

Comprehensive assessment of immunogenicity risk of host cell proteins in biologics and vaccines using in silico and in vitro methods

Mitchell McAllister¹, Kirk Haltaufderhyde¹, Brian Roberts¹, Andres Gutierrez¹, Jacob Tivin¹, Matthew Ardito¹, William Martin¹, Anne S. De Groot¹

¹EpiVax, Inc., Providence, RI

CONTACT INFORMATION: info@epivax.com

aaps
National
Biotechnology
CONFERENCE

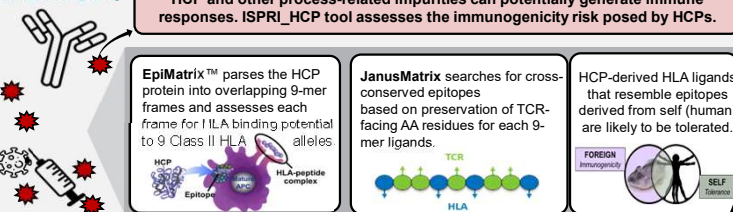
PURPOSE

Host cell protein (HCP) impurities are a critical quality attribute (CQA) because they may trigger undesired immune responses with the potential to compromise the safety and efficacy of biologics. The presence of immune responses to Chinese hamster ovary (CHO)-derived HCPs has contributed to the suspension of clinical trials in the past. EpiVax has developed a web-based tool to evaluate the immunogenicity of HCP impurities. This tool, the Interactive Screening and Protein Re-engineering Interface for Host Cell Proteins (ISPRI-HCP) has been adapted from a base tool, ISPRI, which has been in use by industry clients and collaborators since 2003. ISPRI-HCP evaluates the immunogenic potential of proteins based on T cell epitope content and their similarity to the human proteome. We used ISPRI-HCP to perform an in-silico immunogenicity risk assessment of 140 HCPs identified by a consortium of monoclonal antibody producers. EpiVax will further evaluate and validate the ISPRI-HCP platform by performing in-vitro T cell assays for this set of commonly found HCP impurities.

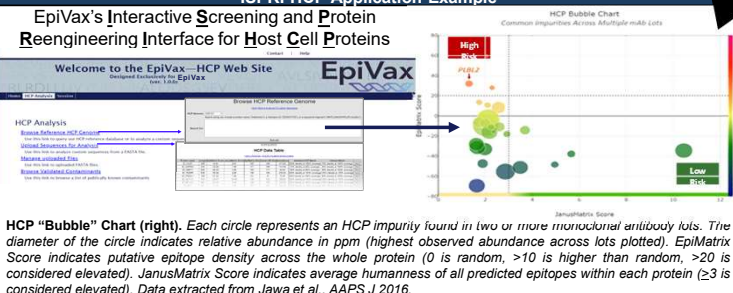
OBJECTIVE(S)

To offer therapeutic developers the ability to determine which host cell protein impurities may be of highest risk for immunogenicity through a quick and easy web-based tool.

METHODS



ISPRI-HCP Application Example

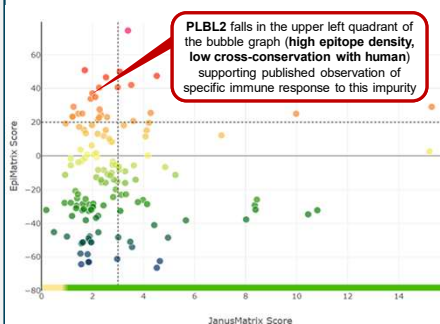


RESULTS

Common "high risk" copurifying HCP

The BioPhorum Development Group (BPDG) compiled a list of frequently seen high-risk HCPs based on information gathered through literature searches, company experiences, and surveys.

Using ISPRI-HCP, we assessed the immunogenicity of the 140 commonly found CHO HCP impurities. Shown is a subset of the calculated EpiMatrix (EMX) and JanusMatrix (JMX) scores for each protein.



- CHO HCPs shown in this graphic have a wide range of potential immunogenicity risk, as assessed by EpiMatrix and JanusMatrix.
- Phospholipase B-Like 2 Protein (PLBL2) is a CHO protein found with high frequency in monoclonal antibody lots.
- PLBL2 was associated with HCP-related immunogenicity in patients treated with Lebrikizumab [Fischer et al.].

Biologics



Vaccines

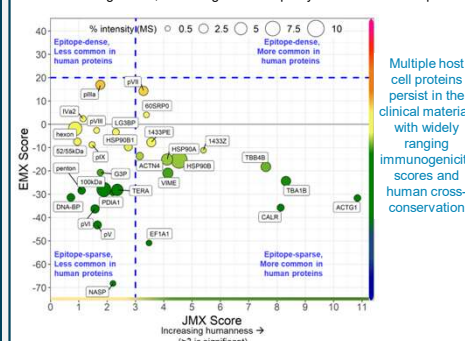


COVID Vaccine HCP and vector proteins: Krutzke et al.

