophilic interaction chromatography coupled to high-resolution mass spectrometry, Anal. Chem. 91 (2019) 873–880.
[41] T. Wohlschlager, K. Scheffler, I.C. Forstenlehner, W. Skala, S. Senn, E. Damoc, J. Holzmann, C.G. Huber, Native mass spectrometry combined with enzymatic dissection unravels glycoform heterogeneity of biopharmaceuticals, Nat. Commun. 9 (2018) 1713.
[42] G. van Schaick, C. Gstöttner, A. Büttner, D. Reusch, M. Wuhrer, E. Domínguez-Vega, Anion exchange chromatography – mass spectrometry for monitoring multiple quality attributes of erythropoietin biopharmaceuticals, Anal. Chim. Acta 1143 (2021) 166–172.
[43] European Medicines Agency, Guideline on similar biological medicinal products containing monoclonal antibodies – non-clinical and clinical issues. EMA/CHMP/BMWP/403543/2010. 2012, 16.
[44] European Medicine Agency, Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues. 2014. EMA/CHMP/BWP/247713/2012. 2014.
[45] J. Daller, Biosimilars: a consideration of the regulations in the United States and European union, Regul. Toxicol. Pharmacol. 76 (2016) 199–208.
[46] V.L. Van de Wiele, A.S. Kesselheim, A. Sarpatwari, Barriers to US biosimilar market growth: lessons from biosimilar patent litigation, Health Aff. (Millwood) 40 (2021) 1198–1205.
[47] ImClone LLC, E. L. and C., ERBITUX (cetuximab), FDA (2004).
[48] R. Anour, Biosimilars versus 'biobetters'—a regulator's perspective, Generics Biosimilars Initiat. J. 3 (2014) 166–167.
[49] A. Beck, Biosimilar, biobetter and next generation therapeutic antibodies, mAbs 3 (2011) 107–110.
[50] F. de Mora, Biosimilar: what it is not, Br. J. Clin. Pharmacol. 80 (2015) 949–956.
[51] M. Kesik-Brodacka, Progress in biopharmaceutical development, Biotechnol. Appl. Biochem. 65 (2018) 306–322.
[52] C. Wang, X. He, B. Zhou, J. Li, B. Li, W. Qian, S. Hou, H. Wang, Y. Shi, Y. Guo, Phase 1 study of anti-epidermal growth factor receptor monoclonal antibody in patients with solid tumors, mAbs 3 (2011) 67–75.
[53] Y. Shi, J. Li, J. Xu, Y. Sun, L. Wang, Y. Cheng, W. Liu, G. Sun, Y. Chen, L. Bai, Y. Zhang, X. He, Y. Luo, Z. Wang, Y. Liu, Q. Yao, Y. Li, S. Qin, X. Hu, F. Bi, R. Zheng, X. Ouyang, CMAB009 plus irinotecan versus irinotecan-only as second-line treatment after fluoropyrimidine and oxaliplatin failure in *KRAS* wild-type metastatic colorectal cancer patients: promising findings from a prospective, open-label, randomized, phase III trial, Cancer Commun. 39 (2019) 28.
[54] R.M. Rifkin, S.R. Peck, Biosimilars: Implications for Clinical Practice, J. Oncol. Pract. 13 (2017) 24s–31s.
[55] W. Fiedler, S. Cresta, H. Schulze-Bergkamen, Dosso De, S. Weidmann, J. Tessari, A. Baumeister, H. Danielczyk, A. Dietrich, B. Goletz, S. Zurlo, A. Salzberg, M. Sessa, C. Gianni, L, , Phase I study of tomuzotuximab, a glycoengineered therapeutic antibody against the epidermal growth factor receptor, in patients with advanced carcinomas, ESMO Open (2018) 3.
[56] X. Wang, J. Guo, X. Deng, Y. Huang, C. Ye, H. Liang, J. Rao, W. Yang, Comparative safety assessments of the biosimilar APZ001 and Erbitux in pre-clinical animal models, Acta Cir. Bras. 33 (2018) 690–702.
