Comprehensive Assessment of Immunogenicity Risk of Host Cell Proteins in Biologics Using In Silico and In Vitro Methods

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Kirk Haltaufderhyde[1], Brian Roberts[1], Frances Terry[1], Andres Gutierrez[1], Jacob Tivin[1], Matthew Ardito[1], William Martin[1], Anne S. De Groot[1]

[1] EpiVax, Inc., Providence, RI

CONTACT INFORMATION: info@epivax.com

aaps National Biotechnology CONFERENCE

PURPOSE

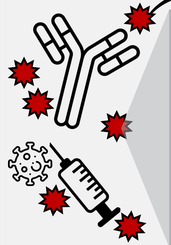
Host cell protein (HCP) impurities are a critical quality attribute (CQA) because they may trigger undesired immune responses with the potential to compromise the safety and efficacy of biologics. The presence of immune responses to Chinese hamster ovary (CHO)-derived HCPs has contributed to the suspension 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 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

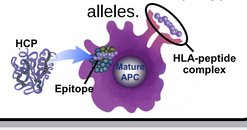
To offer therapeutic developers the ability to determine which host cell protein impurities may be of highest risk for immunogenicity through a quick and easy web based tool.

METHODS

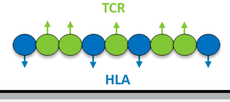
HCP and other process related impurities in biotherapeutics can potentially generate immune responses. ISPRI_HCP tool assesses the immunogenicity risk posed by HCPs.



EpiMatrix™ will parse each HCP protein into overlapping 9-mer frames and assess 9-mer frames for HLA binding potential with respect to nine Class II HLA supertype alleles.



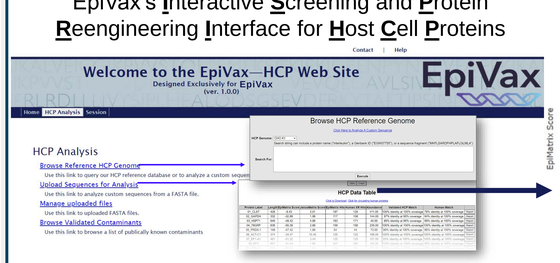
JanusMatrix searches for potentially cross-reactive sequences based on the preservation of the TCR-facing residues and established HLA binding preferences of given 9-mer ligands.

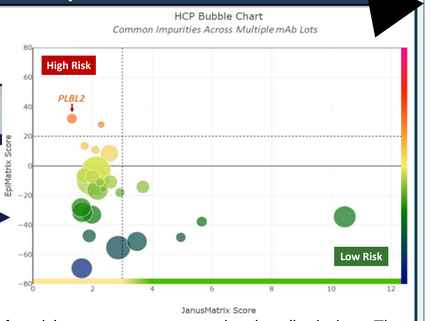


Predicted ligands from an HCP that share the same HLA restriction and TCR-facing contour as epitopes derived from self (human) are presumed to be tolerated.

ISPRI-HCP Application Example

EpiVax's **I**nteractive **S**creening and **P**rotein **R**eengineering **I**nterface for **H**ost **C**ell **P**roteins





HCP Bubble Chart (right). *Each circle represents an HCP impurity found in two or more monoclonal antibody lots. The diameter of the circle indicates relative abundance in ppm (highest observed abundance across lots plotted). EpiMatrix Score indicates putative epitope density across the whole protein (0 is random, >10 is higher than random, >20 is considered elevated). JanusMatrix Score indicates average humanness of all predicted epitopes within each protein (≥3 is considered elevated). Data extracted from Jawa et al., AAPS J 2016.*

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.

Review > Biotechnol Bioeng. 2021 Aug;118(8):2870-2885. doi: 10.1002/bit.27808. Epub 2021 May 31.

