Challenges associated with the immunogenicity assessment of pegylated products

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ABSTRACT

PEGylation is the process of covalent attachment of polyethylene glycol (PEG) polymer chains to another molecule, such as a therapeutic protein. The covalent attachment of PEG can reduce immunogenicity and antigenicity and can also prolong drug circulation time by reducing renal clearance. The presence of pre-existing PEG antibodies is known in some patients following exposure to a variety of products. These antibodies can potentially impact the PK profile of drugs and increase the risk of infusion reactions. Even if anti-PEG antibodies remain a concern, their presence is not necessarily associated with adverse events.

The diversity in size and physical properties of the PEG molecules makes it difficult to draw conclusions regarding the impact of PEGylation. When using anti-PEG assays, the observed incidence of antibodies may be influenced by several factors such as assay sensitivity. Therefore, in some instances, anti-PEG Abs may be present but not detected.

In this study, a direct ELISA format, in which the anti-PEG antibodies are captured with plate-bound PEG, was developed. The detection reagents were chosen based on isotype specific and required several rounds of optimization. Their performance was evaluated based on several parameters including sensitivity, dynamic range, and signal produced from IgG and IgM positive controls. The assay MRD was selected using a comparative approach allowing the generation of a provisional cut point. The evaluation of several additional parameters such as selectivity and drug tolerance were also evaluated. The direct ELISA format developed to detect anti-PEG antibodies proved to be reproducible, sensitive and drug tolerant.

ASSAY DESIGN

Due to the repetitive nature of the PEG, anti-PEG IgG antibodies bind to the repeating epitopes of a single chain of PEG resulting in the absence of bridging. However, the bridging may be possible for the IgM antibodies but the assay will be limited to the detection of anti-PEG IgM antibodies.

The assay preferred format selected was the Direct ELISA detected using species specific anti-isotype HRP antibodies or protein A/G HRP.

CASE STUDY RESULTS

An immunogenicity method was developed to detect antibodies to PEG induced by several pegylated compounds containing different PEG molecular weights (550 Da and 2000 Da)

- Peg molecular weights were used in assay development to mimic the PEG linked to the different drug compounds.

- The goal was to develop a single method that could be used with various pegylated compounds.

Selection of streptavidin assay plate

Optimization of coating conditions

Optimization of detection conditions

Sensitivity Assessment

Binding of PC to PEGs of different length

ELISA using secondary Ab detection

ELISA using Protein A/G detection

Drug tolerance

Additional parameters tested prior to assay validation

Pre-validation parameters

Table 1: The drug tolerance for each compound was as follows:

Drug A: 125 ng/mL tolerated at PC 250 ng/mL; no tolerance at PC 50 ng/mL

Drug B: 250 ng/mL tolerated at PC 50 ng/mL; 25 ng/mL tolerated at PC 50 ng/mL

Drug C: 125 ng/mL tolerated at PC 250 ng/mL; 75 ng/mL tolerated at PC 50 ng/mL

Concluding remarks:

- Screening and confirmation should be done using the whole PEGylated drug (CCP to be determined for each particular drug)

- Further characterization may be done by competition with the whole PEG molecule or the construct minus PEG

- Anti-PEG IgM may not be detected when smaller MW PEG is used for coating.

- CCP and drug tolerance need to be evaluated with each individual compound

- Anti-PEG antibody data are highly dependent on the selected assay format and the selection of critical reagents

- Standardization of the anti-PEG assays and the development of positive control antibodies are needed to have a good understanding of the real anti-Peg antibody incidence in the population.