In-silico prediction of HLA-DP and -DQ epitope content is poorly correlated with clinical immunogenicity of therapeutic proteins.

A.S. De Groot[1,2], R. Tassone[1], L. P. Cousens[1], F. Terry[1], R. Martin[1], M.T. Ardito[1], W.D. Martin[1]

[1]EpiVax, Inc., Providence, RI USA, [2]University of Rhode Island, Providence, RI USA,

Abstract

Purpose: Protein drug developers and immunogenicity screeners generally agree that HLA class II-restricted T helper cells can drive anti-drug antibody (ADA) responses to biologic therapeutics. The link between ADA and T cell responses has contributed to the proliferation of epitope prediction tools used by biologics developers. However, a consensus has yet to be reached regarding the set of HLA alleles most relevant for the prediction of clinical immunogenicity. The purpose of this commentary is to extend our previous analysis of the association between in silico predictions of HLA-DR epitope content and observed immunogenicity[1] by examining the contribution of HLA-DP and -DQ T cell epitope content to immunogenicity predictions.

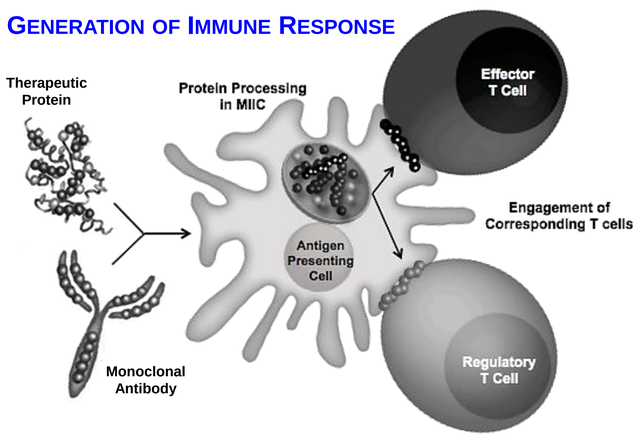
Methods: To expand on our previous analysis, we employed HLA-DR, -DP and -DQ epitope prediction tools (available on the IEDB website) to measure the T cell epitope content of 20 licensed monoclonal antibodies (mAbs). Using linear regression analysis, "hit" counts were then correlated with the percentages of treated patients who produced anti-drug antibodies.

Results: Consistent with our previous findings, HLA-DR epitope content, as predicted using the IEDB Consensus model, did not predict observed immunogenicity. In order to establish a significant correlation it was necessary to adjust the IEDB Consensus model for the presence of regulatory T cell epitopes as predicted by our ISPRI immunogenicity screening system. Further, we found that HLA-DP and -DQ allele epitope content, as assessed by these available tools, did not correlate with published immunogenicity, nor did the inclusion of predicted HLA-DP and -DQ epitope content improve the accuracy of HLA-DR-based immunogenicity predictions. Finally, one model of HLA-DP restricted content was significantly negatively correlated with immunogenicity. This observation deserves further exploration.

Conclusions: Based on these findings, we suggest that, at least until available DP/DQ models can be correlated with observed immunogenicity, estimates of protein immunogenicity should be based on HLA-DR rather than DP/DQ epitope content. Furthermore, we underscore the importance of adjusting for regulatory T cell epitope content in the prediction of immunogenicity.

Background

Generation of Immune Response



Protein Therapeutic

epitope | epitope | epitope

1 + 1 + 1 = Response

T cell response depends on:

T cell epitope content + HLA of subject

Protein immunogenic potential can be ranked

Monoclonal Antibody

epitope | epitope | epitope

1 + 1 - regulatory T cell epitope = Response

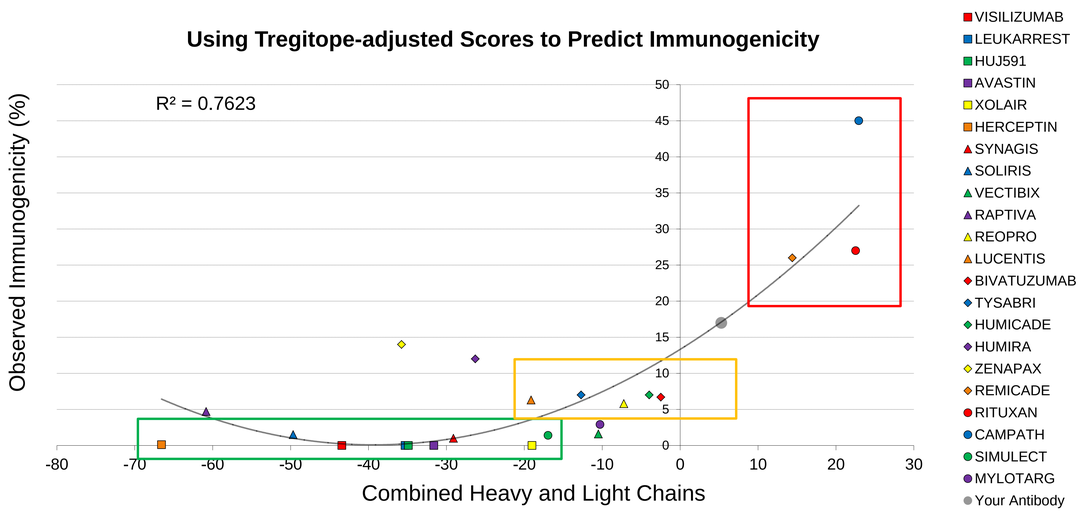
T cell response depends on:

