In silico immunogenicity risk assessment for fusion proteins and novel antibody modalities

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Abstract

Purpose. The potential for immunogenicity and anti-drug antibody (ADA) generation is a hurdle that drug developers face in the process of bringing biologics innovations to the clinic. At the same time, new biologics formats have enabled developers to engineer products with improved function and specificity. The creation of an integrated in silico screening and re-engineering platform called ISPRI has made it possible to rapidly assess the overall immunogenic potential of a biologic and to identify individual T cell epitope clusters that may contribute to immunogenicity in the clinical setting. Here we present several examples of the ISPRI-driven assessment of novel format biologics such as fusion proteins, bispecific antibodies and antibody-drug conjugates.

Methods. We retrieved the amino acid sequences of **Enbrel**, a fusion protein consisting of TNF-receptor and the Fc domain of human IgG1, **Hemlibra**, a bispecific antibody targeting blood factors X and IXa, and **Lumoxiti**, an antibody drug conjugate consisting of a single chain variable fragment (scFv) targeting CD22 fused to a modified PE38 exotoxin. Each of these products has a different format and function. Setting aside the clinical functions of these molecules, we used ISPRI to assess their immunogenicity.

Using models for a globally representative set of HLA-DR supertype alleles, putative T cell epitopes were mapped for each complete protein sequence and constituent domain using the EpiMatrix algorithm, and each predicated HLA ligand was screened against an internally curated database of human antibody isolates and germline sequences. In addition to evaluating for potential T effector epitopes, the ISPRI platform also considers the contribution of regulatory T cell epitopes (Tregitopes) to immunogenic potential. For each input sequence and domain, ISPRI provided Tregitope-adjusted Immunogenicity Scores, which are normally distributed around zero. ISPRI normalizes this score so as to determine how the T cell epitope content of the sequence compares to the T cell epitope content of a random protein of equivalent length. Positive scores indicate greater than expected content, while negative scores indicate less-than-random epitope content. The Tregitope-adjusted Immunogenicity Score can be used to predict the ADA response rate for novel antibody products using a regression model based on a set of clinical benchmark monoclonal antibodies whose observed ADA response rates are known.

The JanusMatrix algorithm contained in the ISPRI toolkit was also used to evaluate the conservation of identified T cell epitope clusters (hot spots) with autologous (human) proteins at both the HLA-binding and TCR-interacting face of each epitope. The relative risk of immunogenicity was estimated using the Immunogenicity Scale and calculated using the ISPRI ADA prediction tool. This comprehensive assessment was performed, using ISPRI, for each of the input protein domains and the complete multidomain proteins.

Results. Consistent with observations of low immunogenicity rates, the ISPRI-generated immunogenicity score of the complete Enbrel fusion protein was low (-56.97), as were the scores of each domain. Similarly, the immunogenicity scores of the Hemlibra domains were low individually (between -33.66 and -52.24), and when combined for use in ISPRI's regression model to predict ADA, resulted in an estimate of minimal ADA response, corroborating the observation of low ADA rates in the clinic. On the other hand, immunogenicity scores for the Lumoxiti domains were comparatively high (between -19.37 and 37.75), and applying ISPRI's ADA model to the isolated components of the scFv resulted in an estimate of ADA response in ~32% of exposed patients. This prediction is consistent with the observation of high ADA positivity (59%) for Lumoxiti in clinical trials. Higher immunogenicity than predicted based on the antibody component alone may be attributed to additional fully foreign T cell epitope content in the PE38 domain and the lack of a human constant domain, which generally contains tolerance-bolstering Tregitopes.

Purpose

Detect and predict immunogenicity and Anti-Drug Antibodies (ADA) for biologics

ISPRI, an in-silico toolkit developed by EpiVax, Inc., can rapidly assess the overall immunogenic potential of a biologic and identify T cell epitope clusters that may contribute to it.

