Case Assignment on Quality Comparability of Biosimilar

Wisit Tangkeangsirisin, PhD

The case study examples describe the comparative analysis of a biosimilar mAb candidate with its RBP. The development of a biosimilar mAb can easily require the consideration of 50 or more quality attributes. A subset of 17 illustrative quality attributes was chosen.

Table 1 summarizes these quality attributes and the methods used for their determination. They cover of attributes of glycosylation, physico-chemical modifications and different biological functions. It is well known that the glycosylation of the Fc domain in an IgG1 type mAb may have an impact on the Fc functionality and is therefore potentially relevant for mAbs targeting membrane bound targets. A prominent example in this context is the correlation between the non-fucosylated G0 glycan and the antibody dependent cytotoxicity (ADCC) in some mAbs. An example chromatogram of the glycan mapping used for the quantitative determination of the N-glycans is shown in Fig. 1 and the peak labels are listed in Fig. 2 accordingly.

N-glycolylneuraminic acid (NGNA) was selected as an attribute that is considered as a risk factor for immunogenicity, although low levels in currently marketed products have been acceptable and not associated with untoward clinical outcome. The predominant sialic acid made by human cells is N-acetylneuraminic acid (NANA) whereas mammalian cell lines used for manufacturing synthesize NANA and also some amount of NGNA which is the reason for the discussion of its potential contribution to immunogenicity in humans. In the case study, NGNA content is stated as the relative percentage of NGNA as part of the total sialic acid content, which consists of the sum of NANA, NGNA and other sialic acids. The high mannose glycan Man5 may have an impact on clearance.

Deamidation, oxidation, formation of dimers and higher aggregates are common degradation pathways which are also assumed for the case study as stability relevant quality attributes under real time storage conditions. The level of deamidation and oxidation is stated as the amount of modified amino acid most susceptible to deamidation and oxidation and which therefore serves as a sensitive marker for these types of degradation events.

Dimers and aggregates are considered to have a potential to increase the risk of immunogenicity and are typically rated as critical quality attributes which need to be tightly controlled.

The biological function of a mAb is primarily linked with its ability to bind the target with the complementary determining region (CDR). It is important to understand that the biological binding assays described below are very sensitive in comparing biologics (both RBP and SBP candidates as well as pre- and postmanufacturing change products). Clinical trials are much less sensitive to resolve differences seen in binding assays. Documented differences in those assays are therefore better resolved during manufacturing process development of the product. The Fc region may also play an important role in mediating complement dependent cytotoxicity (CDC) and both CDC and ADCC and can be part of the mode of actions for mAbs targeting cell membrane bound targets.

To simplify the case study, other quality attributes not listed above are assumed to be indistinguishable between the SBP candidate and the RBP. For example, the primary

structure is identical and higher order structure is considered to be the same as measured by circular dichroism (CD) and nuclear magnetic resonance (NMR). The residual amounts of process related impurities such as host cell proteins, DNA, or toxic process reagents were confirmed to be compliant to available guidelines and represent low levels typical for today's state-of-the-art in manufacturing.

For this exercise we assumed that the quality attribute ranges were generated from 10 RBP and 10 SBP candidate batches which were analyzed at various time points within their respective shelf life.

Case 2

Following data are quality attribute ranges of a monoclonal antibody called **SBP candidate 2** and **RBP A**, targeting a cell membrane bound target and where Fc functionality is an important part of the clinical mode of action.

RBP A is a monoclonal IgG1 type antibody used for treatment in oncology and autoimmune disorders. The mAb is designed to bind a cell surface receptor and subsequently triggers Fc mediated cell killing via CDC, ADCC and apoptosis. The treatment is highly effective, however, in some indications and patient populations, up to 20% of the patients develop neutralizing antibody against RBP A, resulting in a loss of efficacy for those patients. The etiology or structure function relationships for this immunogenicity are not known.

Points to consider:

- **Determine which quality attributes are critical.**
- **Rate each quality attribute as positive, neutral or negative when compared to RBP.**
- **Discuss whether the candidate could be qualified as SBPs at the quality level.**
- **How remaining questions could be addressed by further studies or by the nonclinical and clinical parts of the development program?**

Figure 1 Glycan mapping chromatogram of an IgG1 type mAb using hydrophobic interaction liquid chromatography.

