Comparative Analytical Assessment of Biosimilars Based on Fingerprint-Like Analysis Using *Intelli.b*™ Technology

Summary

The development of biosimilars have significantly accelerated in the past several years due to the expiry of patent protection for several biotherapeutic mAbs, introducing in the market more affordable alternatives that can alleviate costs in the healthcare system and increase patient access while providing safe and effective medicine. A biosimilar is defined as a biopharmaceutical drug designed to elicit clinical performance that is similar to that of an already existing original reference product. Due to the complex nature of biotherapeutic mAbs, the FDA recommends to "quantify the similarity or differences between the two products using a meaningful fingerprint-like analysis algorithm that covers a large number of additional product attributes."[1] Therefore, there is a high demand for innovative analytical technologies for assessing comparative studies based on fingerprint-like analysis of biologics to support biosimilar process development and approval.

Background

Challenge 1: How to generate a fingerprint-like profile for comparative analytical assessment of therapeutic proteins?

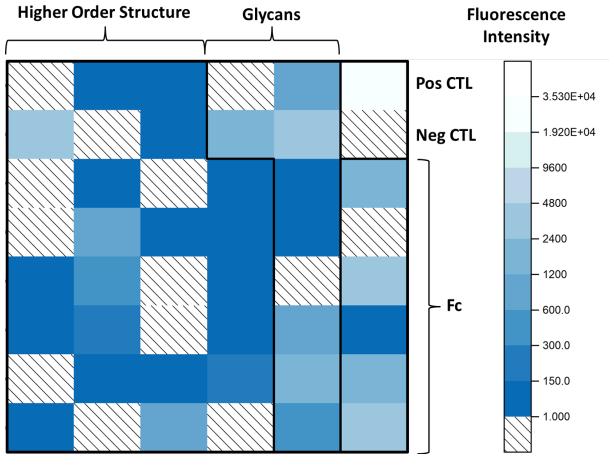

Fig. 1 Fingerprint-like profile for Trastuzumab (Herceptin) showing protein higher order structure, glycans, and Fc profiles generated using Intelli.b™ technology.

The Intelli.b™ technology uses an antibody microarray to generate higher order structure, glycan and Fc profiles that together provides a meaningful fingerprint-like profile for a specific biologic that can be used to demonstrate similarity in protein structure, post-translational modifications, and in-vitro

functional bioactivity (**Fig. 1**). The microarray is composed of several glycan binding proteins such as lectins that can selectively recognize different glycan epitopes, Fc receptors (FcγRI, FcγRIIA/B, FcγRIIIA, FcRn, and C1q), and antibodies targeting different epitopes on different regions of the biologics to provide information on protein higher order structure (HOS) and functionality. In addition, the target molecule is also present on the microarray platform to evaluate the therapeutic's mechanism of action. A key aspect of the technology is the use of antibodies generated on chicken models to achieve maximum surface coverage, including towards conserved epitopes, allowing comparison of biologics with significantly higher resolution. The Intelli.b™ platform can help sponsors establish confidence that a biosimilar product will elicit similar human clinical results as the originator, reducing manufacturing costs and significantly expediting regulatory approval processes.

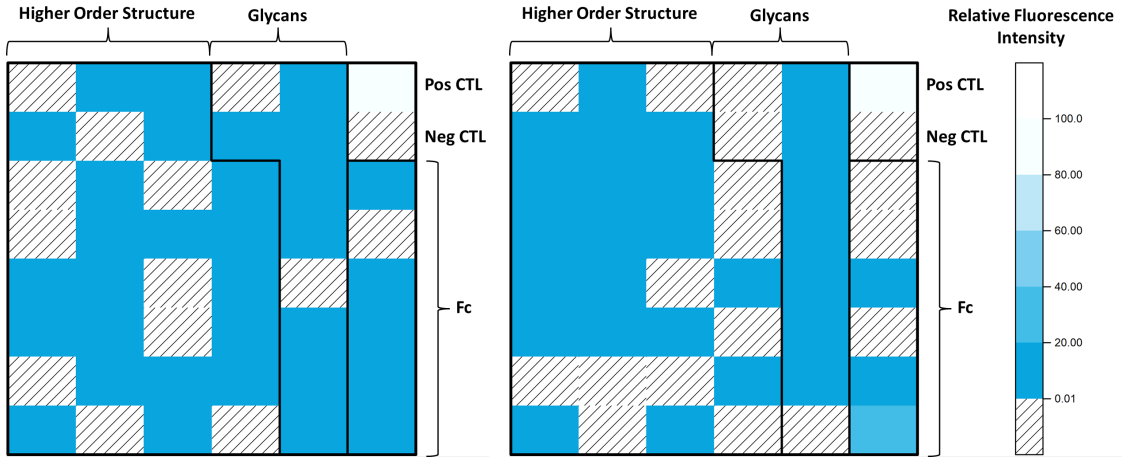

Fig. 2 Fingerprint-like profile for (left) Trastuzumab (Herceptin) and (right) Bevacizumab (Avastin) obtained using Intelli.b™ technology.

