

Biosimilar, biobetter and next generation therapeutic antibodies

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On November 18, 2010, the European Medicine Agency (EMA) released a draft guideline on similar medicinal products containing monoclonal antibodies (mAbs), following a workshop organized by the agency in London on July 2, 2009.¹ The guideline discusses relevant animal model, non-clinical and clinical studies that are recommended to establish the similarity and the safety of a biosimilar compared to an originator mAb approved in the European Union (EU). The end of consultation and the deadline for comments is May 2011. Legislation establishing an abbreviated approval pathway for biosimilars was signed into law in March 2010 in the US and the Food and Drug Administration (FDA) is currently working to define the rules for its implementation.²

To contribute to the debate, the Editor-in-Chief and the Associate Editor of *mAbs* encourage the submission of manuscripts on biosimilar mAbs including views, commentary and research reports from regulatory authorities, originator, generic laboratories, academic scientists and patent attorneys of different regions. These should explain various situations and standpoints as recently discussed during the 6th European Antibody Congress³ and encourage additional dialog. To start the process, in this issue of *mAbs*, Mark McCarmish (Sandoz) details the advantages of strategies aiming to produce highly similar antibodies. Future issues will contain position papers from a US originator company, an Asia biosimilar company and others.

Biosimilars, follow-on biologics, biogenerics, biobetters, biosuperiors, second and third generation antibodies are terms used

in a sometimes confusing way.⁴ To contribute to the clarification of the debate, the aim of this editorial is to propose definitions and provide examples for these different categories of mAbs (Table 1).

Biosimilar antibodies are “generic” versions of “innovator” (or “originator”) antibodies with the same amino acid sequence, but produced from different clones and manufacturing processes. As a consequence, biosimilar mAbs may include possible differences in glycosylation and other microvariations such as charge variants that may affect quality, safety and potency.^{5,6} Biosimilars are known as follow-on biologics in the US but this term is misleading because it could also refer to second and third generation antibodies (that will be defined and illustrated below) and should be avoided. In contrast to the low-cost generic versions of small molecules that are off patent, it is currently not possible to produce exact copies of large proteins and glycoproteins, such as antibodies, owing to their structural complexity, and so the term biogeneric should also be avoided. Nevertheless, tremendous progress has been made in bioproduction and analytical sciences, and it is now possible to produce proteins and glycoproteins that are similar to reference products. The EMA has pioneered the regulatory framework for approval of these products. Since 2005, EMA has initiated regulatory pathways for biosimilar products, resulting to date in marketing authorization in Europe for 13 recombinant drugs encompassing three product classes (human growth hormone, granulocyte colony-stimulating factor and erythropoietins). Specific guidelines are also available in Europe for biosimilar insulin,

interferon and low molecular weight heparins (LMWH).

Outside Europe, the first wave of biosimilar antibodies were copies of current important therapeutic antibodies such as a biosimilar rituximab, which is approved in India (Reditux; Dr. Reddy's Laboratories) and a biosimilar abciximab, which is approved in South Korea (Clotinab; Abu Abxis). Rituximab and abciximab were originally discovered and developed by Genentech/Roche/BiogenIdec (Rituxan/Mabthera) and Centocor (Reopro), respectively.⁷ Further biosimilar candidates currently in development include copies of infliximab (Remicade; Centocor/Merck), trastuzumab (Herceptin; Genentech/Roche), cetuximab (Erbix; ImClone/Lilly & Merck-Serono), bevacizumab (Avastin; Genentech/Roche) and etanercept, an Fc-fusion protein (Enbrel; Amgen/Wyeth).

