In Silico Tools for Immunogenicity Risk Assessment

4th European Workshop on Protein Aggregation and Immunogenicity

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Director, Institute for Immunology and Informatics
Professor (Research) Univ. Rhode Island

Immunogenicity:
A Critical Challenge to Protein Therapeutics

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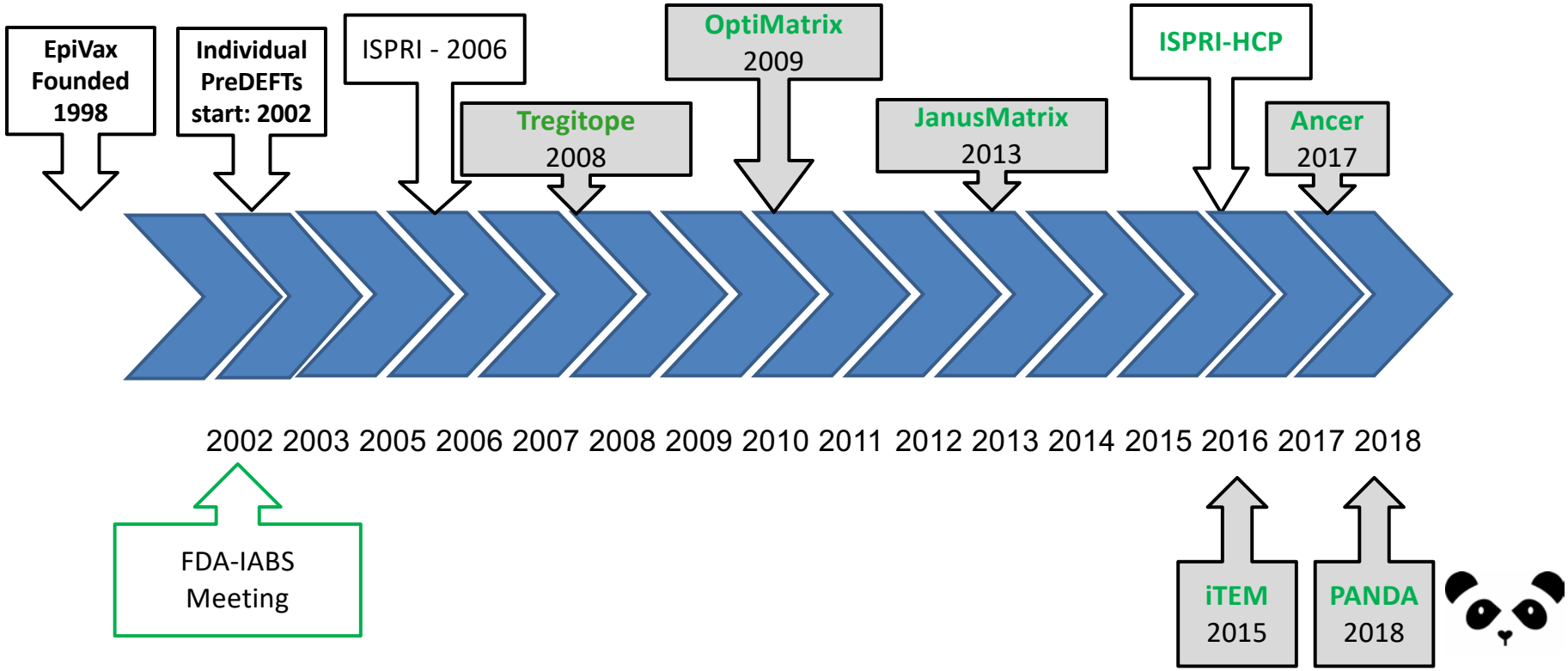
- Safety
  - Hypersensitivity
  - Cross-reactivity – Adverse Effects
- Efficacy
  - Neutralization
  - Change in PK
- Mechanisms
  - Antibodies - ADA
  - Cell-mediated immunity – focus of this talk
  - Innate immunity

20 years of Comprehensive In Silico and In Vitro Immunogenicity Risk Assessment for Biologics and Vaccines



The Epi-People who Make it Happen!

