The ISPRI Toolkit Rapidly Screens Biologic Product Candidates for Immunogenic Potential Driven by T cell Epitope Content and Foreignness

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ABSTRACT

Purpose: We have developed an interactive in silico screening and optimization platform called ISPRI which predicts the overall immunogenicity of a biologic as well as identifies individual T cell epitope clusters contributing to its potential to drive anti-drug antibody (ADA) response. Recent updates to the ISPRI system allow for the analysis of T cell epitopes derived from immunogenic (>5% ADA) and non-immunogenic (<5% ADA) licensed products. We demonstrate that inflammatory sequences carry elevated putative T cell epitope content and that these sequences share limited homology with human antibody isolates, germline sequences and other self proteins.

Methods: HLA-DR-restricted T cell epitopes from each monoclonal antibody in our dataset were mapped using the EpiMatrix algorithm, and each predicted HLA ligand was screened against our curated database of human antibody isolates and germline sequences. 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. Using the JanusMatrix algorithm, we also evaluated each T cell epitope cluster for conservation with human self proteins at both the HLA-binding and TCR-interacting face of each peptide ligand.

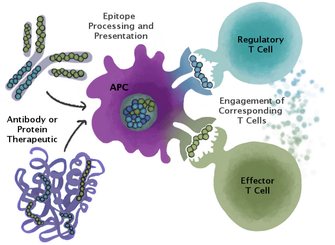
Results: In comparison with non-immunogenic products, the immunogenic products studied here were shown to contain: higher numbers of putative T cell epitopes (p=0.047), lower numbers of Tregitopes (p=0.035), lower representation of putative epitopes in human germline sequences (p=0.0075), lower representation of putative epitopes in human antibody isolates (p=0.0001), and lower representation in the wider human genome (p=0.0003).

Conclusions: We have developed an interactive tool capable of relating antibody T cell epitope content to observed immunogenicity with a high degree of correlation. It is becoming evident that T cell epitope content alone is not sufficient to predict immunogenicity of biologics; the phenotype of the epitopes discovered is also critically important for accurate predictions. High-throughput capacity enabled by this cloud-based system will be essential to future biologic pipeline grooming. Results from this tool support deimmunization, humanization and other approaches to tolerizing monoclonal antibody therapeutics. This application will also allow drug developers to move biologic candidates towards the clinic with improved perspective and reduced risk.

BACKGROUND

T Cell Response

- Phenotype of T cell response can vary depending on epitope stimulus.
- Some highly conserved epitopes in human IgG called Tregitopes are capable of stimulating regulatory T cells, thereby suppressing inflammatory response.



T cell epitopes as predictors of immunogenicity

Protein Therapeutic:

1 + 1 + 1 = response

T cell response depends on:
T effector epitope content

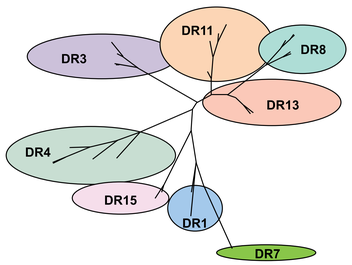
Monoclonal Antibody:

1 + 1 - regulatory T cell epitope = response

T cell response depends on:
T effector epitope content + Tregitope content

- EpiMatrix predicts immunogenicity of mAb sequences as the sum of T effector epitopes, adjusted for Treg epitope (Tregitope) content.*

HLA Diversity



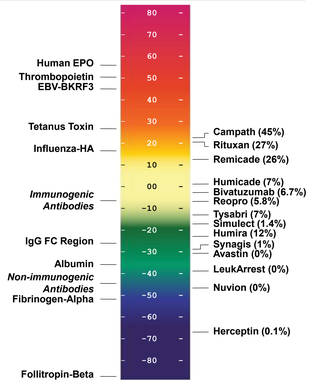
- Immunogenicity is predicted for a set of eight common HLA Class II alleles, covering over 95% of the global human population.**
- Restricting predictions to a highly representative set of alleles simplifies downstream decision making.

Figure adapted from:
Immunogenetics. 2004 Mar;55(12):797-810.