[57] Shanghaï Henlius Biotech, Henlius Novel Anti-EGFR Monoclonal Antibody Demonstrated Favorable Safety and Tolerability Profile in Its Phase 1 Clinical Study.
[58] M.M. Hou, C.L. Ho, H.Y. Lin, W. Jiang, S. Liu, Y. Hong, A. Luk, S.F. Lin, T.C. Hsieh, E. Liu, A novel anti-EGFR antibody HLX07 for potential treatment of squamous cell carcinoma of the head and neck, Ann. Oncol. 30 (2019) ix26–ix27.
[59] Shanghaï Henlius Biotech, Product-Products in R&D.
[60] K. Klinghammer, J. Fayette, A. Kawecki, A. Dietz, P. Schafhausen, G. Folprecht, S. Rottey, P. Debourdeau, J. Lavernia, A. Jacobs, I. Ahrens-Fath, B. Dietrich, H. Baumeister, A. Zurlo, S. Ochsenreither, U. Keilholz, A randomized phase II study comparing the efficacy and safety of the glyco-optimized anti-EGFR antibody tomuzotuximab against cetuximab in patients with recurrent and/or metastatic squamous cell cancer of the head and neck – the RESGEX study, ESMO Open 6 (2021), 100242.
[61] H.E. Xiao-hui, S.H.I. Yuan-kai, Q.I.N. Yan, Y. Sheng, S.U.N. Yan, Phase I study of anti-EGFR monoclonal antibody (CMAB009) in patients with advanced cancer, Natl. Med. J. China 91 (2011) 2333–2335.
[62] H.G. Grabowski, R. Guha, M. Salgado, Regulatory and cost barriers are likely to limit biosimilar development and expected savings in the near future, Health Aff. (Millwood) 33 (2014) 1048–1057.
[63] L. Urquhart, Top drugs and companies by sales in 2017, Nat. Rev. Drug Discov. 17 (2018), 232–232.
[64] E. Moorkens, C. Jonker-Exler, I. Huys, P. Declerck, S. Simoens, A.G. Vulto, Overcoming barriers to the market access of biosimilars in the european union: the case of biosimilar monoclonal antibodies, Front. Pharmacol. 7 (2016) 193.
[65] La Merie publishing. 2022 Sales of Biologics. 2023.

[66] E. Moorkens, A.G. Vulto, I. Huys, An overview of patents on therapeutic monoclonal antibodies in Europe: are they a hurdle to biosimilar market entry? mAbs (2020) 12.
[67] Pharmaceutical. *GlobalData* 2021.
[68] I.H. Cho, N. Lee, D. Song, S.Y. Jung, G. Bou-Assaf, Z. Sosic, W. Zhang, Y. Lyubarskaya, Evaluation of the structural, physicochemical, and biological characteristics of SB4, a biosimilar of etanercept, mAbs 8 (2016) 1136–1155.
[69] P. Kurki, S. Barry, I. Bourges, P. Tsantili, E. Wolff-Holz, Safety, immunogenicity and interchangeability of biosimilar monoclonal antibodies and fusion proteins: a regulatory perspective, Drugs 81 (2021) 1881–1896.
[70] B. Hassett, M. Scheinberg, G. Castañeda-Hernández, M. Li, U.R.K. Rao, E. Singh, E. Mahgoub, J. Coindreau, J. O'Brien, S.M. Vicik, B. Fitzpatrick, Variability of intended copies for etanercept (Enbrel®): Data on multiple batches of seven products, mAbs 10 (2018) 166–176.
[71] Cardianl Health. Biosimilars Report: Tracking market expansion and sustainability amidst a shifting industry. 2023.
[72] D. Ferreri, Indian biosimilar standards: a work in progress, Cent. biosimilars (2021).
[73] P. Cornes, Column: India struggles to meet international biologics standards, Cent. biosimilar (2021).