Process-related impurities in the ChAdOx1 nCoV-19 vaccine

Krutzke et al. analyzed the ChAdOx1 nCoV-19 vaccine for potential HCPs and found that the vaccine contains substantial amounts of both mammalian (HEK)-derived and viral-derived nonstructural proteins.

EpiMatrix and JanusMatrix analysis showed that several viral-vectored proteins had elevated immunogenicity scores and low cross-conservation with the human genome, indicating risk for impurity-driven immune response.



Best case scenario: highly cross-conserved human epitopes are well tolerated in this material. **Worst case scenario:** T cell responses to the co-purifying viral proteins and selected mammalian HCP may stimulate immunogenicity, particularly if response against residual viral vector proteins provides an adjuvant effect.

Hexon, an Adenovirus protein reported to be found in COVID-19 vaccine formulations: Pitkänen et al.

> Thromb. Res. 2021 Dec;208:129-137. doi: 10.1016/j.thromres.2021.10.027. Epub 2021 Nov 6.

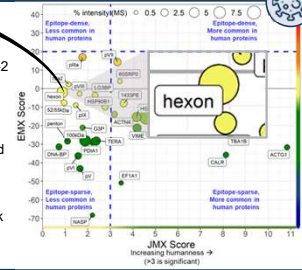
COVID-19 adenovirus vaccine triggers antibodies against PF4 complexes to activate complement and platelets

Hanna H Pitkänen¹, Annuika Joupila², Tuukka Heini³, Vinaya Dulipati⁴, Juha Kotimaa⁴, Seppo Meri⁵, Anu Kantele⁶, Pinja Jalkanen⁷, Ilkka Julkunen⁸, Riitta Lassila⁹

- Hexon protein complexes with platelet factor 4 (PF4) and induces ADA in vaccine-induced thrombotic thrombocytopenia (VITT) subjects.
- This immunological stimulus co-activates the complement system and triggers spontaneous aggregation of healthy platelets [Pitkänen et al.].

Adenoviral hexon protein present in the ChAdOx1 nCoV-19 and Ad26.COV2.S SARS-CoV-2 vector vaccines

ISPRI-HCP Results: Hexon falls in the elevated immunogenicity scores and low human cross-conservation quadrant of our HCP bubble graphs, indicating considerable risk for impurity-driven immune response.



CONCLUSION(S)

Further development of the ISPRI-HCP tool, in collaboration with biologics industry partners, will enable exploration of immune responses in vitro, enhancing the prediction of immunogenic and tolerated T cell epitopes from HCPs, as well as evaluate the correlations with resultant ADA when patient data are available.

REFERENCES

1. Bailey-Kellogg C, et al. CHOPPI: a web tool for the analysis of immunogenicity risk from host cell proteins in CHO-based protein production. Biotechnology and Bioengineering. United States; 2014 Nov 2; :2170-82. PMID: 24888712
2. Koren E, et al. Clinical validation of the "in silico" prediction of immunogenicity of a human recombinant therapeutic protein. Clin Immunol. 2007; 124:26-32. PMID: 17490912
3. De Groot AS, Martin W. Reducing risk, improving outcomes: Bioengineering less immunogenic protein therapeutics. Clin Immunol. United States: Elsevier Inc.; 2009 May; 131(2):189-201. PMID: 19269256
4. Moise L, et al. The two-faced T cell epitope: Examining the host-microbe interface with JanusMatrix. Human Vaccines and Immunotherapeutics. 2013. p. 1577-86. PMID: 235842515
5. Fischer SK, et al. Specific Immune Response to Phospholipase B-Like 2 Protein, a Host Cell Impurity in Lebrikizumab Clinical Material. AAPS J. 2017 Jan;19(1):254-263. doi: 10.1208/s12248-016-9998-7. Epub 2016 Oct 13. PMID: 27739010
6. Jones M, et al. "High-risk" host cell proteins (HCPs): A multi-company collaborative view. Biotechnol Bioeng. 2021 May 31. PMID: 33930190
7. Krutzke L, et al. Research Square 2021. Process-related impurities in the ChAdOx1 nCoV-19 vaccine.
8. Pitkänen HH, et al. COVID-19 adenovirus vaccine triggers antibodies against PF4 complexes to activate complement and platelets. Thromb Res. 2021 Dec;208:129-137. doi: 10.1016/j.thromres.2021.10.027. Epub 2021 Nov 6. PMID: 34768097; PMCID: PMC8571998



EpiVax