METHODS AND RESULTS

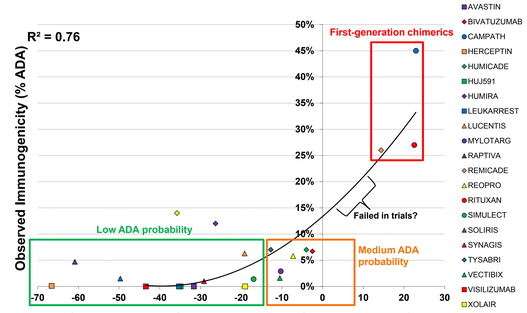
HIGH THROUGHPUT ANTIBODY IMMUNOGENICITY SCREENING

EpiMatrix Protein Immunogenicity Scale



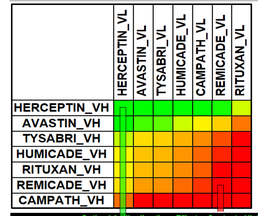
All scores normalized for protein length
For antibodies at right:
(% = exposed patients who developed ADA)

EpiMatrix Antibody Immunogenicity Regression



- Predicted immunogenicity adjusted for Tregitope content correlates with observed immunogenicity.***
- Regression analysis can be used to predict the immunogenic potential of a novel monoclonal antibody sequence.

EpiMatrix Antibody Immunogenicity Grid



- For any input combination of variable domains, Tregitope-adjusted EpiMatrix Scores are calculated and anti-therapeutic response is predicted based on regression analysis (left).

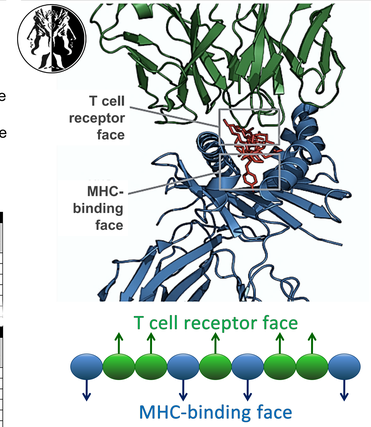
Optimal Antibodies (Low Effector Content - High Tregitope Content)

Antibody	Tregitope-Adjusted EpiMatrix Protein Score[1]	Tregitope Content[2]	Predicted Ab Response	Observed Ab Response
HERCEPTIN	-66.56	75.26	0.00	n.a.
Herceptin	**-66.56**	**75.26**	**0.00**	**0.10**
HERCEPTIN_VH - AVASTIN_VL	-56.24	71.92	0.00	n.a.
HERCEPTIN_VH - TYSABRI_VL	-54.46	56.01	0.00	n.a.
HERCEPTIN_VH - HUMICADE_VL	-48.40	50.94	0.00	n.a.
HERCEPTIN_VH - RITUXAN_VL	-47.16	47.75	0.00	n.a.

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_VH - HUMICADE_VL	1.30	14.62	14.21	n.a.
TYSABRI_VH - REMICADE_VL	4.52	0.00	16.54	n.a.
HUMICADE_VH - REMICADE_VL	8.85	5.35	19.95	n.a.
RITUXAN_VH - REMICADE_VL	9.92	0.00	20.84	n.a.
CAMPATH_VH - TYSABRI_VL	13.03	15.85	23.55	n.a.
Remicade	**14.40**	**2.48**	**24.79**	**26.00**

