Assessing the Variability of an Innovator Molecule N-Glycan Profile

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Abstract

In the development of biologic therapeutic antibodies, matching the innovator molecule N-glycans profile may be critical to proving its bioequivalence. Often the complex and variable compositions of N-glycans, coupled with rapid analytical methods, result in high variability for the N-glycan profile. Characterization of N-glycans is critical to defining a high-performance biosimilar, in particular, to characterize the N-glycan profile variability, and to target development of a biosimilar. This study assessed the N-glycan profile variability for the innovator molecule Rituximab (MabThera® [Roche] and Rituxan® [Genentech]). The results served as a benchmark for the development of a biosimilar to Rituximab.

Introduction

Rituximab is a chimeric monoclonal antibody directed against the surface CD20 antigen expressed on B lymphocytes. It is approved by the FDA for the treatment of several hematological and autoimmune diseases.

Methods

Sample Preparation: Replicates of each lot were prepared using the GlykoPrep 5.7 kit. N-glycans were eluted from the column using 0.5 M sodium acetate (pH 5.8) and 2% acetic acid. The eluted glycans were dialyzed against water, then lyophilized.

Analytical Characterization: GlykoPrep glycoform analysis was performed in Waters ECLIPSE XDB C18 columns (3.5 μm, 2.1 × 150 mm) equipped with an Agilent LC-MSD. Detection limits were 6 ng. GlykoPrep glycoform analysis was confirmed using Waters Alliance 2695 HPLC with a Waters Purosphere C18 column (4.6 × 250 mm).

Exo-enzyme Treatments for Confirmation of N-Glycan Peak ID:

Exo-enzyme digestions of N-glycans were performed using a series of enzymes: 

- Exo-1,2mannosidase (G1F) - Man5 + G1 - Man9 + G0F - Man10 + G0F
- Exo-6mannosidase (G0F) - Man10
- Exo-β-N-acetylhexosaminidase (G0F-N) - Man9 + G0F - Man10 + G0F
- Exo-α-mannosidase (A2F) - Man10
- β-Galactosidase (G2F) - Man5 + G1 - Man9 + G0F - Man10 + G0F

Comparison of N-Glycan Profiles Among Lots:

A total of 17 replicates for each lot of Rituximab were prepared using the GlykoPrep 5.7 kit. Each replicate contained 1 μl of InstantAB-labeled N-glycans (aqueous sample as eluted from the column). The glycans were digested using a series of exo-enzymes, then analyzed using GlykoPrep glycoform analysis. GlykoPrep coupled with rapid analytical methods, was chosen because of its ability to provide precise and accurate results for a small number of samples in a short timeframe.

Results and Discussion

Exo-enzyme digestions were performed to confirm the N-glycan peak identification. Table 1 lists the number of replicates for each lot of Rituximab, each day they were prepared. GlykoPrep coupled with rapid analytical methods, was chosen because of its ability to provide precise and accurate results for a small number of samples in a short timeframe.

The results for each lot for each day showed a large degree of variability, with the most abundant species in these two innovator molecules, even with a limited spread of less than 1% relative abundance. The results for each lot for each day showed a large degree of variability, with the most abundant species in these two innovator molecules, even with a limited spread of less than 1% relative abundance.

Conclusion:

1. Use of an N-glycan profile, as produced by GlykoPrep and the rapid analytical methods, may serve as a high-performance benchmark for the development of a biosimilar.

2. Exo-enzyme digestions were performed to confirm the N-glycan peak identification. Table 1 lists the number of replicates for each lot of Rituximab, each day they were prepared. GlykoPrep coupled with rapid analytical methods, was chosen because of its ability to provide precise and accurate results for a small number of samples in a short timeframe.

Future work:

Improvements to the analytical methods are under development, including better reproducibility of high-performance liquid chromatography and electrospray ionization mass spectrometry (HPLC-ESI-MS), limits of detection and quantitation. In addition, automated liquid handling is under development to increase the precision and the throughput of the multiple sample sets.

Table 1 - Table of Replicates for Rituximab

Table 2 - Table of Replicates for Bevacizumab

Table 3 - Table of Replicates for Avastin

Table 4 - Table of Replicates for Bevacizumab

Table 5 - Table of Replicates for Avastin

Table 6 - Table of Replicates for Avastin

Table 7 - Table of Replicates for Avastin

Table 8 - Table of Replicates for Bevacizumab

Table 9 - Table of Replicates for Bevacizumab

Table 10 - Table of Replicates for Bevacizumab

Table 11 - Table of Replicates for Bevacizumab

Table 12 - Table of Replicates for Bevacizumab

References: