In Vitro Immunogenicity Assays for Evaluating Generic Peptide Drug Products

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Abstract

Background and purpose: The number of abbreviated new drug application (ANDA) submissions for peptide products has been growing in recent years. The FDA's draft guidance on ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin (October 2017) provided recommendations for demonstrating equivalence of generic peptide products to their respective reference listed drugs (RLDs). Using in vitro methods to evaluate sameness of impurity profiles and associated immunogenicity risk between the generic and RLD peptide products is recommended in this guidance. However, there has not been a comprehensive overview on various types of in vitro assays for evaluating comparative immunogenicity risk. Therefore, the purpose of this work is to provide an overview of approaches, challenges, and recommendations for best practices for various in vitro assays for assessing immunogenicity.

Method: Currently available methods for immunogenicity assessment are summarized from published literature. In vitro immunogenicity assays, submitted as part of ANDAs for peptide products, are summarized and analyzed. Method condition, optimization and key parameters during method development for detecting both adaptive and innate immune activity were evaluated.

Results: Multiple in vitro methods are available to assess the immunogenicity risks of peptide products. Depending on the purpose of assessment, in vitro immunogenicity assays can be divided into two groups, assessing adaptive and innate immune responses. Currently, there is no standard method to evaluate immunogenicity risks. Various immune cell-based assays have been demonstrated to correlate with the rate of clinical immunogenicity through combinations of immune cells, broad HLA genotypes, multiple assay readouts, etc. However, upon examining these assays for how these methods are typically developed, it is important to point out key parameters that should be focused on for optimization. These parameters include sensitivity and specificity of the assays, test product concentrations, cell viability or metabolic activity, positive standard selection and excipient effects.

Conclusion: Immunogenicity assessment of peptide products is recommended when differences in the impurity profiles of peptide drug products are observed. In vitro assays for assessing the immunogenicity risks may be acceptable approaches, if they are well validated, optimized, and are suitable for use.

Materials and Methods

Published methods for immunogenicity assessment are summarized from published literature. Method conditions, optimization and key parameters during method development for detecting both T-cell epitopes and innate immune activity were collated. These parameters include coverage of human leukocyte antigen (HLA) genotypes in the donor set, number of cells, product concentration, incubation time and combined readouts of the assays. In addition, an overview of common in vitro immunogenicity assays and associated common deficiencies submitted in ANDAs for certain peptide products are also summarized.

Results and Discussion

Multiple in vitro methods have been published in literature to assess the immunogenicity risks of peptide products. In vitro immunogenicity assays can be divided into two groups based on the immunogenicity response system: assessing innate (Table 1) and adaptive immune responses (Table 2). Currently, there is no standardized in vitro methods and assay procedures for conducting immunogenicity risks evaluations. Various immune cell-based assays have been demonstrated to correlate with the rate of clinical immunogenicity through combinations of immune cells, broad HLA genotypes, multiple assay readouts, etc. However, it is important to point out key parameters that should have been focused on for optimization (Figure 1). These parameters include sensitivity and specificity of the assays, test product concentrations, cell viability or metabolic activity, positive standard selection and excipient effects.