Our singular focus: Use of Immunoinformatics Tools To Better Understand Human Immune Response

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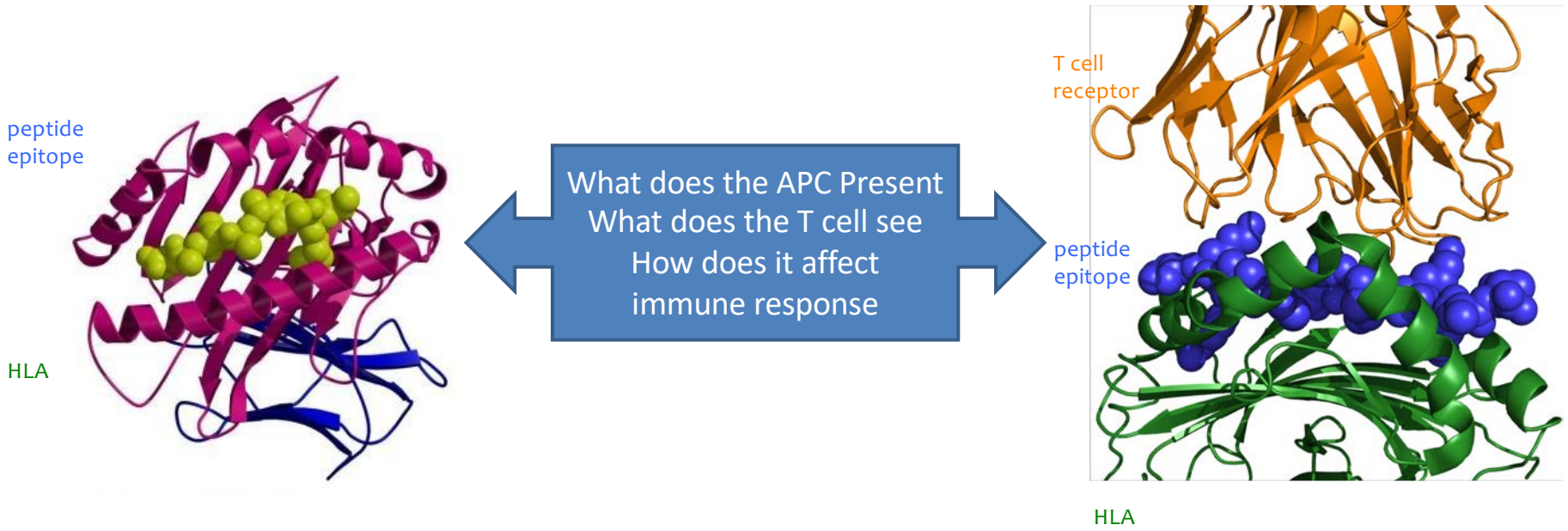

T cell epitope and immunogenicity analysis for biologics and vaccines

Goals for this Talk

In silico tools were developed for assessing the potential immunogenicity and T cell epitope content of vaccine antigens and biologics and are best used in conjunction with in vitro assays.

I will describe ISPRI (the toolkit developed by my team at EpiVax)

- ISPRI is available for commercial license
- Academic projects are encouraged and many are ongoing

In silico tools improve our understanding of the underlying factors driving immunogenicity as they relate to T cell epitopes, including:

HLA restriction
T effector epitopes
T reg epitopes

These tools are actively in use by our team and by our clients for de-risking biologics, designing vaccines, and developing immune-modulating therapies for human diseases such as autoimmunity and allergy.

New Tools to be Discussed: iTEM and J-iTEM

This is the year of Personalized Immunogenicity Risk Assessment!

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- **Personalized Immunogenicity Risk Assessment = *PIMA***
- **Includes: HLA-restricted immunogenicity risk assessment**
  - ***Individualized T cell Epitope Measure* = *iTEM***
- **Treg identification and validation**
  - ***JanusMatrix* – *searches for conservation at the TCR face***
- ***J-iTEM* (combines iTEM and JanusMatrix)**
  - ***for more precise prediction of immunogenicity at the individual level***

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Immunogenicity is Personal

HLA- and Genotype-Based Risk Assessment Model to Identify Infantile Onset Pompe Disease Patients at High-Risk of Developing Significant Anti-Drug Antibodies (ADA)

De Groot AS[1*], Kazi ZB[2], Martin RF[1], Terry FE[1], Desai AK[2], Martin WD[1], Kishnani PS[2*]

