In Silico High Throughput Pre-clinical Determination of Monoclonal Antibody Immunogenicity

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

[1]EpiVax, Inc., Providence, RI, USA

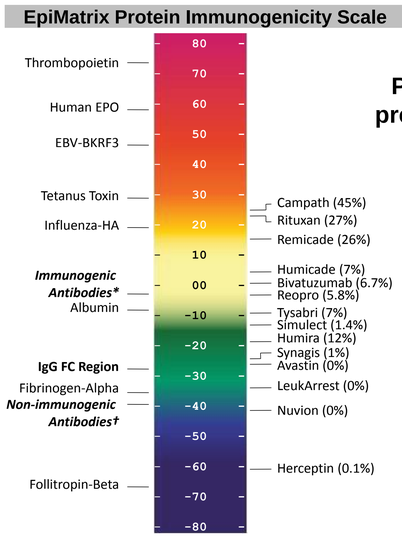
[2]Institute for Immunology and Informatics, University of Rhode Island, Providence, RI, USA

Abstract

One of the great surprises of the biologics revolution has been the discovery that recombinant human proteins, including humanized and fully-human monoclonal antibodies (MAb), can be immunogenic when administered to immune-competent subjects. Preclinical and clinical evaluations of the immunogenic potential for biologic drugs primarily focus on humoral immune responses; as a result, the critical contribution of T cells to the development of anti-drug antibodies (ADA) has been somewhat overlooked. Using the EpiMatrix T cell epitope mapping system, we have developed an interactive in silico screening and optimization platform (ISPRI) that evaluates the overall immunogenic potential of a biologic as well as identifies individual T cell epitope clusters contributing to its immunogenicity. In contrast to other immunogenicity prediction tools, our platform considers the contribution of regulatory T cell epitopes (Tregitopes) to immunogenic potential. Tregitopes are highly conserved T cell epitopes derived from IgG that we and others have shown activate regulatory T cells and promote tolerance induction to associated antigens. Here we demonstrate the correlation of available clinical immunogenicity data with Tregitope-adjusted immunogenicity scoring for twenty approved MAbs. Further, we present a high-throughput platform from which these scores can be used to triage large pools of candidate MAbs during the discovery phase of antibody development.

Background

EpiMatrix Protein Immunogenicity Scale

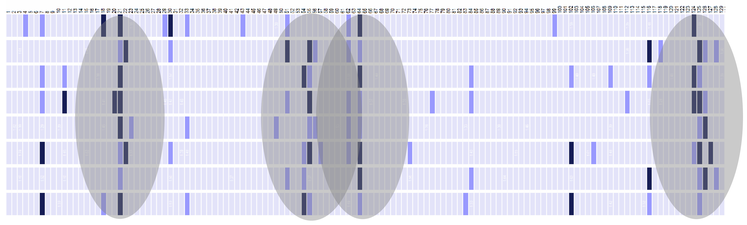


All scores normalized for protein length
For antibodies at right:
(% = exposed patients who developed ADA)

Protein T cell epitope content predicts immunogenic potential.

- Protein sequences parsed into overlapping 9-mer frames.
- **EpiMatrix** assessment: binding potential to 8 "Supertype" Class II HLA alleles.
- EpiMatrix Protein Immunogenicity Score reflects aggregate T cell epitope content.
- Protein Scores >20 = potentially immunogenic.
- Antibodies tend to score low due to presence of Tregitopes.

T cell epitopes tend to cluster regionally.