T cell epitope content + Tregitope content + HLA of subject

Antibodies must be ranked differently

- Therapeutic proteins including monoclonal antibodies are processed through APC.
- T cell epitopes derived from these proteins are presented to T cells in an HLA-specific context.
- We have developed algorithms to score and rank proteins for immunogenic potential based on T cell epitope content.[2]

Correlation of EpiMatrix Scores with Clinical Immunogenicity



Protein T cell epitope content predicts immunogenic potential.

- Anti therapeutic response rates for 20 licensed mAbs were collected from literature/FDA package inserts.
- Protein sequences were collected from patents/public databases and parsed into overlapping 9-mer frames.
- EpiMatrix assesses binding potential of each 9-mer frame to 8 "Supertype" Class II HLA-DR alleles.
- EpiMatrix Protein Immunogenicity Score reflects aggregate T cell epitope content (all scores are adjusted for the presence of Tregitopes).
- These scores were highly correlated to observed immunogenicity using a polynomial regression (R^2=0.76).
- Without adjusting for Tregitope content, monoclonal antibody immunogenicity and raw EpiMatrix Protein Immunogenicity Scores are not well correlated (R^2=0.17).

Methods and Results

Calculation of HLA-DP/-DQ Epitope Content

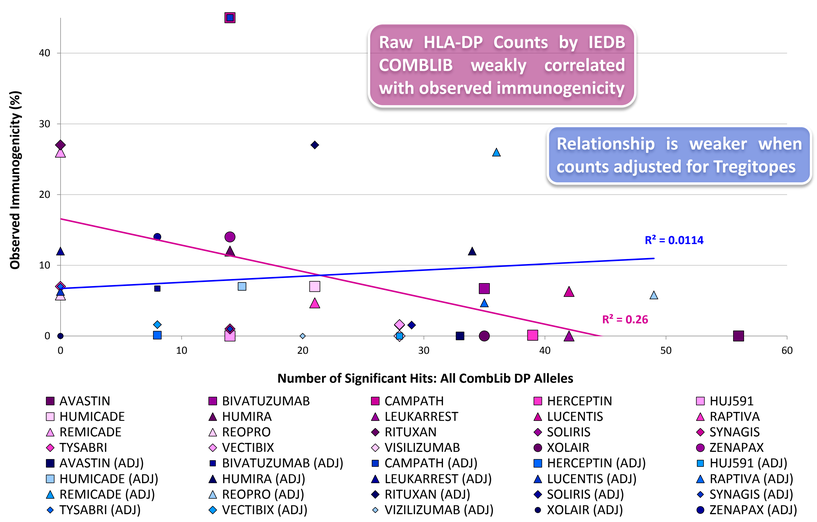
- T cell epitopes restricted to HLA-DP or HLA-DQ alleles were predicted using multiple algorithms available at the IEDB resource.[3]
- IEDB-based epitope prediction was also completed where algorithms were available for the HLA-DR supertype alleles.
- Counts of epitopes ranked in the top 5% of predicted binders for each mAb were correlated with observed immunogenicity either with (Adjusted) or without (Raw) adjusting for the presence of Tregitopes.