PIMA: Personalized Immunogenicity Risk Assessment (Pompe, other "replacement" proteins)
ANCER – Mutanome analysis for development of personalized cancer vaccines
D-ANCER – Donor Organ HLA analysis for prediction of Donor Specific Abs in Transplantation

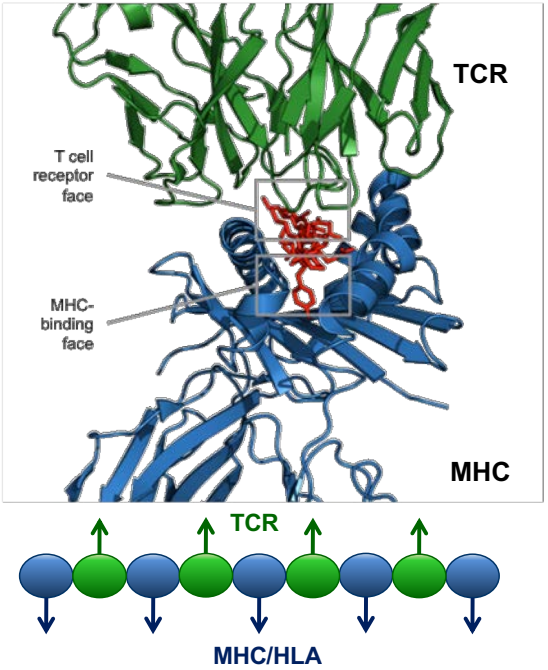
JanusMatrix 2013

A New Way to Search for Homology with Self

Each MHC ligand has two faces:

1. The MHC-binding face (agretope) and
2. The TCR-interacting face (epitope)

JanusMatrix is designed to predict the potential for cross-reactivity between epitope clusters and the human genome, based on conservation of TCR-facing residues in their putative HLA ligands.



JanusMatrix

Find predicted 9-mer ligands with:

- **Identical T cell-facing residues**
- **Same HLA allele and minimally different MHC-facing residues**

1/28/19

Moise L et al. Hum Vaccin Immunother. 2013 Jul;9(7):1577-86

Most Recent In Silico/In Vitro News: Peptide ANDA (PANDA) and FDA Contract

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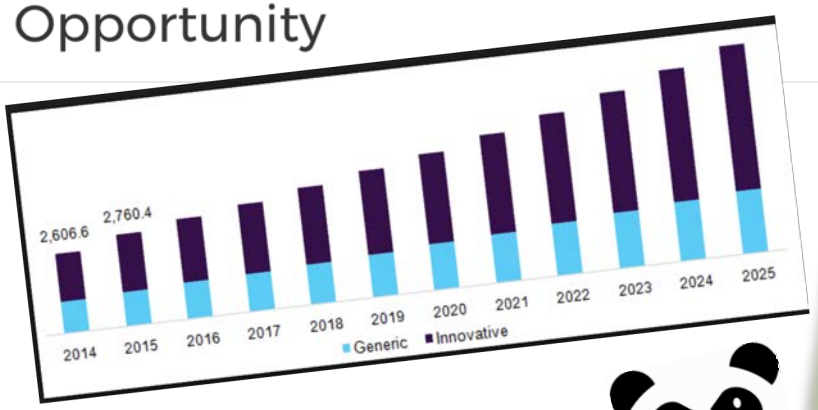
Global Peptide Therapeutics Market, Dosage, Price & Clinical Trials Insight 2024 - 101 Marketed Drugs with a $50 Billion Opportunity



Breaking News: FDA awards $1 million to EpiVax, CUBRC, to assess generic peptide drugs

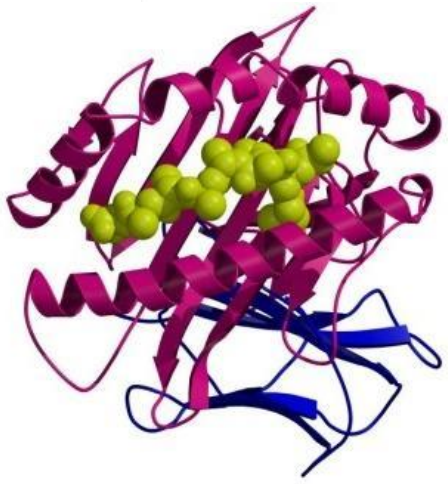
by Kathleen Gonzalez | Oct 5, 2018 | News |

This original article was published on October 2nd, 2018 in ConvergenceRI.