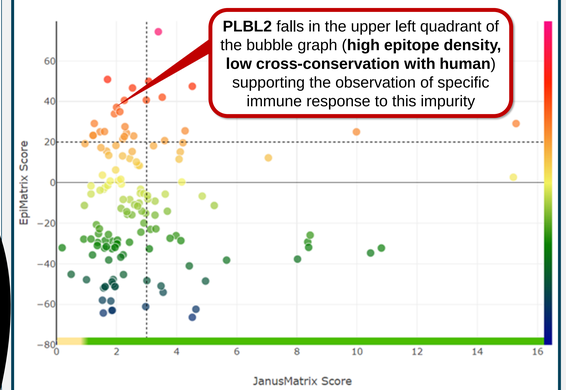
"High-risk" host cell proteins (HCPs): A multi-company collaborative view

Marisa Jones [1], Nisha Palackal [2], Fengqiang Wang [3], Georgeen Gaza-Bulseco [4], Karen Hurkmans [5], Yiwei Zhao [3], Carmelata Chitikila [6], Severine Clavier [7], Suli Liu [8], Emily Menesale [8], Nicole S Schonenbach [9], Satish Sharma [10], Pascal Valax [11], Thomas Waerner [12], Lei Zhang [10], Trish Connolly [13]

Affiliations + expand

PMID: 33930190 DOI: 10.1002/bit.27808

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



- The immunogenicity of HCPs can vary dramatically.
- Phospholipase B-Like 2 Protein (PLBL2) is an example of a CHO protein found with high frequency in monoclonal antibody lots.
- PLBL2 was later observed to generate a specific **immune response in patients treated with Lebrikizumab**, whose clinical material contained the CHO HCP impurity [Fischer et al.].

COVID Vaccine HCP and vector proteins: Krutzke et al.

Process-related impurities in the ChAdOx1 nCov-19 vaccine

Krutzke L, Roesler R, Wiese S, Kochanek S

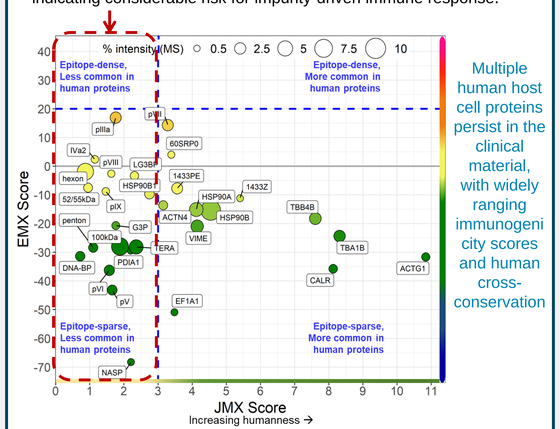
Preprint from Research Square, 04 May 2021

DOI: 10.21203/rs.3.rs-477964/v1 PPR: PPR336678

Preprint

Krutzke et al. analyzed the ChAdOx1 nCov-19 vaccine using biochemical and proteomic methods. They found that in addition to the adenovirus vector, the vaccine contains substantial amounts of both human and nonstructural viral proteins.

Using ISPRI_HCP, we found that several viral vector proteins have elevated immunogenicity scores and low human cross-conservation, indicating considerable risk for impurity-driven immune response.



Multiple human host cell proteins persist in the clinical material, with widely ranging immunogenicity scores and human cross-conservation

Best case scenario, highly cross-conserved human epitopes are well tolerated in this material. **Worst case scenario**, however, it is possible that immune responses against the copurifying human host cell proteins or other human epitopes with which they are cross-conserved, might be capable of stimulating auto-immunity, particularly if response against residual viral vector proteins provides an adjuvanting 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.

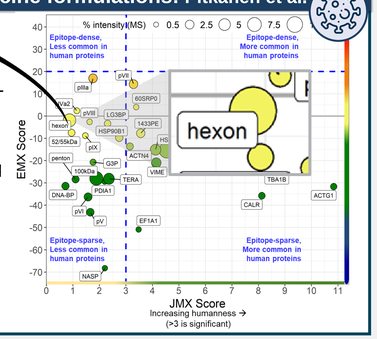
COVID-19 adenovirus vaccine triggers antibodies against PF4 complexes to activate complement and platelets

Hanna H Pitkänen [1], Annukka Jouppila [2], Tuukka Helin [3], Vinaya Dulipati [4], Juha Kotimaa [4], Seppo Meri [5], Anu Kantele [6], Pinja Jalkanen [7], Ilkka Julkunen [8], Riitta Lassila [9]

- 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

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.

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