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

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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)

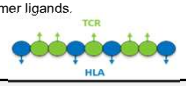
To offer therapeutic developers the ability to determine which host cell protein impurities may be of highest risk for immunogenicity through a quick and easy web-based tool.

METHODS

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

EpiMatrix™ parses the HCP protein into overlapping 9-mer frames and assesses each frame for I LA binding potential to 9 Class II HLA alleles.

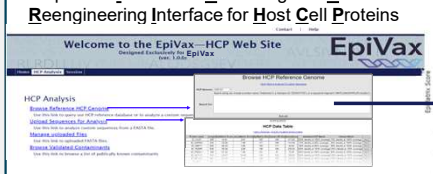
JanusMatrix searches for cross-conserved epitopes based on preservation of TCR-facing AA residues for each 9-mer ligands.

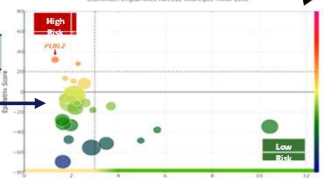


HCP-derived HLA ligands that resemble epitopes derived from self (human) are likely to be tolerated.

ISPRI-HCP Application Example

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





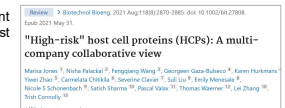
HCP "Bubble" Chart (right). *Each circle represents an HCP impurity found in two or more monoclonal antibody lots. The diameter of the circle indicates relative abundance in ppm (highest observed abundance across lots plotted). EpiMatrix Score indicates putative epitope density across the whole protein (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

Biologics

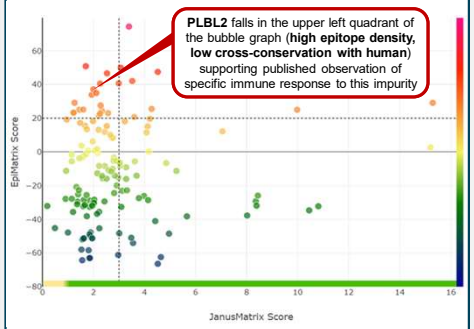
Common "high risk" copurifying HCP

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.



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

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



PLBL2 falls in the upper left quadrant of the bubble graph (**high epitope density, low cross-conservation with human**) supporting published observation of specific immune response to this impurity

- 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.].

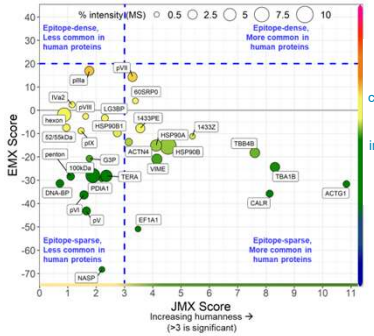
Vaccines

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.



Multiple 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:** 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.

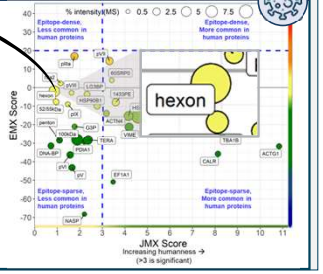
COVID-19 adenovirus vaccine triggers antibodies against PF4 complexes to activate complement and platelets

Hanna H 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(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.

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