PROVIDENCE, RI – The U.S. Food and Drug Administration announced on Tuesday, Oct. 2, that the federal agency has awarded EpiVax and CUBRC a $1 million contract over two years to establish best practices and procedures to evaluate generic peptide drugs for immunogenic potential and related impurities.

FDA will use EpiVax tools in 2018-2019 for Immunogenicity Assessment

What does the T cell See? Linear Epitopes Strominger, Chicz (and others)



Published July 1, 1993

Specificity and Promiscuity among Naturally Processed Peptides Bound to HLA-DR Alleles

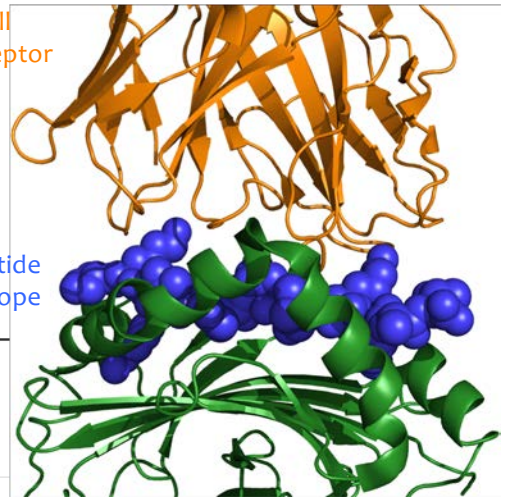
By Roman M. Chicz, Robert G. Urban, Joan C. Gorga, Dario A. A. Vignali, William S. Lane,* and Jack L. Strominger

*From the Department of Biochemistry and Molecular Biology and the *Harvard Microchemistry Facility, Harvard University, Cambridge, Massachusetts 02138*

