In Vitro Assays for the Evaluation of Innate Immunogenicity in Generic Peptide Drug Products

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PURPOSE

The FDA finalized the guidance titled "ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin[1]" in May 2021, with the aim of facilitating submission and approval for generic peptide drugs. This guidance outlines the FDA's recommendations for demonstrating equivalence of synthetically produced generic peptide drug products referencing recombinantly produced reference listed drugs (RLDs). An essential aspect in developing and approving these proposed generic peptide products involves assessing immunogenicity risks using in vitro assays. When there are differences in impurities, generic applicants may conduct additional non-clinical assays, such as cell-based or functional binding assays, to ensure their proposed test product does not increase the potential immunogenicity risk.

In vitro immunogenicity assays can assess both adaptive and innate immune responses and immunogenicity risks. However, there is currently no standardized methods or regulatory guidance for assessing immunogenicity risks, posing challenges for generic applicants in providing necessary data. In order to address these challenges, we conducted a study to analyze the current practices, methods, and regulatory recommendations. We systematically compared innate immunogenicity data submitted by generic applicants while analyzing the approval criteria for U.S. generic peptide drugs. Furthermore, the gaps in the current regulatory guidelines are explored. Our study provides the basis for recommending the best practices in conducting various in vitro assays to evaluate innate immunogenicity.

METHOD(S)

We conducted an analysis of abbreviated new drug applications (ANDAs) submitted to the U.S. FDA. This involved cross-comparing conditions, optimization, and key parameters assessed during the method development process by ANDA applicants. These parameters include cell type and donor population, agonists, types of suitability controls, and combined assay readouts.

Manual data extraction techniques were employed to analyze the FDA database, with information organized and compiled in an Excel spreadsheet. Furthermore, common deficiencies were collated for the in vitro immunogenicity assays submitted as part of the ANDAs. The specific submission details, including application numbers, applicants, and specific drug names, were intentionally blinded.

Opportunities with FDA's Office of Generic Drugs (OGD)

Looking for a post-doctoral opportunity?
https://www.zintellect.com/Opportunity/Details/FDA-CDER-2023-1240
Looking for funding for generic drug research?
https://www.fda.gov/drugs/generic-drugs/generic-drug-research-collaboration-opportunities

Table 1. Cross-comparison of Common In Vitro Cell-Based Methods for Assessing Innate Immune Responses

Assay	Cells	Donor Population	Agonists	Positive Control	Negative Control	Readouts
Cell based assay	Reporter cell lines (THP1, RAW-BLUE, HEK-BLUE (hNOD1 and hNOD2), etc.)	N/A	THP1 cells: FLA-BS, LPS-EB, MDP, Pam3CSK4, Poly(I:C) HMW, and Zymosan Raw-Blue cells: CpG ODN 1826, Imiquimod (R837), FSL-1, CL075 HEK-Blue-hNOD1/hNOD2: M-TriDAP	Agonists	Cell culture media	Traces of IIRMIs were detected using NF-κB/AP-1-inducible SEAP reporter systems. THP1 pattern-recognition receptors (PRR): TLR1/2, TLR3, TLR4, TLR5, TLR2/Dectin, and MDP-NOD2 Raw-Blue PRR: TLR2/6, TLR7, TLR7/8, and TLR9 HEK-Blue (hNOD1 and hNOD2) PRR: NOD1 and NOD2
PBMC activation (stimulation) assay	PBMC and whole blood	>20	Zymosan, LPS, FLA-ST (Flagellin), Pam3CSK4, CpG-ODN, Imiquimod (R837), Poly (I:C), IE-DAP, MDP, PGN-Sandi, FSL-1, CL075, etc.	SEB (staphylococcal enterotoxin B), LPS, zymosan, etc.	PBS, Cell culture media, and PADRE (pan-DR epitope)	Cytokine/chemokine analysis: IL-1β, IL-2, IL-4, IL-6,IL-8, IL-10, IFNγ, TNF-α, MCP-1, MIP-1α, MIP-1β, MMP-2, RANTES, IP-10, and GM-CSF

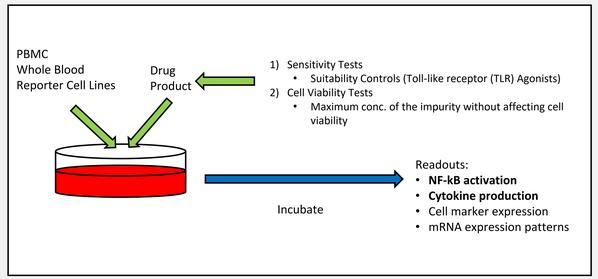

Figure 1. Schematic of In Vitro Assays for Innate Immunogenicity

Table 2. Overview of PRRs Based on IIRMI

Examples of Suitability Controls	Pattern Recognition Receptors (PRRs)
B. subtilis flagellin	TLR5
FSL-1	TLR2/TLR6
ODN2006 Class B	TLR9
Poly(I:C) HMW	TLR3
Poly(I:C) LMW	TLR3
Zymosan	TLR2/Dectin 1
CL075	TLR7/TLR8
MDP	NOD2
ODN2216	TLR9
E. coli O111:B4 LPS	TLR4
Imiquimod	TLR7
Pam3CSK4	TLR1/TLR2