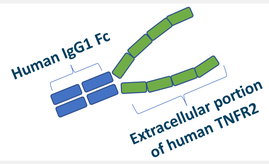
Novel Format Biologics

As biologic formats become more complex to allow for more specific and improved function, ISPRI-driven assessment of novel format biologics such as fusion proteins, bispecific antibodies and antibody-drug conjugates revolutionizes the approach to biologics development.

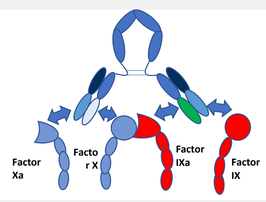
Objective

Use ISPRI to assess immunogenic potential of three proteins with distinct formats and functions and compare results to clinical observations.

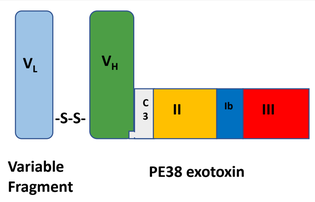
1. **Enbrel**, a fusion protein consisting of TNF-receptor and the Fc domain of human IgG1.



2. **Hemlibra**, a bispecific antibody targeting blood factors X and IXA. It acts like Factor VIIIa by bridging FIXa and FX to activate FX.



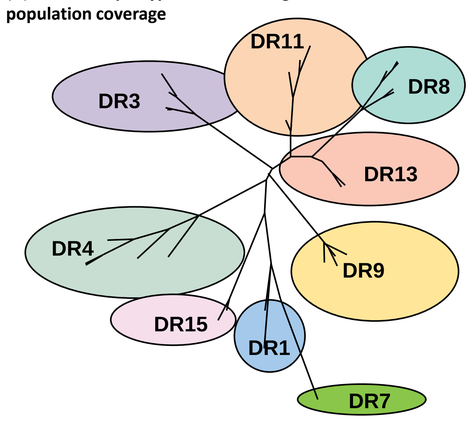
3. **Lumoxiti**, an antibody drug conjugate consisting of single chain variable fragment (scFv) targeting CD22 fused to a modified PE38 exotoxin.