T cell receptor

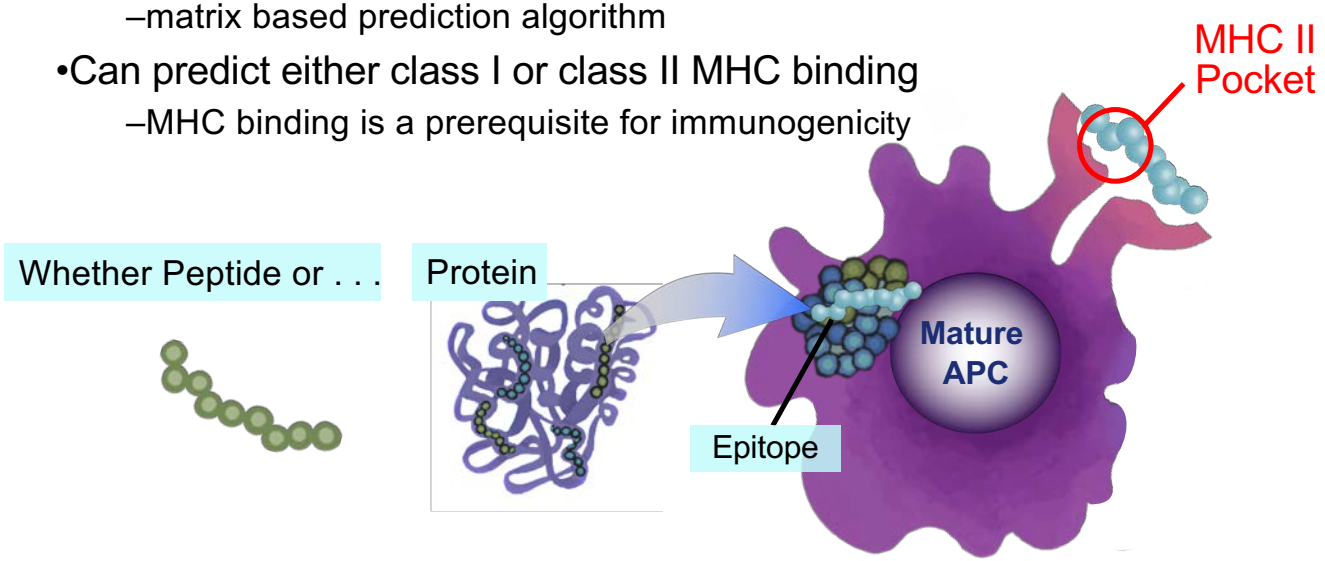
peptide epitope

HLA



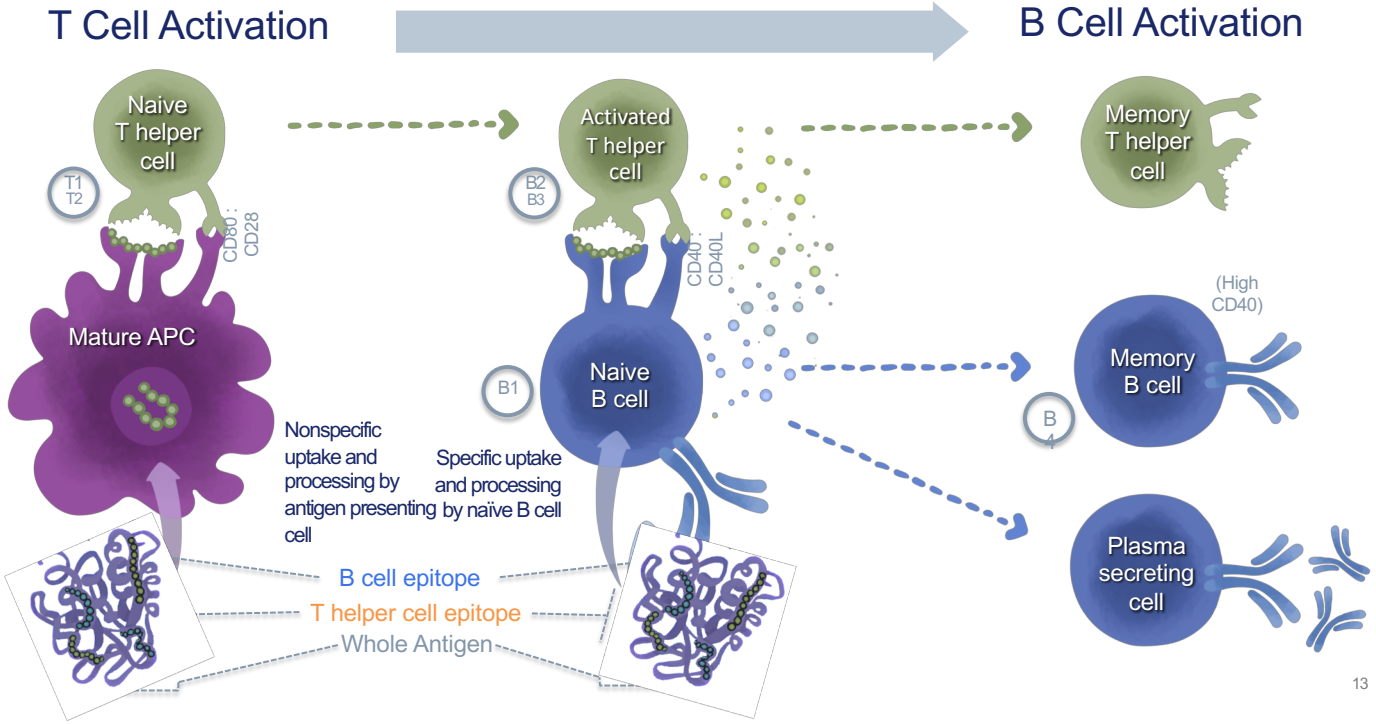
Identifying T cell epitopes Is key to assessing Immunogenicity Risk

- EpiVax uses **EpiMatrix** to predict epitopes
  - –matrix based prediction algorithm
- Can predict either class I or class II MHC binding
  - –MHC binding is a prerequisite for immunogenicity