JanusMatrix



ANTIBODY CHARACTERISTIC ANALYSIS

EpiMatrix Scores

EpiMatrix Report
File: Your File - Sequence: Your Protein

Frame Start	AA Sequence	Frame Stop	DRB1*0101 Z-Score	DRB1*0301 Z-Score	DRB1*0401 Z-Score	DRB1*0701 Z-Score	DRB1*0801 Z-Score	DRB1*1101 Z-Score	DRB1*1301 Z-Score	DRB1*1501 Z-Score	Hits	AbDB[1] Frequency (%)	Germline[2] Count
1	QVQLQESGP	9	0.42	0.73	0.33	0.12	0.05	0.26	0.55	0.68	0	7.57	55
2	VQLQESGPG	10	1.93	1.86	1.78	1.10	2.10	1.84	1.46	2.26	6	7.66	55
3	QLQESGPGL	11	1.42	-0.05	0.73	0.39	-0.56	0.18	-0.16	-0.10	0	10.25	62
4	LQESGPGLV	12	1.58	1.91	0.88	2.46	0.58	1.17	0.46	1.59	2	10.31	62
5	QESGPGLVR	13	-0.06								0		0
6	ESGPGLVRP	14	-2.27								0		0
7	SGPGLVRPS	15	-0.35								0		0
8	GPGLVRPSQ	16	-0.36								0		0
9	PGLVRPSQT	17	0.44								0		0
10	GLVRPSQTL	18	0.52								0		0
11	LVRPSQTLS	19	0.67	1.14	0.67	0.28	0.62	0.12	1.78	0.71	1	0.31	0
12	VRPSQTLSL	20	2.02	2.32	2.12	2.54	0.99	1.67	2.10	2.13	7	0.30	0
13	RPSQTLSLT	21	-0.78	-0.55	-0.64	-0.90	-0.53	-0.23	0.05	0.44	0	0.28	0
14	PSQTLSLTC	22	0.27	-1.37	0.09	0.77	-0.30	-0.81	-0.38	0.65	0	7.30	24
15	SQTLSLTCT	23	-0.10	-0.74	-0.39	-0.39	0.03	-0.31	-0.50	0.49	0	2.81	14
16	QTLSLTCTV	24	1.09	-0.04	0.41	1.92	-0.86	0.80	-0.48	0.09	1	2.77	14
17	TLSLTCTVS	25	0.32	-0.24	1.26	-0.41	0.32	0.04	-0.01	-0.33	0	9.05	35
18	LSLTCTVSG	26	1.60	1.00	1.95	0.82	1.77	0.45	1.79	1.29	3	8.84	35
19	SLTCTVSGF	27	-1.27	-0.50	-0.01	-0.41	0.25	-1.11	-0.44	-1.73	0	0.02	0
20	LTCTVSGFT	28	1.28	-0.13	0.65	2.12	0.82	0.31	-0.39	1.77	2	0.00	0
21	TCTVSGFTF	29	-0.32	-1.16	-1.23	-0.10	-1.46	-0.54	-0.99	-0.46	0	0.00	0
22	CTVSGFTFT	30	-0.59	-1.03	-0.79	0.06	-1.22	-1.21	-1.34	-0.99	0	0.00	0
23	TVSGFTFTD	31	-0.08	-0.53	-0.63	-0.29	0.08	-0.55	-1.03	0.02	0	0.00	0
24	VSGFTFTDF	32	-1.09	-0.53	-0.29	-0.22	-0.22	-0.90	0.70	-0.23	0	0.00	0
25	SGFTFTDFY	33	-0.29	-0.47	-0.32	-1.10	-1.87	-1.49	-0.17	-0.99	0	0.00	0
26	GFTFTDFYM	34	-1.47	-1.70	-1.45	-1.55	-2.18	-1.24	-0.51	0.50	0	0.00	0
27	FTFTDFYMD	35	0.12	-0.62	0.15	0.92	0.06	0.08	-0.13	0.34	0	0.00	0
28	TFTDFYMDW	36	-1.27	-0.97	-1.08	-1.20	-1.93	-2.16	-2.97	-1.57	0	0.00	0
29	FTDFYMDWV	37	-0.15	-0.74	0.15	0.38	-0.29	0.15	-0.27	0.65	0	0.00	0
30	TDFYMDWVR	38	-0.97	-1.25	-1.20	-0.94	-0.69	-0.65	-0.45	-0.16	0	0.00	0
31	DFYMDWVRQ	39	0.25	-0.50	0.63	0.58	-0.11	0.18	-0.64	0.25	0	0.00	0
32	FYMDWVRQP	40	0.64	0.76	1.29	0.96	0.93	1.75	0.32	-0.60	1	0.00	0
33	YMDWVRQPP	41	0.17	0.55	0.71	0.92	0.83	1.19	0.33	0.42	0	0.00	0
34	MDWVRQPPG	42	0.92	0.76	0.37	-0.21	1.15	1.34	1.06	0.91	0	0.03	0
35	DWVRQPPGR	43	-0.25	-0.75	-1.10	-1.18	0.45	-0.38	0.42	-0.46	0	0.01	0
36	WVRQPPGRG	44	2.23	1.20	1.91	1.21	2.55	2.28	0.82	1.19	4	0.06	0

[1]AbDB Frequency (%) describes the percent of antibody chains in EpiVax's curated human antibody database which contain a given 9-mer.