Table 1. In Vitro Cell-Based Methods to Assess Innate Immune Response

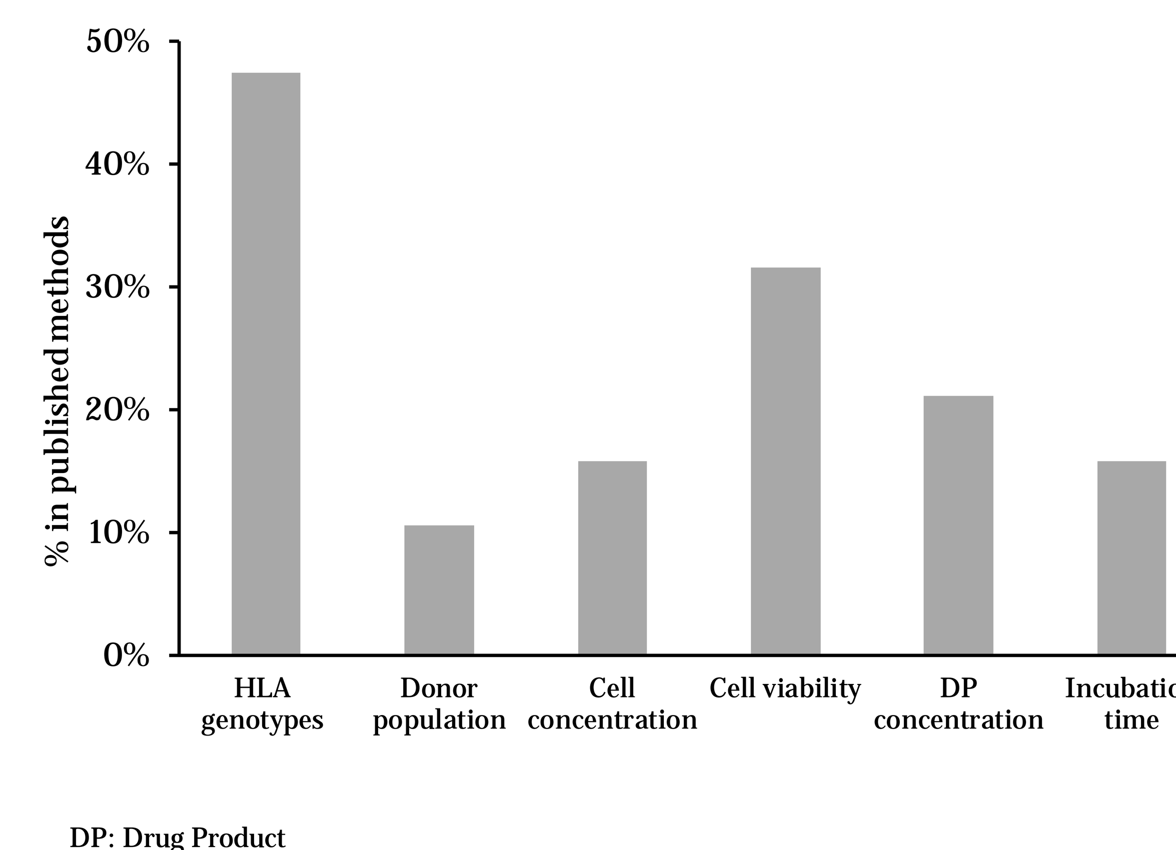
Assay	Cells	HLA genotypes	Population of donors	Readouts
Innate immune response modulating impurity cell-based assay	Macrophage cell lines (RAW-BLUE, MM6)	N	N/A	RAW-BLUE: SEAP reporter construct inducible by NF-κB. MM6: mRNA levels of IL-6 and IL-8
Innate immune response to stress-induced aggregates	Human monocyte-like cell lines THP-1 and MM6.	N	N/A	The concentrations of inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-8, IL-10 and IL-12)
In vitro assay to monitor DC maturation	Monocyte-derived DCs	N	12	DC activation markers and cytokine release
PBMC stimulation assay	PBMC	Y	22	Cytokine analysis (The cytokines include IL-1β, IL-6, IL-10, MCP-1, MIP-1α, MIP-1β, MMP-2, and TNF-α.)
Peripheral Tissue Equivalent (PTE) module	A three-dimensional endothelial cell / collagen matrix culture system, co-cultured with PBMC.	N	N/A	Production of cytokines and chemokines, such as interleukins 1a/b, 6, 8 and 10 and TNF-α.

N/A: Not Applicable

Table 2. In Vitro Cell-Based Methods to Assess Adaptive Immune Response

Assay	Cells	HLA genotypes	Population of donors	Readouts
In Vitro Comparative Immunogenicity Assessment (IVCIA) assay	PBMC without CD8+ T cells	Y	50	T cell proliferation, IL-2 concentration & secreting cells
DC-T cell assay	Monocyte-derived dendritic cells and CD4+ T cells	Y	50	CD4+ T-cell proliferation, IL-2 secretion via ELISpot assay
In vitro PBMC derived T cell assay	PBMC from naïve healthy donors	Y	39	IFNg secretion via ELISpot assay.
In vitro T cell: PBMC assay	Purified CD4+ T cells co-cultured with irradiated PBMCs (1:2)	Y	26	T cell proliferation and IL-2 secretion.

Figure 1. Key Parameters That Are Not Optimized in Published Methods



DP: Drug Product

Common deficiencies identified in ANDA submissions related to immunogenicity assessments

Innate immune response	• Not investigating innate immune response when there are no new impurities found
	• Not providing rationale for the selected cytokine signal readouts
	• Insufficient demonstration of assay sensitivity
	• Not providing sufficient detail on the methodology
	• Not examining formulation effects
Adaptive immune response	• Did not demonstrate that assays are suitable for the intended purpose
	• Did not demonstrate that selected PBMC population was representative of US patient population, such as HLA class diversity and donor population size
	• Insufficient optimization of the conditions, such as duration of assay, number of cells per well, concentration of drug product, suitable controls, etc.
	• Insufficient method validation: assay sensitivity and specificity
	• Insufficient information and justification for how to determine the thresholds for various levels of risk

Conclusion

- The immunogenicity risk of generic synthetic peptide products (i.e., the five peptides listed in aforementioned guidance) should be comparatively assessed to their respective recombinant RLD products.
- Multiple in vitro cell-based methods to assess immunogenicity risk have been reported in literature and could be used in assessing immunogenicity for generic peptide drug products.
- Insufficient method optimization and validation have been identified among common deficiencies in ANDA submissions related to immunogenicity assessments. Key method parameters should be optimized and validated.

Disclaimer

The poster reflects the views of the authors and should not be construed to represent FDA's views or policies.

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