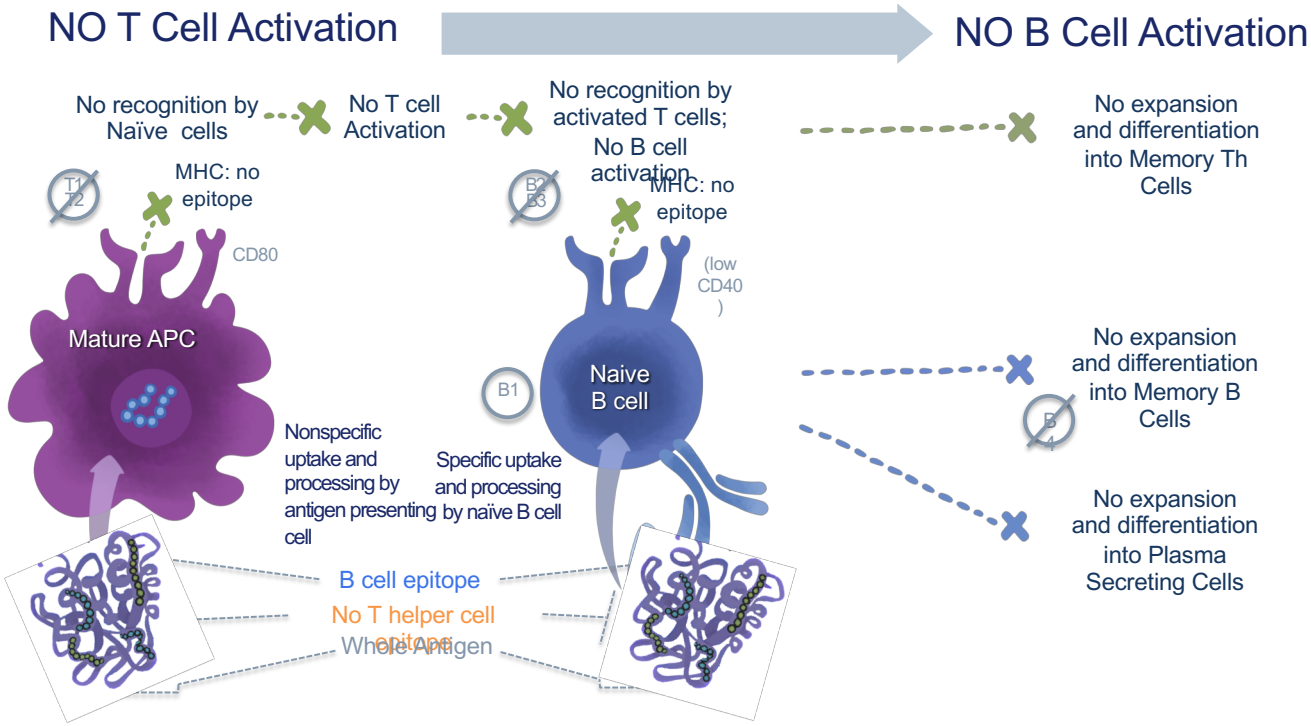
- –Full suite of HLA-based predictions; Class II usually used for biologics.
- –Cloud-based tool used by most **large Biotech companies: ISPRI**
- –Separate website available for **vaccine design: iVAX**

Presence of T cell epitopes drives ADA



Activation of CD4 T cells and the T-dependent antibody response

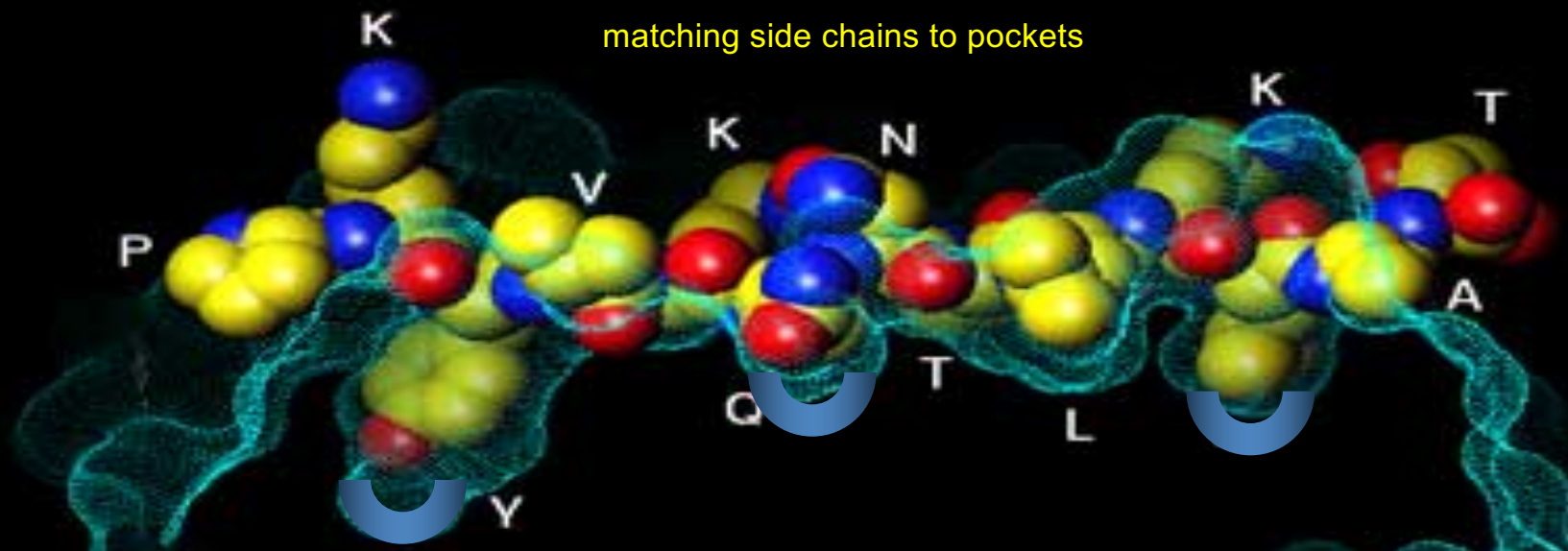
Taking it one step further: Absence of T cell epitopes reduces ADA



