Effector and Regulatory T Cell Epitope Content, and not Humanization nor Biophysical Properties, is Predictive of Antibody Immunogenicity

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Abstract

Monoclonal antibodies (mAbs) are 'humanized' to reduce their potential to drive the development of anti-drug antibodies (ADA) that may interfere with efficacy. Since T cell epitopes are key drivers and modulators of immunogenicity, EpiVax has developed immunoinformatics tools to discover T effector and regulatory T cell epitopes in mAb sequences and methods to determine the risk of ADA. Here, we evaluated 137 mAb sequences to determine whether Observed or Predicted ADA responses were associated with defined measures of "humanness" and biophysical properties that are commonly applied to assess antibody "developability".

We analyzed 137 mAbs whose key biophysical properties were recently published, and two subsets of the above: 19 mAbs with cancer indications (CmAbs), and 23 mAbs for which ADA responses were available (KmAbs). The latter two subsets had no overlap with each other. Each mAb sequence was analyzed for the presence of putative effector and regulatory T cell epitopes using the EpiMatrix system. We also calculated four humanization measures. The relationship between the humanization measures and the variables of predicted immunogenic potential, including observed ADA response, were assessed using Pearson correlation coefficients. The same type of assessment was performed to evaluate the relationship between 12 biophysical properties that had been published for the entire set of 137 antibodies, predicted immunogenic potential and observed ADA response.

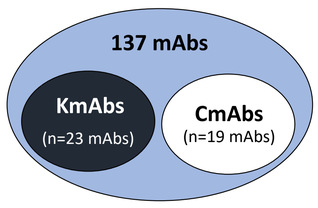
For the set of 23 mAbs with known immunogenicity (KmAbs), effector T cell epitope content adjusted by Tregitope content (the Tregitope-Adjusted EpiMatrix Score) and the corresponding EpiMatrix-predicted ADA response were significantly correlated with observed ADA response. None of the 12 biophysical properties nor the humanization measures were correlated with observed ADA response for the KmAbs set. We also did not find any significant correlation between biophysical properties and our ISPRI metrics for assessing immunogenic potential when exploring these relationships for the comprehensive dataset (137 mAbs), for the CmAbs subset, and for the KmAbs subset.

This analysis suggests that calculations based on the T effector and regulatory T cell epitope (Tregitope) content of monoclonal antibodies appear to be better correlated with clinical immunogenicity results than biophysical properties and standard humanization measures.

Methods

Analyzed Datasets

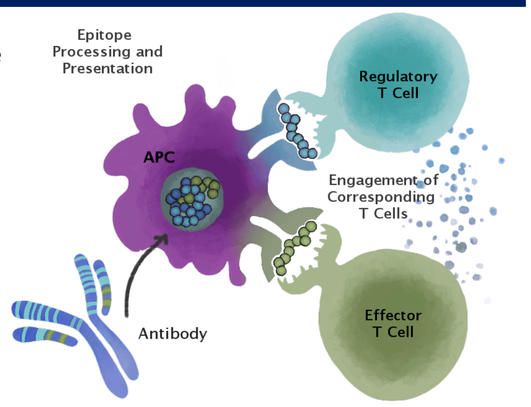
- **137 mAbs:** 137 mAbs whose key 12 biophysical properties were recently published (1).
- Two subsets (with no overlap):
  - **CmAbs:** 19 mAbs with **C**ancer indications (2).
  - **KmAbs:** 23 mAbs with **K**nown ADA responses.



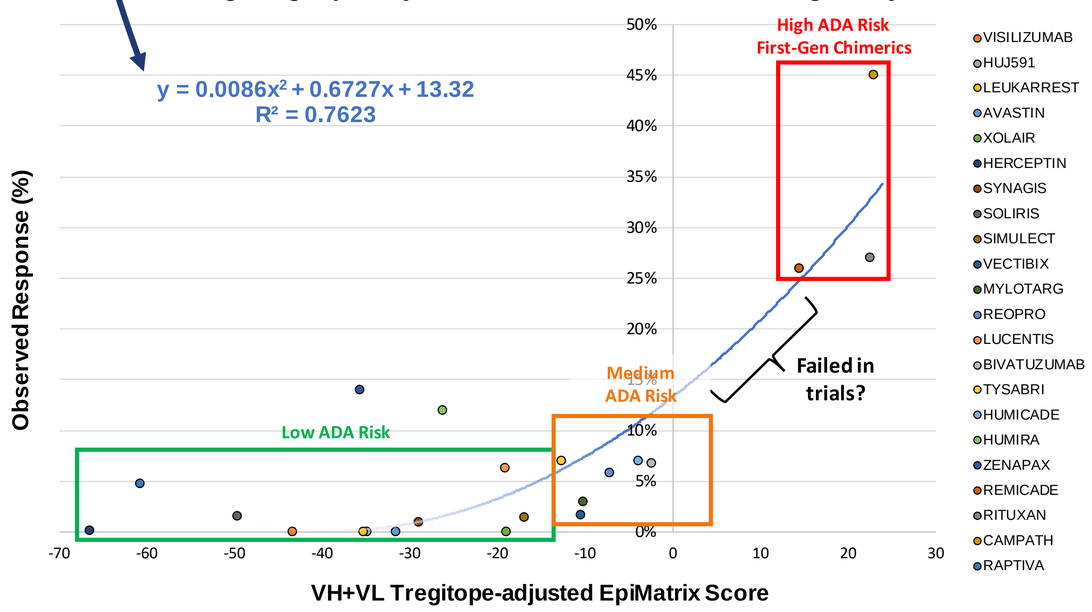
T cell epitope and Immunogenic Potential Assessments