Bio-better antibodies are antibodies that target the same validated epitope as a marketed antibody, but have been engineered to have improved properties, e.g., optimized glycosylation profiles to enhance effector functions or an engineered Fc domain to increase the serum half-life.⁸ Such “Me better” antibodies with controlled and optimized glycosylation have been obtained in glyco-engineered CHO cells or yeast strains, e.g., copies of rituximab and trastuzumab amino acid sequences with afucosylated glycoforms that result in a 40- to 100-fold increase in ADCC,^{9,10} or with increased plasmatic half-life, e.g., copies of rituximab, trastuzumab, bevacizumab that have a mutation of two or three amino acids in the Fc domain resulting in extended pharmacokinetics.¹¹ In these cases, the cost of

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Table 1. Selected examples of first generation, Biosimilars, Biobetters, second and third generation monoclonal antibodies and alternatives formats

1 st generation mAbs	Biosimilars	Biobetters	2 nd generation	3 rd generation	Alternative formats
CD20					
Rituximab (1997) chIgG1 (CHO) (Rituxan/Mabthera)	Reditux (2007, Dr. Reddy) chIgG1 (CHO)	“Rituximab” GS4:0 aFuc hzIgG1 (<i>Pichia pastoris</i>) Same epitope	Ofatumumab (2009) hIgG1 (CHO) Different epitope and mechanism of action (MOA) (Arzerra)	Obinutuzumab (PhIII) aFuc hIgG1 (CHO) Different epitope and MOA	TRU-015 (PhIIb) SMIP
	TL011 (PhI, Teva/Lonza) chIgG1 (CHO)		Ocrelizumab (PhIII) hzIgG1 (CHO) Same epitope		
TNFα					
Infliximab (1998) chIgG1 (SP2/0) (Remicade)	TNFmab (Pre-clin, LGLS)		Adalimumab (2002) hulgG1 (CHO) (Humira)		Certolizumab (2008) Fab-PEG (<i>E. coli</i>) (Cimzia)
	CT-P13 « infliximab » (PhIII, Celltrion)		Golimumab (2009) hulgG1 (CHO) s.c. every 4 wk (Simponi)		
Etanercept (1998) TNFR-Fc (CHO) (Enbrel)	TNFcept (PhI, LGLS)				
HER2					
Trastuzumab (1998) chIgG1 (CHO) (Herceptin)	CT-P6 « trastuzumab » (PhIII, Celltrion)	Trastuzumab s.c. for- mulation every 4 wk (PhIII)	Pertuzumab (PhIII) hulgG1 (CHO) Different epitope (targets HER2 and HER3)		Trastuzumab emtansine (PhIII) Antibody Drug Conjugate (targets HER2 and tubulin)
		“Trastuzumab” GF15:0 aFuc hzIgG1 (<i>Pichia pastoris</i>) Same epitope			2 in 1, bispecific (targets HER2 and VEGFA)
EGFR					
Cetuximab (2004) chIgG1 (SP2/0) (Erbix)		CMAB009 (PhI) chIgG1 (CHO)	Necitumumab (PhIII) hulgG1 (CHO) Same epitope		
		Xtend EGFR, M428L/ N434S hzIgG1 (CHO) Fc-engineered (longer half life) Same epitope			
VEGF-A					
Bevacizumab (2004) hzIgG1 (CHO) (Avastin)		Xtend VEGF, M428L/ N434S hzIgG1 (CHO) Fc-engineered (longer half life) Same epitope			Ranibizumab Fab, affinity matu- rated (<i>E. coli</i>) (Lucentis)
					2 in 1, bispecific (targets HER2 and VEGFA)
RSV—Prot F					
Palivizumab (1998) hzIgG1 (NS0) (Synagis)			Motavizumab (PhIII, stopped) hzIgG1 affinity matu- rated (NS0) (Numax)	Motavizumab- YTE, long-lasting Fc-engineered version (PhI)	

Note: Adapted from reference 4 and updated from reference 22 (6th European Antibody Congress 2010).

treatment is expected to decrease because of lower cost of the products, less frequent administration regimens or lower dosages.

Cetuximab, a chimeric mouse-human IgG1 targeting epidermal growth factor receptor (EGFR), is approved for use in the EU and US as a treatment for colorectal cancer and squamous cell carcinoma of the head and neck.¹² A high prevalence of hypersensitivity reactions to cetuximab has been reported in some areas of the US. Among 76 cetuximab-treated subjects, 25 had a hypersensitivity reaction to the drug.¹³ The IgE antibodies thought to be responsible for the reaction were shown to be specific for an oligosaccharide, galactose- α -1,3-galactose, that is present on the Fab portion of the cetuximab heavy chain when the molecule is produced in the murine SP2/0 cell line used for commercial manufacturing, but not in the CHO cells used as control. The mechanism underlying a hypersensitivity reaction to cetuximab involves pre-existing IgE antibodies that target an oligosaccharide present on the recombinant molecule produced in the SP2/0 cell line. These results have implications for evaluating risks associated with antibody-based therapeutics and for understanding the relevance of IgE antibodies specific for post-translational modifications of natural and recombinant molecules. The second N-glycosylation site in the Fab portion on heavy chain Asn88 of cetuximab is of prime importance. For the marketed version of cetuximab produced in SP2/0 cells, 21 different glycoforms were identified with around 30% capped by at least one α -1,3-galactose residue, 12% capped by a N-glycolylneuraminic acid (NGNA) residue and traces of oligomannose.¹⁴ Importantly, both α -1,3-galactose and NGNA were found only in the Fab moieties, rather than the Fc fragment for which only typical IgGs G0F, G1F and G2F glycoforms were identified. In cases of cetuximab-induced anaphylaxis, pre-existing IgEs specific for this α -1,3-galactose epitope were detected in patients treated with cetuximab. Using a solid phase immunoassay, these IgEs were found to bind to SP2/0 produced cetuximab and F(ab')₂ fragment, but not to the Fc fragment. Interestingly, no IgE immunoreactivity was found against

a CHO-produced version of cetuximab (CHO-C225). A bio-better version of cetuximab produced in CHO cells is currently in development in China.¹⁵

Second-generation antibodies are follow-up antibodies with improved variable domains (such as humanized or human variable domains or affinity matured CDRs).⁴ Blockbuster antibodies, such as rituximab, infliximab, trastuzumab and cetuximab, are directed against the now highly clinically validated targets CD20, tumor necrosis factor (TNF), human epidermal growth factor receptor 2 (HER2)¹⁶ and EGFR, respectively. Second-generation antibodies directed against these same antigens have alterations, such as improved variable domains to decrease immunogenicity, target distinct epitopes with higher or lower affinity for their antigens, or have different antibody formats, e.g., conjugation of the Fab domain to polyethylene glycol (PEGylation) and Fc-fusion proteins. These antibodies have been investigated in the clinic and recently approved for use in several diseases. Examples include panitumumab (Vectibix; Amgen), which followed cetuximab; ofatumumab (Arzerra; Genmab/GlaxoSmithKline), which followed rituximab and adalimumab (Humira/Trudexa; Abbott), certolizumab pegol (Cimzia, UCB) and golimumab (Simponi; Centocor), all of which followed infliximab. These successful first generation antibodies are also being followed by antibody-drug conjugates derivatives, e.g., trastuzumab emtansine¹⁷ and bispecific versions, e.g., antibody targeting both HER2 and VEGF.¹⁸

In addition, third-generation antibodies, targeting different epitopes, triggering other mechanisms of action and that are often engineered for improved Fc-associated immune functions or half-life, have also reached Phase 1 to 3 clinical trials.¹⁹ For example, the third-generation CD20-specific antibody obinutuzumab (GA101; Glycart/Roche/BiogenIdec) is less immunogenic than rituximab, has a different mechanism of action and is glycoengineered to trigger increased cytotoxicity.^{20,21} Another example is the respiratory syncytial virus-specific palivizumab (Synagis; MedImmune/Abbott), which was followed by the second-generation

antibody motavizumab (MEDI-524; MedImmune), which has affinity matured complementarity determining regions (CDRs), and then by the third generation antibody MEDI-557 (MedImmune), a version of motavizumab with engineered Fc domains for a longer serum half-life, that is in a Phase 1 study.

The development of legal and regulatory pathways for biosimilar mAbs will continue to raise much debate among lawmakers, regulators, originator and generic industry, patent attorneys, academia and health care professionals, and *mAbs* looks forward to contributing to the discussions.

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