Lack of T cell epitopes abrogates activation of CD4 T cells and T-dependent antibody response

Epitope binding to HLA involves

matching side chains to pockets



Side chains of amino acids (R group) anchor the peptide in place.

The side chains are anchored into specific pockets

Pockets are conserved in evolution - - -

http://www.umassmed.edu/pathology/graphics/sternfig1.jpg *(malaria epitope in DRB1*0101)*

T cell epitope Prediction
HLA Pocket Profiles – Are Redundant Sturniolo et al.1999

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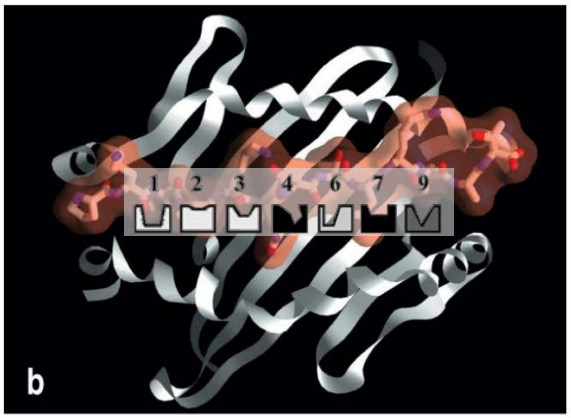
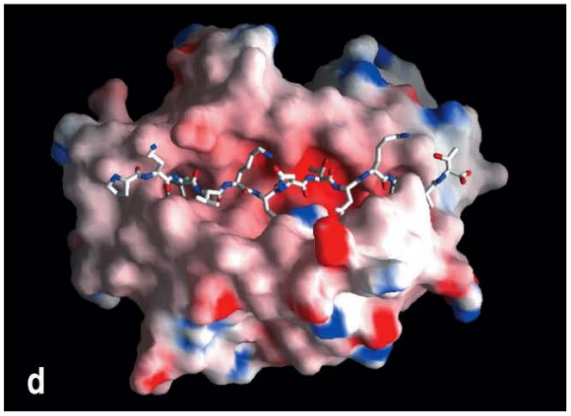
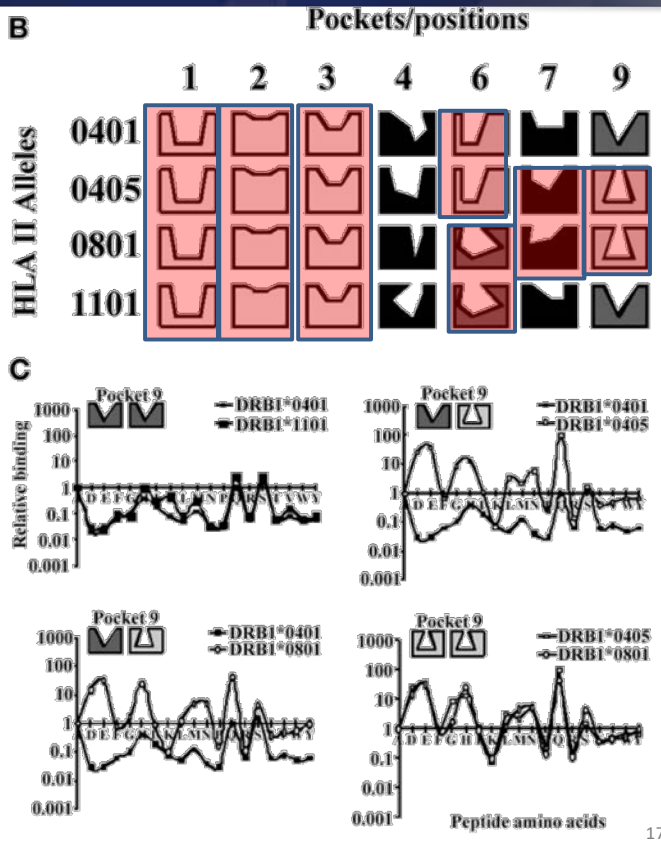
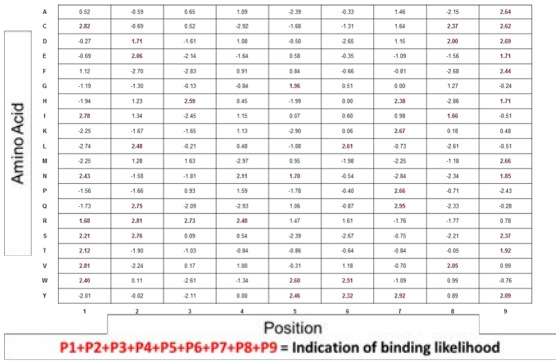