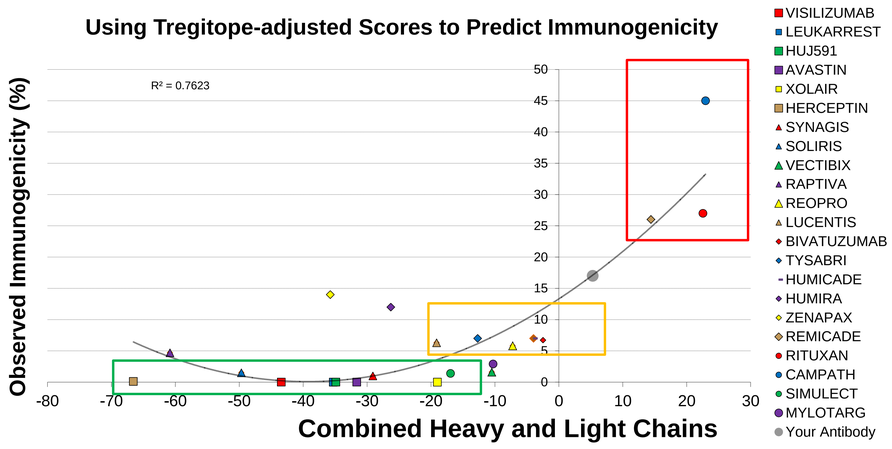
- We can identify regions of unusually high T cell epitope density (promiscuous HLA-ligands) contained within input proteins.
- In classical antigens, these T cell epitope clusters are potent drivers of immune response, even in otherwise low-scoring proteins.
- Effector CD4 T helper cells responding to these clusters can proliferate and stimulate B cells to produce anti-drug antibodies.

Not all T cell epitope clusters are danger signals.

- In human antibodies, we have identified several highly conserved, highly promiscuous T cell epitope clusters which we call Tregitopes.
- For antibodies, regulatory T cell epitopes (Tregitopes) relate to natural regulatory T cells and help to dampen the immune potential of the antibody sequence.
- We and others have demonstrated this phenomenon in the laboratory.[1]
- When calculating Protein Immunogenicity Scores based on aggregate T cell epitope content, we exclude known Tregitopes and report a Tregitope-adjusted EpiMatrix Score.

Methods

Clinical Immunogenicity



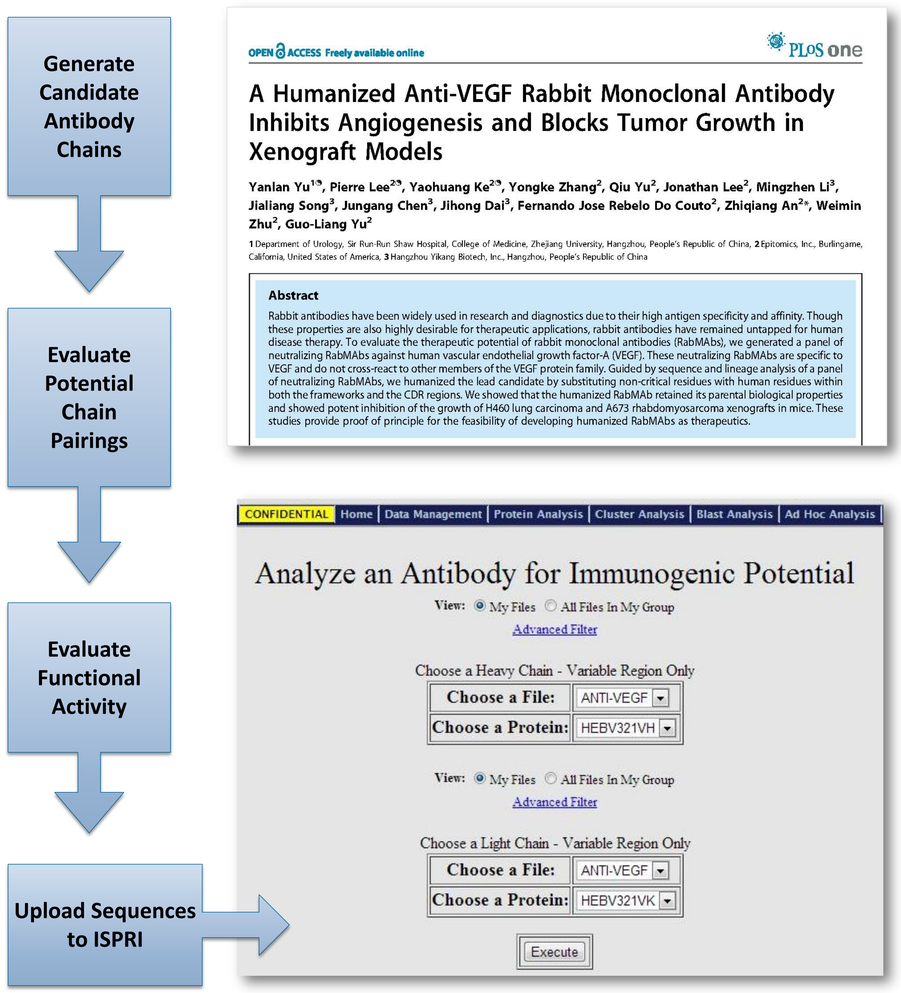
Anti-therapeutic response figures from published literature and FDA package inserts were collected in order to model observed immunogenicity of 20 licensed monoclonal antibodies as a function of Tregitope-adjusted EpiMatrix scores.[2] The Tregitope-adjusted scores of licensed antibodies are well correlated with observed anti-therapeutic response[3].

