Comprehensive Characterization of Remicade and Its Biosimilar—Remsima Using Mass Spectrometry

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**Purpose**

The impending “patent cliff” for billion dollar monoclonal antibody drugs (mAbs), the enactment of the Biologics Price Competition and Innovation Act, and the newly issued US FDA guidance on biosimilar products has opened the door for numerous biosimilar applications. Remsima, a copy of Remicade, was the first biosimilar mAb approved in Europe, and is also approved in South Korea and Canada and currently under review by the FDA. In the EU, Remsima is approved for all indications based on clinical data in ankylosing spondylitis and rheumatoid arthritis. In Canada, the indications for ulcerative colitis and Crohn's disease were not approved by extrapolation due to the differences in Fcγ-IIIa receptor binding and antibody directed cell-induced cytotoxicity (ADCC) assays (Feagan B. et al. Biologicals, 42, 2014).

Analytical characterization of mAbs is challenging due to their large size (~150 kDa) and complex nature owing to intrinsic and process driven heterogeneities. Mass spectrometry (MS) has been used extensively to characterize mAbs and to provide highly detailed information of individual amino acids variants/modifications as well as some structural information. The aim of this project is to perform a MS comprehensive comparison between infliximab innovator, Remicade, and its biosimilar, Remsima and to relate product differences to the biological activity.

**Methods**

Multiple lots of Remicade were purchased from the University of Michigan Pharmacy and lots of Remsima were acquired from Celltrion, Korea. The samples were then digested with a combination of Lys-C and trypsin (Promega) at mildly acidic pH, to minimize proteolysis induced deamidation. The samples were then processed by LC-MS/MS and analyzed using Byologic software (Protein Metrics). Alternatively, the samples were digested by IdeS enzyme (Genovis-FabRicator) and analyzed by reverse phase UPLC (RP-UPLC) and ion mobility mass spectrometry (IM-MS).

**Results**

Peptide sequencing by MS showed that both products were identical. Sequence variants were < 0.2% for both. Remsima displayed more C-terminal lysine (8%) relative to Remicade (6%). Levels of oxidation and deamidation were comparable for the multi-lot analysis of the two products. Of note were heavy chain HC-Met-34 and HC-Met-255 which showed ~15% oxidation and HC-Asn-31 which showed ~4% deamidation. Key differences were observed in the glycation levels, with Remsima showing considerably higher levels of glycation (Figure 1A). The mAbs samples were digested with IdeS, which cleaves the mAb into Fc and F(ab)'2, and analyzed by RP-UPLC and IM-MS. RP-UPLC showed Remsima was more hydrophilic (Figure 1B) and IM-MS showed Remsima fragments had higher average mass confirming its glycation. Finally the levels of N-glycoforms at Asn-300 also varied between the two formulations: Remicade has higher levels of G0F while Remsima has higher levels of G1F (Figure 2).

**Conclusion**

In this study, mass spectrometry analysis was successfully applied to comprehensively characterize between innovator product Remicade and its biosimilar, Remsima. Both products show similar levels of sequence variants, oxidation, and deamidation. However they had different glycosylation and glycation patterns, which could potentially affect Fcγ-IIIa receptor binding, and ADCC and result in reduced efficacy in treatment of ulcerative colitis and Crohn's disease.