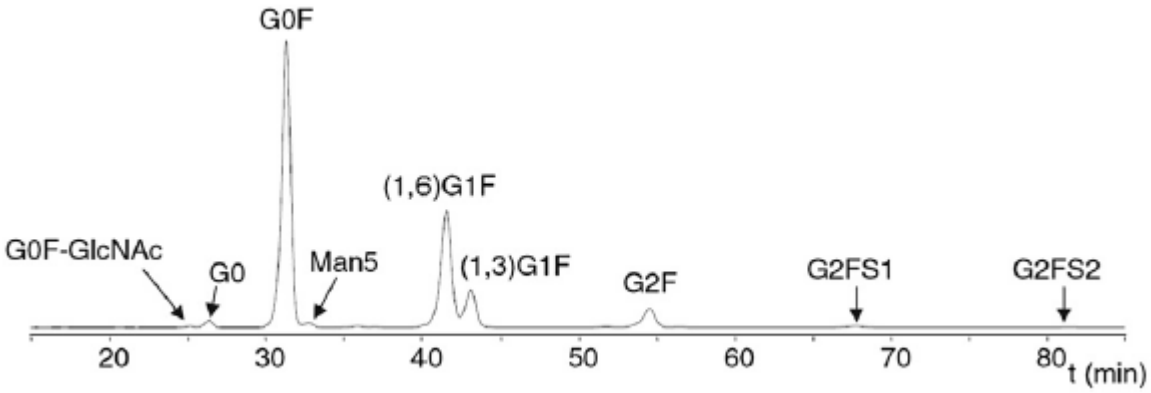

Figure 2 Glycan labels

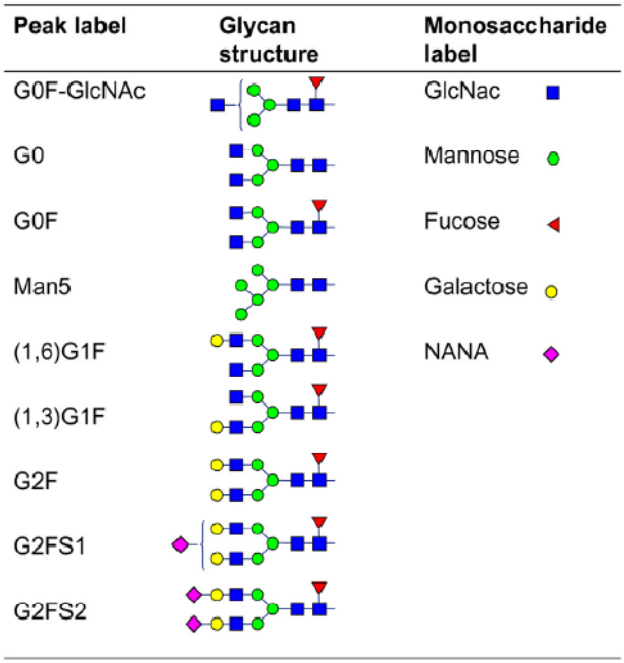

Table 1 Quality attribute ranges of SBP candidate 2 and RBP A, targeting a cell membrane bound target and where Fc functionality is an important part of the clinical mode of action. The lengths of the bars show the relative widths of the quality attribute ranges. For attributes,

showing a black line on the left, this black line represents the point of origin (i.e. 0%).

Quality attribute	Product	Min/max ranges	Bar diagram
G0F-GlcNac	RBP A SBP candidate 2	0.05 – 0.3 % 0.00 – 0.2 %	
G0	RBP A SBP candidate 2	0.4 – 2.1 % 9.2 – 12.0 %	
G0F	RBP A SBP candidate 2	20.3 – 32.1 % 25.3 – 33.0 %	
Man5	RBP A SBP candidate 2	0.0 – 0.9 % 0.2 – 0.5 %	
(1,6)G1F	RBP A SBP candidate 2	2.5 – 4.1 % 3.0 – 4.2 %	
(1,3)G1F	RBP A SBP candidate 2	1.6 – 2.3 % 1.5 – 2.0 %	
G2F	RBP A SBP candidate 2	10.5 – 25.7 % 11.0 – 24.4 %	
G2FS1	RBP A SBP candidate 2	35.3 – 39.1 % 36.2 – 38.5 %	
G2FS2	RBP A SBP candidate 2	11.4 – 13.2 % 11.4 – 13.0 %	
NGNA	RBP A SBP candidate 2	3.3 – 5.5 % 3.4 – 5.8 %	
Deamidation	RBP A SBP candidate 2	0.9 – 2.4 % 0.3 – 1.5 %	
Oxidation	RBP A SBP candidate 2	1.2 – 4.3 % 1.0 – 4.1 %	
Dimer	RBP A SBP candidate 2	0.0 – 2.1 % 0.0 – 0.8 %	
Higher aggregates	RBP A SBP candidate 2	0.0 – 0.8 % 0.0 – 0.2%	
Binding assay	RBP A SBP candidate 2	91 – 108 % 93 – 105 %	
CDA activity	RBP A SBP candidate 2	84 – 110 % 90 – 111 %	
ADCC activity	RBP A SBP candidate 2	82 – 118 % 380 – 475 %	

Table 2 Overview of the quality attributes and analytical methods which were used for the case study exercise

Quality attribute	Analytical methodology
N-Glycans as shown in Figs. 4 and 5	Glycan mapping of enzymatically released and fluorescence labeled glycans using hydrophilic interaction liquid chromatography (HILIC)
N-glycolylneuraminic acid (NGNA)	Weak anion exchange chromatography of enzymatically released and fluorescence labeled sialic acids
Deamidation oxidation	Peptide mapping and quantification of lead peptides containing the amino acids known to be most susceptible for deamidation and oxidation
Dimers and aggregates	Size exclusion chromatography
Binding	Functional cell based assay which measures the binding to the antibody target
Complement dependent cytotoxicity (CDC)	Functional CDC assay
Antibody dependent cell-mediated cytotoxicity (ADCC)	Functional ADCC assay