Characterization and comparison of commercially available TNF receptor 2-Fc fusion protein products

Letter to the Editor

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Dear Dr Reichert,

The emerging area of biosimilars to large complex protein molecules such as antibodies brings with it many challenges, both for biotechnology manufacturers as well as regulatory authorities. We read with interest your recent article by Tan et al.1 that, for us, highlighted these challenges previously referred to as a knowledge gap between the innovator and the biosimilar developer.2 It is of considerable importance for patients that biosimilars become available on the market, however it is equally imperative that these biosimilars meet the same standards of safety and efficacy as the innovator products. With this in mind, we have a number of comments on the article by Tan et al.

The authors performed an analysis of two purported etanercept biosimilars which they obtained from the Chinese market. The analysis included primary sequence, peptide mapping, intact mass, charge variants, glycosylation, bioactivity, and affinity. The comparisons were made against Enbrel® as the innovator molecule. Analyses were performed on four lots of “biosimilar 1,” one lot of “biosimilar 2,” and one lot of Enbrel®.

Etanercept drug substance (DS) has been commercially manufactured since 1998, and marketed as the drug product (DP) Enbrel®. The manufacturing process for Enbrel® has been optimised over time and extensive biochemical characterization analyses have been performed to demonstrate comparability and ensure consistency. This long manufacturing history has allowed Pfizer to develop a comprehensive, detailed, and extensive set of product quality data to ensure a consistent safety and efficacy profile for Enbrel®.

While the characterization profile for the etanercept molecule has been demonstrated to be comparable and consistent throughout its lifetime, a feature of etanercept manufactured from all processes is the heterogeneity observed in the relative proportions of a subset of common peptide and carbohydrate isoforms. This heterogeneity is common to the manufacture of large biomolecules, and thereby complicates their analysis and characterization.

The extensive manufacturing data available to Pfizer for etanercept includes specific details for cell line development and genetic construct, raw materials, cell culture conditions, purification parameters as well as formulation and drug delivery. This information is proprietary and therefore not available to the manufacturers of any potential biosimilar product, which presents as a knowledge gap. Due to the complexity of biological systems, and the nature of biotechnological manufacturing, any attempt to copy an originator molecule cannot result in an identical product.

Production of biosimilars is further complicated, as subtle changes to production conditions, such as the extent of structural conformation, glycosylation pattern or degree of aggregation, can have a profound effect on the properties of a large protein molecule. These parameters can in turn influence the therapeutic effect and safety profile of the biologic. For these reasons, the need for clinical trials in order to demonstrate the safety, immunogenicity, and efficacy profiles of biosimilars for each indication has been identified.2-4

It is vital that any etanercept biosimilar developer performs comprehensive in vitro biochemical characterization studies as well as safety, immunogenicity, and efficacy trials that demonstrate comparable clinical performance to Enbrel®. These data are paramount to ensuring patient safety and enable an assessment of risk benefit of the biosimilar product. What is most notable from a number of recent publications, are the specific omissions in the scope of the analyses performed for key product quality attributes.

Pfizer consider a number of parameters to be of critical importance for demonstration of biochemical comparability for large, complex biomolecules, such as etanercept. Detailed structural biochemical and in vitro characterization studies must be completed which may potentially impact the assessment of potency, clearance, efficacy, immunogenicity or safety profile of such biologics. The Tan et al. paper contains notable omissions from this summary of analyses that should typically be included in a characterization program:

• Determination that the same structural isoforms are present with no change noted in the primary, secondary or tertiary structures of the molecule. Primary analysis should typically provide full sequence coverage, but in the Tan et al. paper, only ~50% sequence coverage was described and sequence variants were observed.

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Orthogonal tools should also be applied to assess secondary and tertiary structure.

- Identification and relative amounts of aggregate species assessed using orthogonal techniques.
- Comprehensive biochemical studies to characterize the activity and relative potency of the protein biologic, in combination with appropriate in vitro studies using a number of key cellular receptors.
- Characterization of all post translational modifications which can modulate protein clearance and interactions with key cellular receptors. In particular, in depth characterization for all glycan species to confirm identity, structure, relative abundance and sites of location for all species present.
- A successful characterization program will contain data from multiple lots of material derived from the current process and the new process. In this instance, a single lot of innovator material was used for comparison.

Because biosimilars are never exact copies of the innovator medicine, establishing appropriate standards for biosimilarity remains an important area for scientific, legislative and regulatory debate. Regulatory agencies across the globe rightly hold innovator biologic medicines to high standards to ensure patient safety and clinical efficacy. Pfizer believes that the data requirements for biosimilar approval should be sufficiently rigorous to ensure patients receive quality products that have efficacy and safety profiles that are highly similar to those of the innovator reference product. Applying this standard, the comparability assessment performed by Tan et al. does not comprise the necessary characterization data to make an informed evaluation of biochemical comparability.

Disclosure of Potential Conflicts of Interest
All authors are employees of Pfizer.

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