[2]Germline Count describes the number of human germlines with matches to the 9-mer. Click on the cell to show/hide the germline matches.

CDR Residues are highlighted in red in the AA sequence. CDR definitions based on Bioinf.org.uk

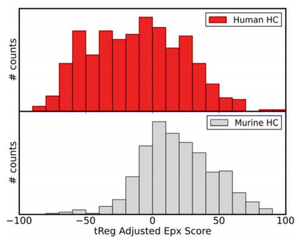
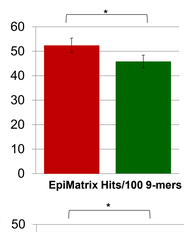


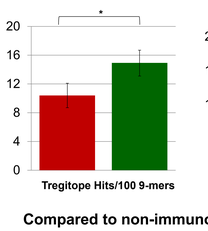
Figure credit: PLoS One. 2013 Oct 21;8(10):e76909.

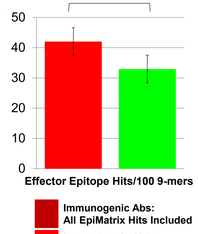
- Immunogenicity of an antibody sequence may depend not only on total epitope content, but also on the features of putative epitopes derived from the sequence.
- Published work has described the variable EpiMatrix scores of human and murine germline antibody sequences (Seeliger, PLoS One, above).
- In particular, those peptides which occur at higher frequencies in the human germline and in EpiVax's curated database of human antibody isolates are less likely to stimulate inflammatory response.

Inflammatory Content

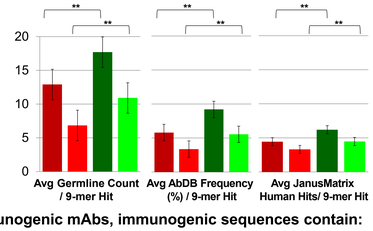


Suppressive Content





Tolerated Content



Compared to non-immunogenic mAbs, immunogenic sequences contain:

- **More inflammatory content**
  - Total predicted epitopes
  - Effector profile epitopes
- **Less regulatory content**
  - Fewer Tregitopes
- **Less tolerated content**
  - Human germline-conserved epitopes
  - Representation among human antibody isolates
  - Epitopes with potential to cross-react with T cells trained on other human sequences (JanusMatrix)

Tolerated content measures are correlated, as expected.

Tregitope content and human cross-reactive content differentiate are exclusively available using the ISPRI Toolkit.

CONCLUSIONS

- ✓ **Relative risk for immunogenicity of protein therapeutics can be predicted by comparing the number of T cell epitopes predicted in a given sequence compared to the number of epitopes expected by random chance.**
- ✓ **For epitopes derived from antibody sequences, frequency among circulating human isolates can affect the phenotype of T cell response.**
- ✓ **Tregitopes are an important class of T cell epitopes derived from IgG, capable of stimulating regulatory response.**
- ✓ **Monoclonal antibody products that stimulate limited immunogenicity in the clinic have been shown to contain fewer predicted epitopes, but more Tregitopes than products that stimulate significant (>5%) immunogenicity in clinical studies.**
- ✓ **Epitopes derived from non-immunogenic products are found more often in human germlines and publicly available human antibody isolates than are epitopes derived from immunogenic products.**
- ✓ **Immunogenic potential for antibody candidates can be predicted on a high throughput basis using the EpiMatrix Protein Immunogenicity Scale, Antibody Immunogenicity Regression and Antibody Immunogenicity Grid.**

REFERENCES

* Weber, CA et al. T cell epitope: friend or foe? Immunogenicity of biologics in context. *Adv Drug Deliv Rev.* (2009) 61(11):965-76.

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