Immunogenicity prediction for monoclonal antibodies in the context of the broader human proteome, supported by clinical observations

Frances E. Terry[1], Andres H. Gutierrez[1], William D. Martin[1], Anne S. De Groot[1,2]

[1]EpiVax, Inc., Providence, RI, United States; [2]Institute for Immunology and Informatics, University of Rhode Island, Providence, RI, United States

Abstract

Background

- Immunogenicity and anti-drug antibody (ADA) generation: challenge for developing new biologic products.
- T cell epitopes are key drivers, or modulators, of immunogenicity.
- Identifying T effector and regulatory T cell epitopes (Tregitopes) in monoclonal antibodies (mAbs) enables a more accurate forecast of immunogenic potential.
- Our published regression model for forecasting immunogenicity was based on the analysis of 22 antibodies. [1] and is now used by most major BioPharma companies to estimate immunogenic risk in new mAbs.
- In the current study, we have updated our regression model based on clinically reported ADA data and updated our immunogenicity scale with benchmarks reflective of key subsets of the human proteome.

Methods

- Annotated human proteome sequences were acquired from UniProt and separated into secreted, intracellular and membrane-associated groups according to annotation labels.
- Immunogenicity Scores were calculated based on T cell epitope content predicted by the EpiMatrix system.
- For mAbs, VH & VL sequences were screened for putative effector T cell epitopes using EpiMatrix.
- Potential Tregitopes, including highly conserved T cell epitopes derived from IgG activate regulatory T cells and promote tolerance induction to associated antigens, were identified in the mAb sequences.
- We calculated the EpiMatrix Score (a summary of all T cell epitope content), Tregitope content (a summary of all Tregitope content), and Tregitope-adjusted EpiMatrix Score (EpiMatrix Score excluding Tregitope content) of the combined light and heavy chains.
- Regression models using EpiMatrix Score, Tregitope content and Tregitope-adjusted EpiMatrix Score to predict ADA response were evaluated.
- Correlation and fit were assessed by Pearson correlation coefficients and root mean square error (RMSE).

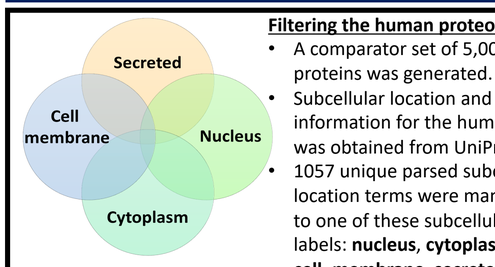
Results

- Secreted human proteins contain fewer putative T cell epitopes per unit length than a comparator set of random proteins or the complete human proteome.
- Membrane-associated proteins contain the highest density of predicted T cell epitopes.
- The EpiMatrix Scores of immunogenic antibodies are significantly higher than those of antibodies with limited observed immunogenicity in the clinic.
- Compared to EpiMatrix Score and Tregitope content, the Tregitope-adjusted EpiMatrix Score had the highest correlation with observed ADA (Pearson correlation coefficient=0.69, p-value<0.001) and the lowest RMSE (6.94).
- An exponential model using Tregitope-adjusted EpiMatrix Score was the best predictor of ADA.

Conclusions

- Our new model for prediction of antibody immunogenicity with almost double the number of mAbs is capable of relating antibody epitope content to observed immunogenicity with a high degree of correlation, consistent with the published model.
- New candidate Tregitopes are now undergoing experimental validation in our laboratory.
- Deimmunization, humanization and other approaches to tolerizing mAb therapeutics in our interactive in silico screening and optimization platform (ISPRI) enable drug developers to move biologic candidates towards the clinic swiftly and with reduced risk.

Methods



Filtering the human proteome

- A comparator set of 5,000 random proteins was generated.
- Subcellular location and signal peptide information for the human proteome was obtained from UniProt.
- 1057 unique parsed subcellular location terms were manually assigned to one of these subcellular location labels: **nucleus**, **cytoplasm**, **cell_membrane**, **secreted**.
- Additional filters were applied to construct unique groups **multi-pass cell membrane, cytoplasm membrane-associated,** and **intracellular.**

