Scientific and Regulatory Considerations for Assessment of Immunogenicity Risk for Generic Peptide and Oligonucleotide Drug Products

Day 1 Workshop Summary
October 7-8, 2024

Introductory Talks

- **Speakers: Anna Schwendeman, Iilun Murphy, Eric Pang**
- Background to adaptive and innate immune response biology
- Relevant FDA guidance
  - *ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin Guidance for Industry* (May 2021)
  - Product Specific Guidance (PSG) for peptide products

Session 1: Adaptive Immunogenicity Risk Mitigation - Product-Related Impurities

- **Chair: Daniela Verthelyi; Speakers: Narasimha Rao, Anne De Groot, Robert Siegel, Sophie Tourdot, Mohanraj Manangeeswaran**
- Discussion focused on the challenges associated with adaptive immune response assay development
- Validation of in-silico, MHC/HLA binding, and T-cell functional assays
- In silico modeling discussed in the context of
  - HLA binding to peptide alleles
  - T-cell receptor binding to peptide epitope bound to HLA
  - Challenge of demonstrating in-silico/in-vitro/in-vivo correlation
  - Limitations in terms of modeling of peptide containing non-natural amino acid residues, covalently attached side chains

Session 1: Adaptive Immunogenicity Risk Mitigation - Product-Related Impurities

- Harmonization of protocols for
  - MHC binding assays
  - MHC-Associated Peptide Proteomics (MAPPs)
  - T-cell proliferation assays
- Challenges
  - Synthesis of test peptides of appropriate purity
  - Choice of cells – population, viability, functionality, # cell per well, e.g., PBMCs
  - # of donors – diversity, healthy vs. patient, ensure broad HLA coverage
  - Selection of controls – peptide length, purity, propensity for aggregation, T-cell epitope content, endotoxin content
  - Defining assay sensitivity, selectivity, and positive signal acceptance criteria
  - Peptide assay concentration
- Discussion of acceptability of current immunogenicity-related impurity thresholds

Session 2: Innate Immunogenicity Risk Mitigation - Process-Related Impurities

- **Chair: Eric Pang; Speakers: Andrew Graves, Jeremy Fry, Sofie Denies, Noel Smith, Seth Thacker, Daniela Verthelyi**
- Discussion focused on the challenges associated with innate immune response assay development
- Cell line assays – choice of cell line and readout, e.g., HEK Blue, RAW Blue, THP-1 Blue cells and NFKb readout
- Cytokine assays – primary PBMC cells, whole blood (fresh or frozen), cytokines: IL-2, 4, 6, 8, 10, IFNγ, TNFα
- Challenges
  - # of donors – usually healthy donors, based on inter-donor variability
  - Cell viability – especially in the context of impact of formulation on assay sensitivity, e.g., presence of preservatives such as phenol
    - Appropriate dilution or concentration
    - Can the drug substance be tested to avoid excipient effect?

Session 2: Innate Immunogenicity Risk Mitigation - Process-Related Impurities

- Challenges cont.
  - Validation of assay sensitivity to IIRMIs that may be present
  - Choice of positive, negative, suitability controls critical to demonstrate assay sensitivity, and to ensure a broad coverage of innate immune response receptors
  - Acceptance criteria
  - Justification of choice of statistical approach for data analysis
- Key questions:
  - Are the detectable levels of IIRMI clinically relevant?
  - Are cell lines sufficient to discern IIRMIs?
  - Why multiple biomarkers? Advantages and disadvantages

Session 3a: Discussion Topic: Adaptive Immunogenicity Risk Mitigation - Product-Related Impurities

- Key Themes:
- Differences in results generated using different in-silico models; in-silico model validation
- DC:T cell assays: culture time, control of cell viability, number of T cells or CD4+T cells and ratio of DC:T cells in the well, establishing assay sensitivity, FBS or defined media, cytokines and growth factors
- Appropriate concentration to test individual impurities
- Improvements to current assays that could be made to better reflect clinical immunogenicity
- Donor selection: determination of number of donors by HLA-coverage, assay variability or both, healthy donors or patient primary cells

Session 3b: Discussion Topic: Innate Immune Response Modulating Impurities

- Key Themes:
- Critical assay attributes for establishment of IIRMI assays, more sensitive/relevant biological readouts of innate immune activation
- Main challenges in implementing IIRMI assays to assess the immunogenicity risk of generic peptides, helpful strategies
- Strategies to overcome excessive dilutions due to formulation inhibitory effects
- Justification of statistical analysis approaches to analyze IIRMI data
- Criteria to accept/select assay runs and IIRMI data, qualifying attributes for donor selection
- How to proceed if drug product batches show a signal in one or more of the IIRMI assay readouts as compared to the RLD, the need to identify the IIRMI(s) in order to implement controls