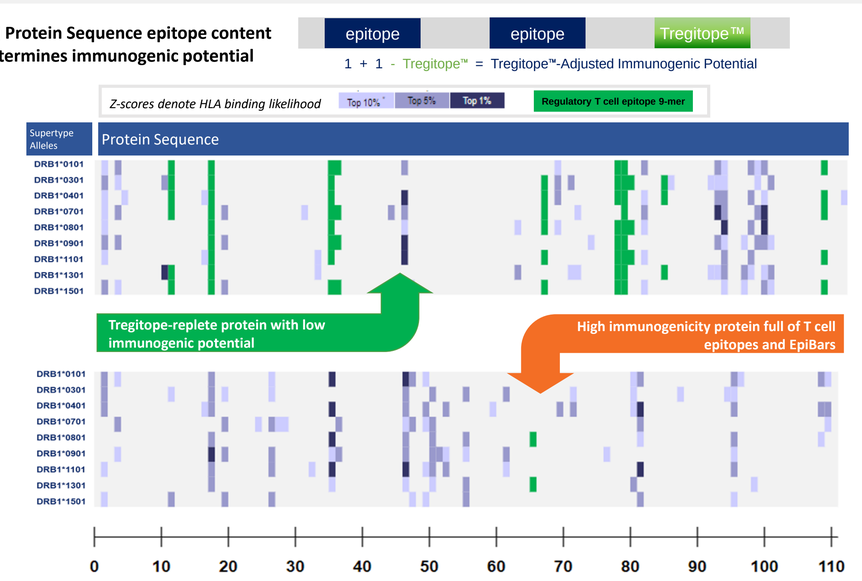
Methods

- All sequences are analyzed using a representative of HLA-DR supertypes that cover >95% of the worldwide human population. **(A)**
- T cell epitopes were mapped for each complete protein sequence as well as their constituent domains using the EpiMatrix algorithm. **(B)**
- ISPRI distinguishes regulatory T cell epitopes (Tregitopes™) from T effector epitopes in the analysis of the immunogenic potential. Janus Matrix™ is able to compare T cell epitope clusters against human proteins to see if they are similar at the 2, 3, 5, 7, and 8 (TCR facing) positions of the nine-mer, which may cause recognition of these nine-mers as self by T cells. "Self-like" regions and Tregitopes™ may promote tolerance, giving a more accurate representation than volume of epitope content alone. **(C)**
- Immunogenicity scores are predicted and compared on a scale created from a large number of random sequences with amino acids at naturally occurring frequencies, normally distributed around zero in order to characterize the T-cell epitope content. Benchmarks are made with well characterized proteins (see below).

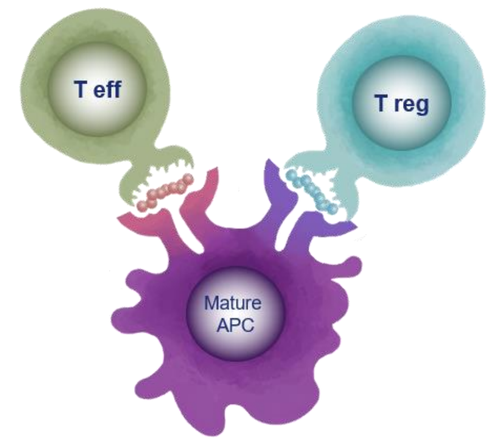
(A) HLA DR Supertypes offer broad global population coverage



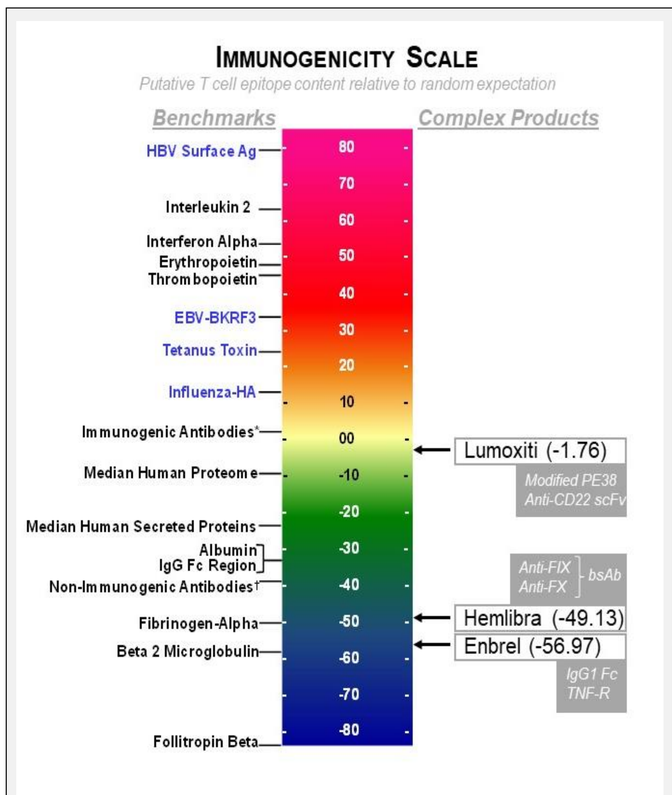
(B) Protein Sequence epitope content determines immunogenic potential



(C) T cell phenotype modulates immune response



Results



*Average of antibodies known to induce anti-therapeutic responses in more than 5% of patients
†Average of antibodies known to induce anti-therapeutic responses in less than 5% of patients

Relative risk of immunogenicity of complex biologics from scale of well characterized monoclonal antibodies and proteins.

Enbrel (Etanercept)

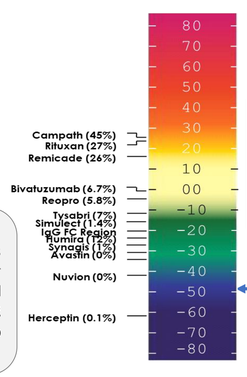
- In line with low observed immunogenicity, the ISPRI-generated immunogenicity score for the complete Enbrel fusion protein was low (-56.97).
- Scores of each domain were also low and **no junctional epitopes** were identified at the point of fusion between domains.