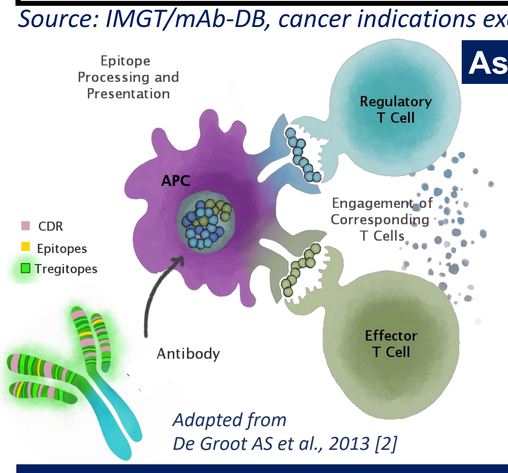
Human proteome data set

Intracellular	1,469	Cytoplasm	2,521
Secreted	1,328	Nucleus	2,979
Multi-pass cell membrane	1,193	Cytoplasm membrane-associated	1,811
Human proteome	20,401	Random	5,000

Monoclonal antibody data set

Antibody (Target)			
1 Prolia (RANKL)	12 Zinbryta (IL-2Rα)	23 Fasenra (IL-5Rα)	34 Bococizumab (PCSK9)
2 Cosentyx (IL-17A)	13 Zenapax (IL-2Rα)	24 Takhzyro (KLKB1)	35 Kevzara (IL-6R)
3 Emgality (CGRP-L)	14 Zinplava (Cd toxin B)	25 Trogarzo (CD4)	36 Dupixent (IL-4Rα)
4 Benlysta (BLyS)	15 Ilumya (IL-23 p19)	26 Taltz (IL-17A)	37 Humira (TNF α)
5 Raptiva (CD11a)	16 Repatha (PCSK9)	27 Synagis (RSV F)	38 Stelara (IL-12/23 p40)
6 Praluent (PCSK9)	17 Visilizumab (CD3E)	28 Simulect (IL-2Rα)	39 Remicade (TNF α)
7 Ajovy (CGRP-L)	18 Ocrevus (CD20)	29 Tremfya (IL-23 p19)	40 Cinqair (IL-5)
8 Siliq (IL-17RA)	19 Nucala (IL-5)	30 Ilaris (IL-1β)	41 Rituxan (CD20)
9 Soliris (C5)	20 Leukarrest (CD11/18)	31 Xolair (IGHE)	42 Lemtrada (CD52)
10 Simponi (TNF α)	21 Aimovig (CGRP-R)	32 Tysabri (ITGα4)	43 Campath (CD52)
11 Crysvita (FGF23)	22 Entyvio (ITGα4β7)	33 Humicade (TNF α)	✪ Novel sequence

Source: IMGT/mAb-DB, cancer indications excluded, immunogenicity data from package inserts and/or literature



Adapted from De Groot AS et al., 2013 [2]

Assessment of T cell epitope and Tregitope Content

VH and VL chain sequences of each mAb were screened for putative effector T cell epitopes using EpiMatrix [1] to calculate:

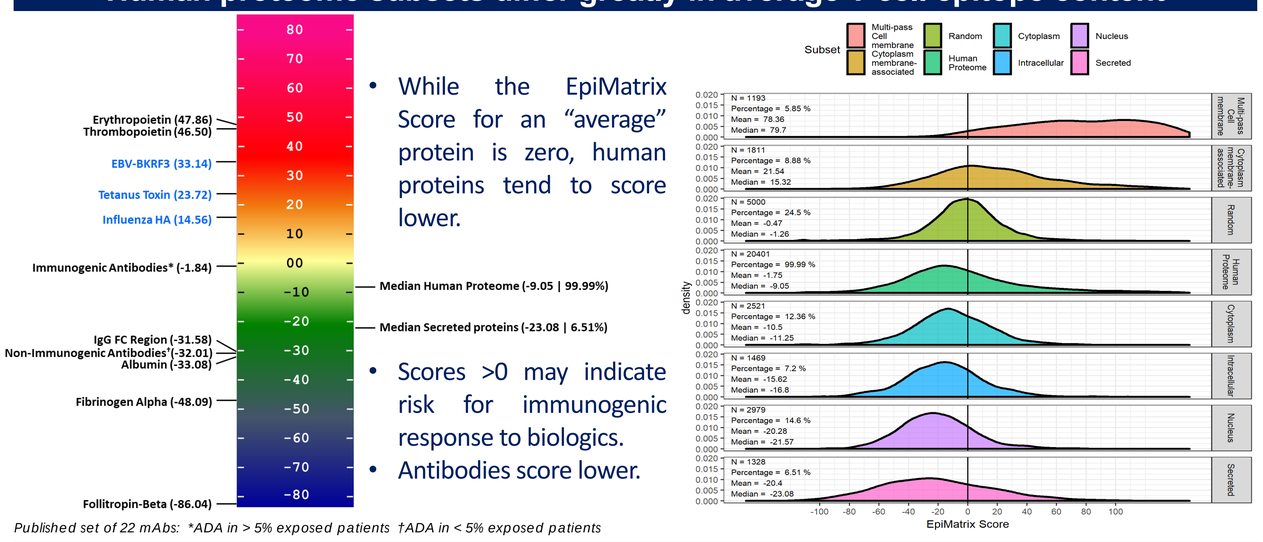
(1) T cell epitope content
9 globally relevant supertype HLA-DR alleles