Table 3. Immunogenicity Risk Assessment: Comments and Guidance Based on Keywords

Keyword	Comments/Recommendations	Keyword	Comments/Recommendations
Drug product	1) Studies should be conducted with at least three batches of the proposed drug product and three batches of the RLD as available. We recommend testing three batches of proposed drug product that includes batches covering at least the midpoint of the proposed shelf-life. 2) Provide a table detailing the manufacturing and testing date for each lot together with the information regarding the drug substance lot(s) used to produce it. For RLD lots provide lot numbers and expiry dates for each lot. 3) The use of non-consecutive lots is recommended.	Drug product concentration	1) The drug product should be tested at the maximal product concentration that does not reduce cell viability or impair responses to IIRMI. 2) The product is recommended not to be diluted more than 10-fold relative to the preparation administered to the patient.
Drug substance	1) Your exhibit batches should be manufactured using at least two batches of your proposed drug substance.		
Impurity identification	1) Identify each peptide-related impurity that is 0.10% of the drug substance or greater. 2) For any impurities that are new or present at increased concentration in your product relative to the RLD, you are asked to provide data supporting the lack of effect on the risk of immunogenicity for the proposed product. 3) This characterization may be performed using in-silico and in-vitro assays to assess whether these impurities contain sequences likely to bind to MHC and/or T cell epitopes that would facilitate the induction of an immune response to the product or facilitate multimeric presentation to B cells.	Sensitivity and range	1) Assay performance should be controlled using suitability controls in the presence of drug product to ensure consistent sensitivity to low levels of a variety of innate immune response modulators capable of triggering diverse innate immune pathways. 2) Provide a table of the suitability controls used including low and high positive controls assessed in appropriate matrix and along with any additional data that confirms the assay consistently performs with the expected sensitivity and range. 3) Calculations on the sensitivity of the assay should account for all dilutions and manipulations of the samples during the testing process. Spiking studies into the drug product prior to any manipulation to demonstrate signal recovery are recommended.

RESULT(S)

Table 1 introduces a cross-comparison of the two most common in vitro cell-based assays for assessing the innate immune response associated with peptide products. Table 2 provides an overview of Pattern Recognition Receptors (PRRs) based on different IIRMIs to assist in understanding the agonists in Table 1. Figure 1 illustrates the schematic of in vitro assays. Regardless of the cells used, the assays should be performed in the presence of the drug product. These assays include sensitivity tests using various Toll-like receptor (TLR) agonists and cell viability tests using the maximum concentration of the impurity that does not affect cell viability. Following incubation, readouts such as NF-kB activation, cytokine production, and cell/mRNA expression are assessed.

Common deficiencies in assessing the innate immune response have been identified across various generic peptide applications. These deficiencies include: 1) failure to investigate the innate immune response in instances where no new impurities are found because the impurities found through innate immune assays are not usually those detected in the impurity profile comparisons; 2) inadequate justification for the selection of cytokine signal readouts; 3) insufficient demonstration of assay sensitivity; 4) lack of a comprehensive description of the methodology employed; and 5) neglect of the examination of potential formulation effects. Table 3 highlights key parameters identified by reviewers, presented as keywords with corresponding comments and recommendations based on these keywords.

CONCLUSION(S)

Immunogenicity risk assessment is critical for some of the complex generic peptide drug applications. When developing assays, it is crucial to identify and prioritize key factors to obtain optimized assays, in order to enable efficient regulatory assessment. The common issues or deficiencies include, but are not limited to assay sensitivity and specificity, donor selection, optimal concentrations for drug products, assessment of cell viability or metabolic activity, selection of appropriate positive standards, and examination of potential effects of excipients. Developing performance standards and identifying best practices for non-clinical immunogenicity assessment are important. The Agency is currently working on improving and standardizing in vitro immunogenicity assays by funding research projects via collaborations with industry and academia to refine and align these assays. In addition, the Agency organizes workshops/webinars to share updated thinking and obtained knowledge. One example is the recent FDA/CRCG workshop titled "Scientific & Regulatory Considerations for Assessment of Immunogenicity Risk for Generic Peptide & Oligonucleotide Drug Products" (Oct 7-8, 2024)[2] focusing on clarifying review standards for in vitro and in silico immunogenicity assays and to communicate best practices to the industry.

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1. FDA's guidance for industry, ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin (May 2021) https://www.fda.gov/media/107622/download
2. https://www.complexgenerics.org/education-training/scientific-and-regulatory-considerations-for-assessment-of-immunogenicity-risk-for-generic-peptide-and-oligonucleotide-drug-products/

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