Each mAb sequence was screened for the presence of putative effector and regulatory T cell epitopes using the EpiMatrix system (3).

To assess the immunogenic potential of each mAb, we calculated:
(1) T cell epitope content
(2) Regulatory T cell epitope (Tregitope) Content
(3) Tregitope-adjusted EpiMatrix score
(4) EpiMatrix predicted ADA response

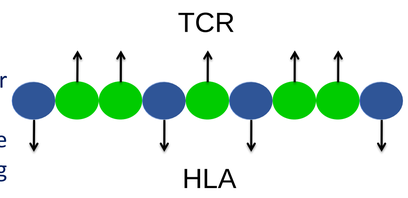


Using Tregitope-Adjusted Scores to Assess Immunogenicity



Humanization Assessment

To assess the humanization measures of each mAb, we calculated:
(1) Average number of human germline matches per constitutive 9-mer peptide.
(2) Average frequency of 9-mer peptide found in a database of human antibodies.
(3) Number of matches at >80% amino acid similarity in the human proteome per constitutive 9-mer peptide.
(4) Average number of potentially cross-conserved T cell epitope matches by virtue of conserved T cell receptor (TCR)-facing residues and compatible HLA-facing residues) per constitutive 9-mer peptide (see right).



Correlation between Humanization, Biophysical Properties, Predicted and Observed ADA

- The relationship between the humanization measures, predicted immunogenic variables and observed ADA response were assessed using Pearson correlation coefficients.
- Pearson correlation was also applied to evaluate the relationship between 12 biophysical properties and predicted and observed ADA response.
- Correlation were significant if P-values were below 0.05.

Results

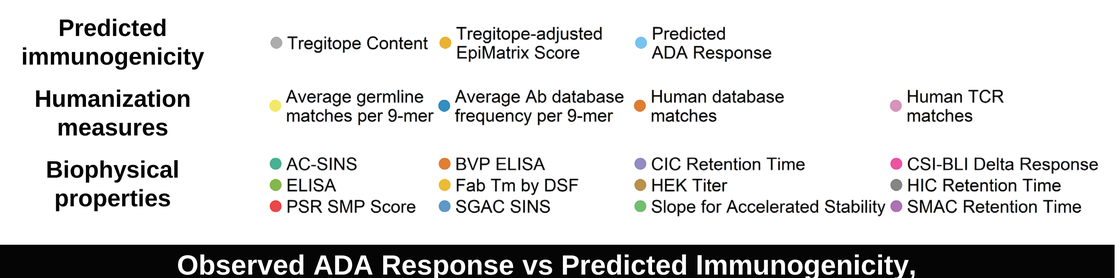
Correlations with Observed ADA Response for 23 KmAbs

*For the set of **23 mAbs with known immunogenicity (KmAbs)**:*

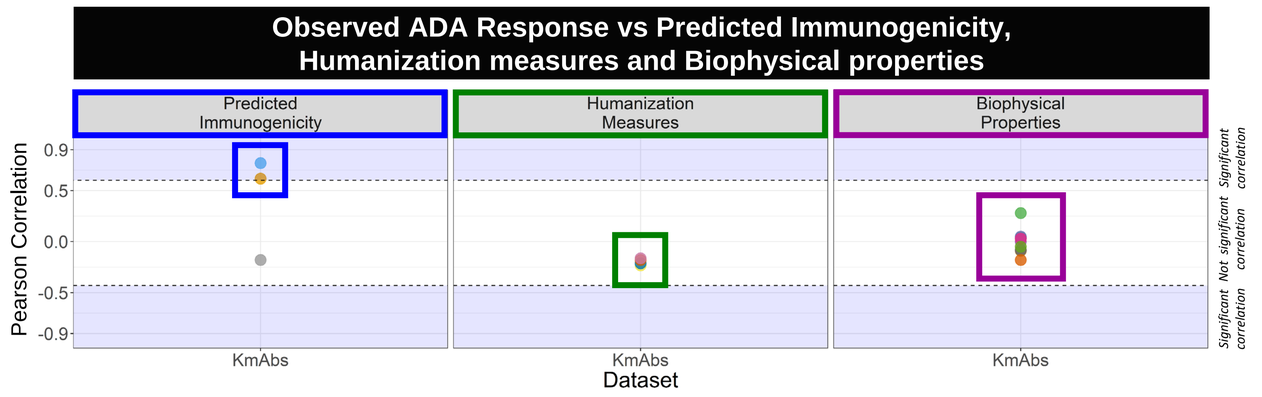
- Humanization measures (based on homology analysis) are not correlated with observed ADA response.