(1) Regulatory T cell epitope (Tregitope) content
Highly conserved T cell epitopes derived from IgG, activate regulatory T cells and promote tolerance induction to associated antigens

(3) Tregitope-adjusted EpiMatrix Score
Excludes putative HLA ligands from Tregitopes

Results

Human proteome subsets differ greatly in average T cell epitope content



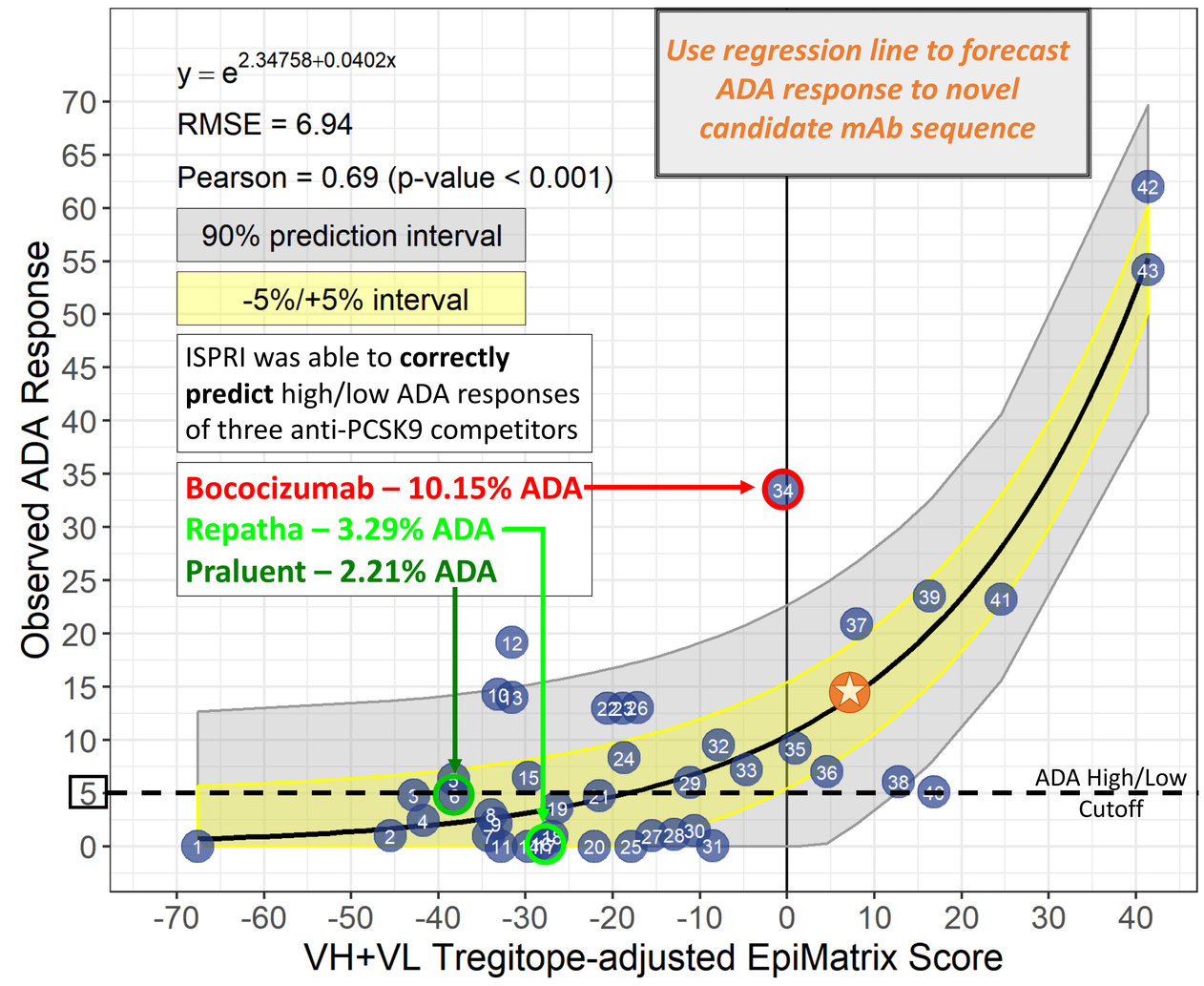
- While the EpiMatrix Score for an "average" protein is zero, human proteins tend to score lower.
- Scores >0 may indicate risk for immunogenic response to biologics.
- Antibodies score lower.