Results 1

Using Intelli.b™ technology, a comparative analytical assessment between two different biotherapeutic mAbs possessing a similar framework could be successfully performed based on fingerprint-like analysis as shown in **Fig. 2**. For this case study, trastuzumab and bevacizumab were chosen as both contain a humanized IgG1 framework.[2] When comparing their higher order structure, roughly 37% divergence was observed based on the antibody-binding profile. Analyzing their glycoprofiles, both trastuzumab and bevacizumab showed similar lectin binding patterns in which binding signals were detected at lectins with binding selectivity to core fucose (*Pisum sativum* and *Lens culinaris*), mannose (*Narcissus pseudonarcissus* and *Canavalia ensiformis*), and GlcNAc oligomer (*potato, Solanum tuberosum* and UDA *Urtica dioica*). No lectin binding was observed for terminal galactose (*Ricinus communis* and *Phaseolus vulgaris*) for Bevacizumab. The overall glycan profiles derived from Intelli.b™ technology are consistent with the reported glycans in IgG1 Fc that are known to have a biantennary, core-fucosylated structure carrying two, one, or no galactose residues.[3] Analyzing their Fc receptor profiles, trastuzumab showed binding on all Fc receptors used with the exception of FcRn and a significant high affinity for FcγRIIIA, which are in agreement with reported Fc and C1q binding activities.[4] Compared to trastuzumab, bevacizumab showed significant high affinity for FcγRI and no binding for FcRn, FcγRIIB, and C1q. Galactosylation of glycans are known to potentially affect binding to C1q and the lack of terminal galactose observed on the glycoprofile for bevacizumab can be attributed to the lack of C1q binding

activity.[5] In this case study, a comparative assessment of two different biotherapeutic mAbs could be performed using Intelli.b™ technology, which demonstrates the similarities and differences in terms of HOS, glycosylation, and Fc activity profiles.

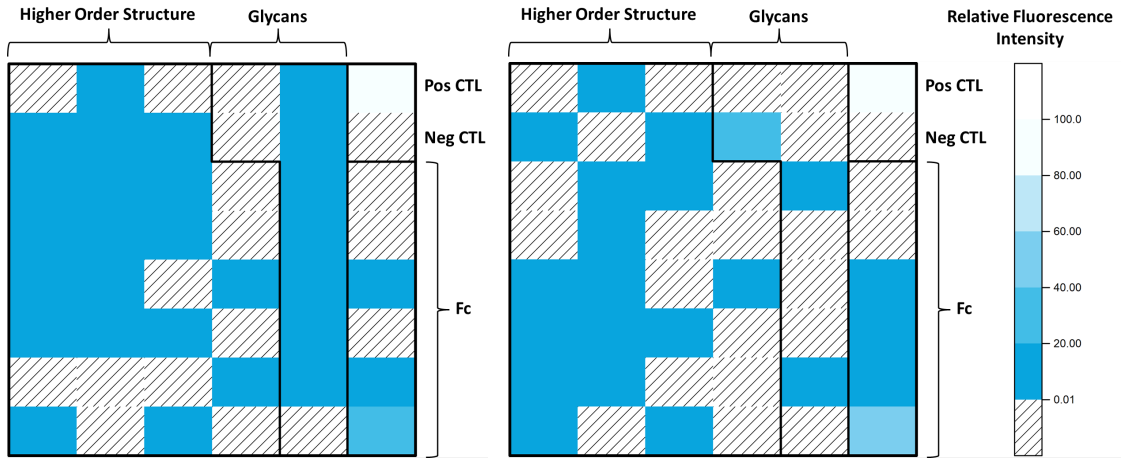

Fig. 2 Comparative analytical assessment of (left) originator bevacizumab (Avastin) and biosimilar Bevatas (right) based on fingerprint-like analysis.

Challenge 2: Can we demonstrate biosimilarity between the originator and biosimilar?

Results 2

Using Intelli.b™ technology, a comparative analytical assessment based on fingerprint-like analysis of an originator and its biosimilar was obtained as shown in **Fig. 2**. The biologics showed 77% of similarity in protein HOS. In addition, analysis of their glycan profiles also revealed a lack of terminal galactose. Although their glycan profiles differed significantly, the results indicate the presence of core fructose, mannose, and GlcNAc oligomers for both biologics. Furthermore, high similarity was observed on their Fc bioactivity profile. Intelli.b™ technology can provide a collective analytical characterization that is sufficient to demonstrate that, while analytical differences do exist, sponsors can determine the degree of similarity and further study the significance and impact of such divergences.

Conclusion: Intelli.b™ technology enables the assessment of analytical comparison of biologics based on fingerprint-like analysis that includes protein higher order structure, glycosylation, and Fc bioactivity profiles in a fast and cost-effective manner.

References

1. Scientific Considerations in Demonstrating Biosimilarity to a Reference Product, *FDA* **2015**
2. **Lu *et al.*** *Journal of Biomedical Science* **2020**, 27:1
3. **Carolin *et al*.** *Proteomics 2009, 882-913*
4. **Jae *et al.*** *BioDrugs* **2019**, 411-422
5. **Neungseon *et al.*** *MABS* **2018**, 678-691