Protein	Length	EpiMatrix Hits	EpiMatrix Score	Tregitope-adjusted EpiMatrix Score
Enbrel (Complete)	467	128	-51.00	-56.97
IgG1 Fc	232	103	-11.61	-23.84
TNFR	235	25	-87.74	-87.74

Hemlibra (Emicizumab)

- Immunogenicity scores for this bispecific antibody's domains were low individually (spanning -52.24 to -33.66).
- When combined for ISPRI's regression model to predict ADA rates, an estimate of minimal ADA response was calculated, corroborating low ADA rates in the clinic.

From package insert:
6.2 Immunogenicity ... The immunogenicity of HEMLIBRA was evaluated using an enzyme-linked immunosorbent assay (ELISA) or an electrochemiluminescence (ECL) assay. **No patients tested positive for anti-emicizumab antibodies in HAVEN 1 and HAVEN 2 (n = 171). Four patients tested positive for anti-emicizumab antibodies in the dose-finding trial (n = 18).**



Summary of EpiMatrix Analysis

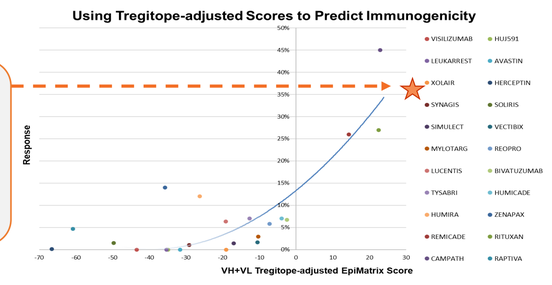
EpiMatrix Analysis		
Target Antibody	EpiMatrix Protein Score	Tregitope-Adjusted EpiMatrix Protein Score
Variable Heavy 1: EMICIZUMAB_DEMO - EMICIZUMAB_1_VH	58.56	-41.79
Variable Heavy 2: EMICIZUMAB_DEMO - EMICIZUMAB_2_VH	-22.30	-52.24
Variable Light 1: EMICIZUMAB_DEMO - EMICIZUMAB_1_VL	7.29	-51.64
Variable Light 2: EMICIZUMAB_DEMO - EMICIZUMAB_1_VL	7.29	-51.64
Complete Antibody	***13.45***	***-49.13***

Hemlibra (0% *predicted* ADA response rate)

Lumoxiti (Moxetumomab pasudotox)

- For this antibody drug conjugate, domain scores were relatively high (spanning -19.37 to 37.75) and when applying ISPRI's ADA model to the isolated components of scFv, the estimated ADA response was shown to be about 32%.
- Clinical observations are consistent with this high ADA frequency prediction, occurring 59% of the time in clinical trials. The gap may be attributed to lack of a human constant domain or foreign T cell epitope content in the PE38 domain.

Tregitope-adjusted EpiMatrix Score of Lumoxiti scFv component indicates high potential for ADA response



Conclusions

ISPRI Immunogenicity and Anti-Drug Antibody Predictions are consistent with clinical observations.

In this study, the immunogenicity risk assessments performed by ISPRI for three out of the three multi-domain molecules evaluated were consistent with clinical observations.

EpiVax's ISPRI Toolkit not only allows for the rapid in silico analysis and assessment of the immunogenicity risk of complex, multidomain biologics, but also estimates ADA rates.

This innovative, ground-breaking technology and tool for assessment will only increase in importance as biologics formats become more complex and it becomes increasingly necessary to consider the immunogenic potential of not only novel constructs as a whole (including at new junctions not found in nature) but also of their individual components where each might have distinct T cell epitope characteristics.

For questions regarding immunogenicity screening, please contact: Katie Porter at 401-272-2123; or at info@epivax.com

www.epivax.com