*Published set of 22 mAbs: *ADA in > 5% exposed patients †ADA in < 5% exposed patients*

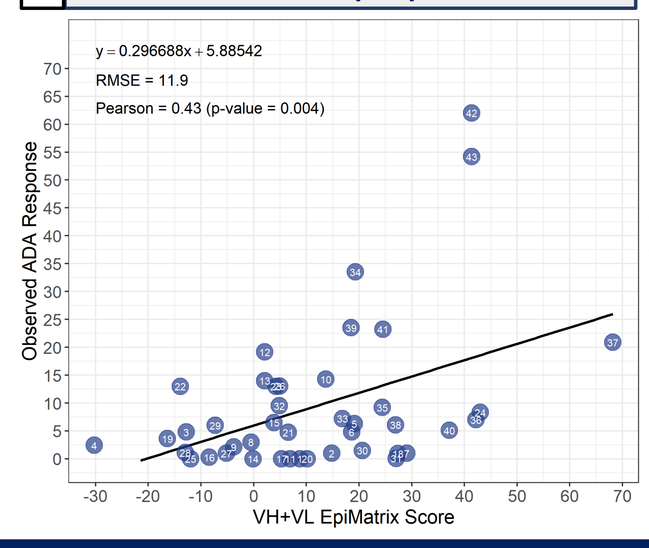
Tregitope-adjusted EpiMatrix Score is the best predictor of Antibody Immunogenicity

- EpiMatrix Score, Tregitope content and Tregitope-adjusted EpiMatrix Score were evaluated as individual predictors of antibody immunogenicity using univariate linear, polynomial, and exponential regression models.
- The fit of the model was assessed using root mean square error (RMSE).
- ADA predicted using the **Tregitope-adjusted EpiMatrix Score** with a set of novel candidate Tregitopes (Fig. A) was **closer** to observed ADA compared to EpiMatrix Score (Fig. B) or Tregitope content (Fig. C) alone.

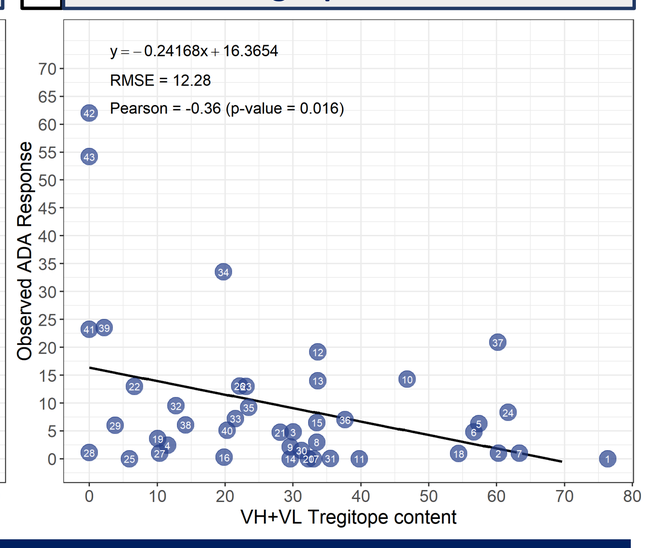
A **T cell effector epitope content excluding Tregitopes**



B **T cell effector epitope content**



C **Tregitope content**



Conclusions

- Secreted human proteins generally contain fewer putative T cell epitopes per unit length than a comparator set of random proteins or the complete human proteome. By contrast, membrane-associated proteins contain the highest density of predicted T cell epitopes.
- The mAb immunogenicity model based on Tregitope-adjusted EpiMatrix Score is the best predictor of anti-therapeutic response (Figures A-C).
- The Tregitope-adjusted EpiMatrix Score is significantly correlated with Observed ADA Responses (Figure A).
- The new model is based on twice as many mAbs as the original [1] and uses one more HLA-DR allele to predict T cell epitopes.
- Once the novel Tregitopes are validated experimentally in our laboratory, this new model will be incorporated into our in silico screening and optimization platform **(ISPRI)** to facilitate the development of mAbs with reduced risk of immunogenicity.

References

[1] De Groot AS, Martin W. Reducing risk, improving outcomes: bioengineering less immunogenic protein therapeutics. Clin Immunol. 2009;131(2):189-201.

[2] De Groot AS, Terry F, Cousens L, Martin W. Beyond Humanization and De-immunization: Tolerization as a Method for Reducing the Immunogenicity of Biologics. Exp Rev Clin Pharm. 2013;6(6):651-52.

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

www.epivax.com