These data were related to observed immunogenicity using a polynomial regression with a resulting correlation (R^2) value of 0.76. This represents a significant improvement over modeling immune response to antibodies based on T cell epitope content alone. Without adjusting for Tregitope content, monoclonal antibody immunogenicity and raw EpiMatrix Protein Immunogenicity Scores are not well correlated (R^2=0.17).

The Antibody Polynomial Regression is designed to identify high-risk sequences based on the balance of effector and regulatory T cell epitope content contained therein. This new analysis method may be used to prospectively evaluate clinical immunogenicity of antibodies – based on amino acid sequence alone – prior to testing in clinical trials.

Online Immunogenicity Prediction

We have integrated our antibody screening tools into our online Interactive Screening and Protein Re-engineering Interface (ISPRI). Here we will use a Rabbit monoconal antibody (RabMAb) example from the literature to demonstrate the tool's utility.



Results

In evaluating the immunogenic potential of antibody sequences, we find it useful to characterize those antibodies according to two separate criteria: namely Tregitope content and effector, or neo-epitope, content.

Optimal: Low neo-epitope, high Tregitope content

- Optimal candidates such as Herceptin can be prioritized, as they are least likely to be immunogenic.

Low risk: Low neo-epitope, low Tregitope content

- Antibodies in this category such as Tysabri are frequently non-immunogenic and produce anti-therapeutic antibody response rates between 5% and 10%.

Mixed: High neo-epitope, high Tregitope content

- One licensed example – Campath – in this category; difficult to characterize.
- These risky candidates can be avoided.
- In some cases, immunogenic potential of neo-epitopes contained in humanized antibodies may be effectively controlled in the presence of significant numbers of Tregitopes.
- In general, we expect antibodies in this category to be more immunogenic than antibodies with fewer neo-epitopes and/or more Tregitopes .

High risk: High neo-epitope content. low Tregitope content

- Most immunogenic, with immunogenicity rates frequently exceeding 10% .
- Chimeric antibodies such as Rituximab tend to fall into this category.

Additional factors not analyzed here, such as dose, route, purity, aggregation, mechanism of synthesis (i.e. mammalian vs. bacterial expression), and target can also affect observed anti-therapeutic antibody response.

RabMAb chain pairs ranked by immunogenic potential.

