Overcoming the Challenge of Rheumatoid Factor Interference in Immunogenicity Assays for Human Monoclonal Antibody Therapeutics

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Introduction

Bridging anti-therapeutic antibody (ATA) assays are vulnerable to matrix factors that can bridge the conjugated therapeutic and generate false positive signals. Rheumatoid factor (RF) and autoimmune antibodies associated with rheumatoid arthritis (RA) pose this problem. The object of this study was to develop a sensitive, drug tolerant and robust assay to detect ATA against therapeutic protein X (TPX) in RA patients. The TPX is a humanized monoclonal antibody for the treatment of RA.

Method

A bridging electrochemiluminescence (ECL) ATA assay was validated in human plasma using a three tier approach screen, confirm and titer.

- Acidified samples are added to a mixture which contains Tris base, biotin-labeled TPX and Sulfo-TAG-labeled TPX.
- This mixture was then allowed to complex with ADA immobilize RF factors. In place of aggregates, whole molecule human IgG (and human IgG Fc) were used
- The plate is washed to remove any non-specific bound complexes, and read buffer is added. The plate is read on the MSD Sector™ Imager 6000.

Results

- Figure 1. Design of immunogenicity assay.
- Figure 2. Proposed mechanism of RF interference.
- Figure 3. Comparison of signal responses of plasma samples from normal subjects and RA individuals.
- Figure 4. Correlation between RF value and ADA assay response.
- Figure 5. Elimination of RF interference by anti-human IgM. Anti-human IgM antibody was tested at 5, 50, 100 and 250 µg/mL. This reagent reduced false positive signal in some samples. However, most samples showed an increased response (Example 3 and 20). Additionally, sample 23 increased its signal with higher concentrations of anti-IgM.
- Figure 6. Comparison of anti-human IgM and anti-human IgM (Fab). Anti-human IgM Fab reduced signal in three of the RA samples tested. However most samples showed either no change or an increase in signal with addition of Fab. Sample 9 saw the most improvement with Fab addition. Overall, whole antibody to IgM performed better than Anti-IgM Fab.
- Figure 7. Elimination of RF interference by human IgG and RFRR. Rafter et al. (2011) reported RFRR per manufacturer, should remove all IgGs up to 15 mg/mL and IgM specific to RF. Addition of this reagent eliminated all signal response from PCs, however 4 out of 13 RA samples still produced high signals. Human IgG was tested at 100 and 250 µg/mL and lowered false signals. Higher concentrations could not be tested due to the low starting concentration of human Fc material.
- Figure 8. Elimination of RF interference by human IgG. IgG aggregates are cited in the literature to interfere with RF factors. In place of aggregates, whole molecule human IgG (and human IgG Fc) were tested. Commercially available whole molecule IgG was tested over a range from 50 to 2000 µg/mL. Whole molecule IgG significantly dropped the signal for RA individuals.
- Figure 9. Elimination of RF interference by human IgG and anti-human IgM. The combination of both whole molecule IgG and anti-human IgM (whole antibody) demonstrated the best combination to reduce false positive signal. The addition of anti-IgG antibody could remove response from anti-light antibodies to the TPX, therefore the IgG and anti-light combination was not selected for the assay.
- Figure 10. Assay sensitivity in normal plasma samples with and without human IgG addition to Mastermix.

Conclusions

- The ability to overcome interference of RF is vitally important for accurate determination of true ATA response.
- Commercially available whole molecule IgG demonstrated significant reduction of false positive bridges and allowed for a simple and easily accessible reagent to overcome this challenge.

References


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