New Dogs/Old Tricks: Methods for Assessing the Immunogenicity of Peptide Drugs and Their Impurities

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Immunogenicity is a term that describes immune responses to protein or peptide biologics. Due to several well-documented adverse events that were attributed to immunogenicity (PRCA, Erythropoietin, for example), immunogenicity risk assessment using in silico and in vitro methods recently achieved "standard practice" status in the biologics preclinical development pipeline. An industry white paper on T cell-dependent immunogenicity was developed by a consortium of researchers and leading biologics manufacturers and published in 2013. The approaches described in the white paper are now widely adopted by biologics developers.

A new draft guidance for generic peptide drugs was recently published, highlighting FDA concern about the potential for peptide drugs and their impurities to drive unwanted immunogenicity and adverse effects (https://www.fda.gov/ downloads/drugs/guidances/ucm338856.pdf]. More specifically, peptide drug-substance (DS) and impurities that are derived from solid phase synthesis may contain T cell epitopes that can presented on the surface of antigen presenting cells by class II HLA molecules, priming T cells and driving unwanted immune responses. Recent updates to this approach now permit the following three-step approach to immunogenicity risk assessment for peptide drugs and their impurities.

Step 1. Immunoinformatics assessment: The potential of the DS to stimulate a T cell response can be rapidly assessed computationally using T cell epitope mapping algorithms. We use EpiMatrix for this purpose and focus on HLA DR (Class II) HLA binding predictions. In a typical DS analysis, the EpiMatrix algorithm is used to screen the primary amino acid sequence of the DS and its impurities, for the presence of HLA DR ligands, which can be considered putative T cell epitopes. Discriminating between potential inflammatory "T effector" epitopes and regulatory "T reg" epitopes is performed with a second algorithm, known as JanusMatrix. The latter algorithm identifies putative Treg epitopes, defined as HLA/epitope complexes that present a human-like outer contour (TCR face). HLA/epitope complexes that do not present an outer contour (TCR face) that is 'human-like' are more likely to drive effector T cell response. Following assessment of T cell epitope phenotype, the next step is to combine the scores for effector and regulatory T cell epitope content, providing an overall assessment of immunogenic potential of the DS and impurities. The resulting Treg adjusted EpiMatrix scores are highly correlated with immune responses in vivo.

Step 2: In vitro - HLA binding: The DS, its impurities, and peptides representing predicted T cell epitopes can be evaluated for binding to human HLA in assays that measure binding affinity in dose-ranging studies, in vitro. HLA binding is used to confirm in the in silico analysis and inform the design of in

vitro assays (see step 3).

Step 3: In vitro assay – Teff Assay: Measurement of de novo T cell response: Cell culture protocols have been developed to emulate in vivo conditions that support differentiation of naïve T cells to effector T cells by antigen stimulation with biologics or their constituent T cell epitopes. In vitro stimulations using the biologic drug and human peripheral blood cells (PBMC) allow for natural antigen processing of the DS and other product components including impurities. In vitro assays using predicted epitopes derived from the DS product impurities provide information about the ability of these defined sequences to drive a T cell response using human T cells. At the end of the culture period, T cell phenotype and/or function are characterized in assays that measure the magnitude and quality of effector T cells that have potential to drive ADA development. Treg Assay: Co-incubation of the putative Treg epitopes with known T effector epitopes (such as Tetanus Toxoid-derived T effector epitopes) allows the assessment of bystander suppression. Bystander suppression is a feature of Treg epitopes.

In summary, in silico and in vitro assessment of novel T effector (inflammatory) and Treg (suppressive) epitopes is necessary to best evaluate the impact of novel impurities on the immunogenicity risk in humans. In addition to the standardized tools described above, we will describe novel in silico methods that have been developed to anticipate well-known synthetic peptide impurities (The What If Machine). Several case studies (Tasuglutide, Salmon Calcitonin) will be provided to illustrate these well-established (and more recently developed) Immunogenicity Risk Assessment methods.

References

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