Humanized Version →

Optimal Antibodies (Low Effector Content - High Tregitope Content)				
Antibody	Tregitope-Adjusted EpiMatrix Protein Score[1]	Tregitope Content[2]	Predicted Ab Response (%)	Observed Ab Response (%)
*** **Herceptin** ***	-66.56	75.27	0.00	0.10
HEBV321_VH - HEBV321_VK	-39.74	58.45	0.17	n.a.
HEBV321_VH - VKI-A20_VK	-39.21	66.94	0.17	n.a.
EBV320_VH - HEBV321_VK	-33.34	34.55	0.45	n.a.
EBV307_VH - HEBV321_VK	-32.51	34.55	0.54	n.a.
EBV320_VH - VKI-A20_VK	-32.26	41.05	0.57	n.a.
EBV302_VH - HEBV321_VK	-32.18	34.55	0.58	n.a.
*** **Avastin** ***	-31.61	67.57	0.65	0.00
EBV307_VH - VKI-A20_VK	-31.37	41.05	0.68	n.a.
EBV302_VH - VKI-A20_VK	-31.01	41.05	0.73	n.a.
HEBV321_VH - EBV321_VK	-29.85	32.97	0.90	n.a.
HEBV321_VH - EBV320_VK	-29.19	32.82	1.01	n.a.
HEBV321_VH - EBV302_VK	-29.19	33.12	1.01	n.a.
EBV321_VH - HEBV321_VK	-23.53	34.55	2.25	n.a.
EBV321_VH - VKI-A20_VK	-21.62	41.05	2.80	n.a.
VH3-21_VH - HEBV321_VK	-20.74	75.86	3.07	n.a.
HEBV321_VH - EBV307_VK	-20.40	33.28	3.18	n.a.
VH3-21_VH - VKI-A20_VK	-18.54	86.14	3.81	n.a.
VH3-21_VH - EBV321_VK	-10.66	49.58	7.12	n.a.
VH3-21_VH - EBV320_VK	-10.07	49.34	7.42	n.a.
VH3-21_VH - EBV302_VK	-9.89	49.81	7.51	n.a.
VH3-21_VH - EBV307_VK	-0.76	50.05	12.81	n.a.
Low Risk Antibodies (Low Effector Content - Low Tregitope Content)				
Antibody	Tregitope-Adjusted EpiMatrix Protein Score[1]	Tregitope Content[2]	Predicted Ab Response (%)	Observed Ab Response (%)
EBV320_VH - EBV321_VK	-23.44	9.07	2.28	n.a.
EBV320_VH - EBV320_VK	-22.80	9.02	2.45	n.a.
EBV320_VH - EBV302_VK	-22.74	9.11	2.47	n.a.
EBV307_VH - EBV321_VK	-22.61	9.07	2.51	n.a.
EBV302_VH - EBV321_VK	-22.28	9.07	2.60	n.a.
EBV307_VH - EBV320_VK	-21.98	9.02	2.69	n.a.
EBV307_VH - EBV302_VK	-21.91	9.11	2.71	n.a.
EBV302_VH - EBV320_VK	-21.65	9.02	2.79	n.a.
EBV302_VH - EBV302_VK	-21.58	9.11	2.81	n.a.
EBV320_VH - EBV307_VK	-13.88	9.15	5.64	n.a.
EBV321_VH - EBV321_VK	-13.67	9.07	5.73	n.a.
EBV321_VH - EBV320_VK	-13.08	9.02	5.99	n.a.
EBV307_VH - EBV307_VK	-13.05	9.15	6.01	n.a.
EBV321_VH - EBV302_VK	-12.93	9.11	6.06	n.a.
EBV302_VH - EBV307_VK	-12.72	9.15	6.16	n.a.
*** **Tysabri** ***	-12.71	15.70	6.16	7.00
EBV321_VH - EBV307_VK	-4.02	9.15	10.75	n.a.
*** **Humicade** ***	-3.97	17.29	10.78	7.00
Mixed Antibodies (High Effector Content - High Tregitope Content)				
Antibody	Tregitope-Adjusted EpiMatrix Protein Score[1]	Tregitope Content[2]	Predicted Ab Response (%)	Observed Ab Response (%)
*** **Campath** ***	22.94	21.72	33.28	45.00
High Risk Antibodies (High Effector Content - Low Tregitope Content)				
Antibody	Tregitope-Adjusted EpiMatrix Protein Score[1]	Tregitope Content[2]	Predicted Ab Response (%)	Observed Ab Response (%)
*** **Remicade** ***	14.40	2.48	24.79	26.00
*** **Rituxan** ***	22.53	0.00	32.84	27.00

[1]Tregitope-adjusted EpiMatrix Score: Values greater than zero represent high effector epitope content.
[2]Tregitope content: Values greater than +20 represent high Tregitope content.

Conclusions / Future Directions

Epitope discovery technology and related *in silico* immunogenicity screening tools are rapidly becoming invaluable components of the biologic product pipeline. We have developed an interactive tool capable of relating antibody epitope content to observed immunogenicity with a high degree of correlation. As shown in the RabMab example, results from this tool support deimmunization, humanization and other approaches to tolerizing monoclonal antibody therapeutics. This application will allow drug developers to move biologic candidates towards the clinic with improved perspective and reduced risk.

References / Acknowledgements

[1] Cousens LP, Tassone R, Mazer BD, Ramachandiran V, Scott DW, De Groot AS. Tregitope update: mechanism of action parallels IVIg. Autoimmun Rev. 2013 Jan;12(3):436-43.

[2] Van Walle I et. al. Immunogenicity screening in protein drug development. Expert Opin Biol Ther. 2007 Mar;7(3):405-18.

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