Correlation of Epitope Counts with Clinical Immunogenicity

HLA allele	Predictive Model: EpiMatrix Raw	EpiMatrix Adjusted	Consensus Raw	Consensus Adjusted	CombLib Raw	CombLib Adjusted	SSM Raw	SSM Adjusted	NN Raw	NN Adjusted
DRB1*0101	0.28	**0.56**	-0.16	0.09	-0.16	-0.16	-0.26	0.01	0.07	0.32
DRB1*0301	0.37	**0.52**	**0.57**	**0.64**	-	-	0.02	0.31	-0.22	-0.20
DRB1*0401	**0.51**	**0.68**	0.03	**0.45**	-	-	0.20	**0.47**	0.05	0.29
DRB1*0701	0.22	**0.5**	0.21	0.21	0.20	0.20	0.25	**0.60**	0.23	0.23
DRB1*0802	0.31	**0.48**	0.11	0.28	-	-	0.36	**0.49**	-0.23	-0.10
DRB1*1101	**0.58**	**0.76**	0.30	**0.59**	-	-	**0.47**	**0.77**	0.18	0.18
DRB1*1302	0.12	**0.52**	0.37	0.37	-	-	0.18	0.18	**0.55**	0.52
DRB1*1501	0.05	0.43	-0.17	0.00	-	-	-0.13	-0.03	-0.15	-0.09
DPA1+01 DPB1+0401	-	-	0.03	0.19	-0.43	0.21	-0.25	0.02	0.22	0.30
DPA1+0103 DPB1+0201	-	-	0.23	0.39	-0.28	-0.02	-0.36	0.17	0.3	0.32
DPA1+0201 DPB1+0101	-	-	-0.09	0.05	-0.30	-0.08	-0.03	**0.59**	0.04	0.13
DPA1+0201 DPB1+0501	-	-	0.33	0.57	-0.36	-0.04	0.13	0.41	0.33	0.35
DPA1+0301 DPB1+0402	-	-	-0.17	0.29	-0.34	0.27	-0.01	0.38	0	0.28
DQA1+0101 DQB1+0501	-	-	-0.22	0.26	-0.23	-0.05	-0.22	0.34	-0.09	0.34
DQA1+0102 DQB1+0602	-	-	0.07	-0.25	-0.26	0.31	0.13	-0.27	0.2	-0.09
DQA1+0301 DQB1+0302	-	-	0.33	-0.02	**0.63**	0.07	0.12	-0.19	0.2	-0.15
DQA1+0401 DQB1+0402	-	-	-0.15	0.11	0.16	0.25	-0.2	-0.17	-0.31	-0.06
DQA1+0501 DQB1+0201	-	-	**-0.51**	-0.08	-0.27	0.05	**-0.53**	-0.16	-0.06	-0.16
DQA1+0501 DQB1+0301	-	-	0.22	-0.15	-0.33	0.04	0	0.00	0.14	-0.23
All DR	**0.45**	**0.73**	0.36	**0.76**	0.03	0.03	0.32	**0.69**	0.07	0.33
All DP	-	-	0.05	0.41	**-0.51**	0.13	-0.18	0.41	0.27	0.43
All DQ	-	-	-0.2	0.08	-0.13	0.19	-0.32	-0.04	0.03	-0.05
All DP/DQ	-	-	-0.07	0.35	**-0.45**	0.26	-0.3	0.31	0.21	0.30
All DR/DP/DQ	-	-	0.24	**0.67**	-0.02	-0.41	0.09	**0.54**	0.18	0.39

Bold underline indicates statistically significant (p<0.05) correlation / Positive correlations in blue, negative correlations in red / - indicates predictive model was not available

Tregitopes Confound HLA-DP Correlation with Immunogenicity



None of the models of HLA-DQ epitope content were associated with observed immunogenicity.

Only one of four models of HLA-DP epitope content was consistently associated with observed immunogenicity. Negative correlation may indicate that lack of DP content leads to immunogenicity. This relationship was nullified by adjusting for regulatory epitope content.

Conversely, raw HLA-DR epitope content was a positive predictor of immunogenicity in 25/32 (78%) of allelic model-to-ADA response assessments, and Tregitope adjustment enhanced this relationship.

Conclusions

- **HLA-DP/DQ predictive models available at IEDB are weakly or negatively correlated with observed immunogenicity of licensed mAbs and do not add any incremental predictive power over HLA-DR epitope prediction algorithms used alone.**
- **Tregitope-adjustment enhanced statistically significant positive correlations of EpiMatrix predictions with ADA overall and in all but one allele-specific model, and enhanced overall correlation of IEDB HLA-DR models.**
- **Adjusting for Tregitopes had mixed effects on correlation of HLA-DP/DQ epitope content, and negated the statistical significance of the only algorithm whose raw content was initially well-correlated.**
- **Until evidence of HLA-DP/DQ T cell epitope contribution to immunogenicity of therapeutic proteins can be conclusively demonstrated, in silico assessment should focus on HLA-DR alleles and include consideration of validated regulatory T cell epitopes such as Tregitopes.**

References / Acknowledgments

1) De Groot A.S. and Martin W. Reducing risk, improving outcomes: bioengineering less immunogenic protein therapeutics. Clin Immunol. 2009 May;131(2):189-201. doi: 10.1016/j.clim.2009.01.009. Epub 2009 Mar 6.
2) De Groot A.S. and L. Moise. Prediction of immunogenicity for therapeutic proteins: State of the art. Current Opinions in Drug Development and Discovery. May 2007. 10(3):332-40.
3) Wang P, Sidney J, Kim Y, Sette A, Lund O, Nielsen M, Peters B. Peptide binding predictions for HLA DR, DP and DQ molecules. BMC Bioinformatics. 2010; 11:568. Published online 2010 November 22. doi: 10.1186/1471-2105-11-568. PMCID: PMC2998531