- Tregitope-adjusted EpiMatrix Score and predicted ADA response (based on our polynomial regression) are significantly correlated with observed ADA response.
  - **This suggests that EpiMatrix predictions of effector and regulatory epitope content accounts for a portion of the variability in clinical results that cannot be explained by homology to human sequences.**

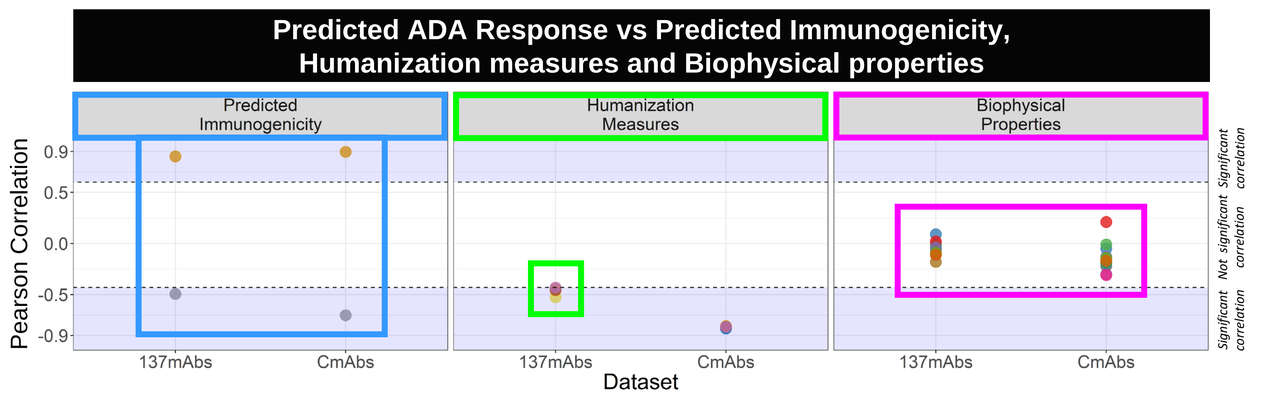
- We do not find any significant correlation between the 12 biophysical properties measured for mAbs and observed ADA response.
- The low correlation for biophysical properties and observed ADA response suggests lack of association between these variables.



Observed ADA Response vs Predicted Immunogenicity, Humanization measures and Biophysical properties



Predicted ADA Response vs Predicted Immunogenicity, Humanization measures and Biophysical properties



Correlations with Predicted ADA Response for 137 mAbs and 19 CmAbs

*For the complete dataset (**137 mAbs**) and the set of mAbs with Cancer indications (**CmAbs**):*

- As expected, Tregitope-adjusted EpiMatrix and Tregitope content are correlated with predicted ADA response. The negative correlation between predicted ADA response and Tregitope Content indicates that in our model, the presence of Tregitopes decreases ADA responses.

- For the 137 mAbs and compared to the other metrics of predicted immunogenicity, predicted ADA response has the weakest correlation with the humanization measures (**-0.46**). Based on this result, **we conclude that our prediction of ADA response take into consideration more than just sequence homology to human sequences.**

- We do not find any significant correlation between the 12 biophysical properties measured for mAbs and predicted ADA response.

Conclusions

- Neither the humanization measures nor the 12 biophysical properties measured for mAbs were correlated with observed ADA response.
- Tregitope-adjusted EpiMatrix Score and predicted ADA response were significantly correlated with observed ADA response.
- For this analysis, Tregitope-adjusted EpiMatrix Score was a better predictor of antibody immunogenicity than either humanness or biophysical properties.
- Our *in silico* predictions of effector and regulatory epitope content account for a portion of the variability in clinical immunogenicity results that cannot be explained by biophysical properties, sequence homology with human germlines, antibodies or proteome alone.

References

1) Jain T, Sun T, Durand S, Hall A, Houston NR, Nett JH, Sharkey B, Bobrowicz B, Caffry I, Yub Y, Cao Y, Lynaugh H, Brown M, Baruah H, Gray LT, Krauland EM, Xu Y, Vásquez M, Wittrup KD. Biophysical properties of the clinical-stage antibody landscape. PNAS 2017:114(5):994-949.
2) Almagro JC, Daniels-Wells TR, Perez-Tapia SM, Penichet ML. Progress and Challenges in the Design and Clinical Development of Antibodies for Cancer Therapy. Front. Immunol. 2018;8:1751.
3) De Groot AS, Martin W. Reducing risk, improving outcomes: bioengineering less immunogenic protein therapeutics. Clin Immunol. 2009;131(2):189-201.

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