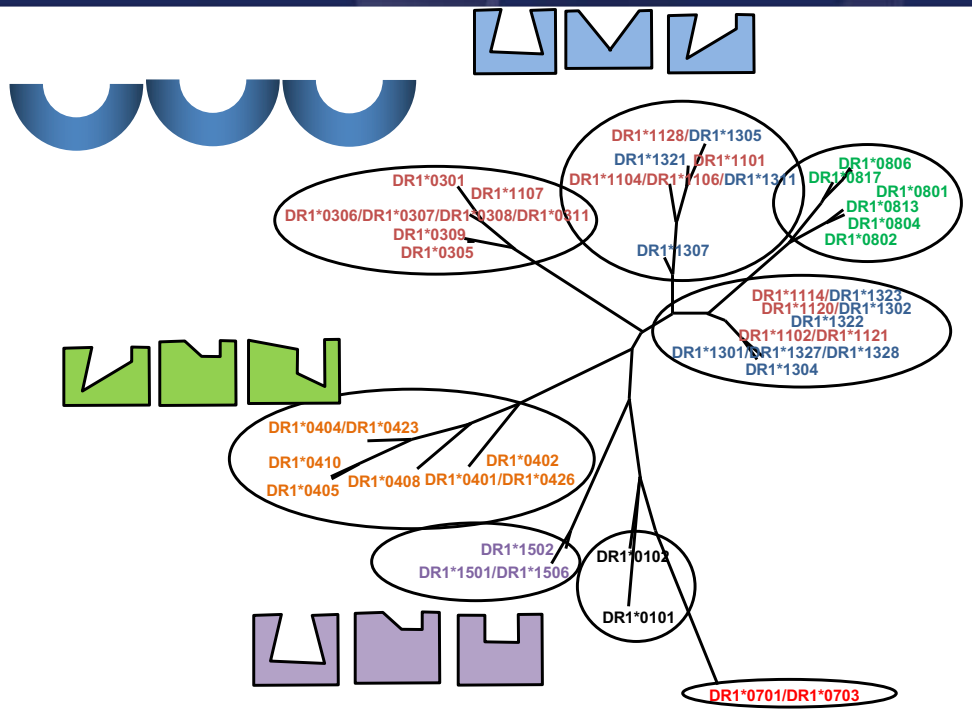




We maintain a set of allele specific models of MHC-ligand binding.
We refer to these models collectively as the EpiMatrix System.
"Matrix" - models are driven by a 20x9 set of coefficients (one for each binding position and amino acid).
Matrices can be combined with pocket profiles to develop new prediction tools.

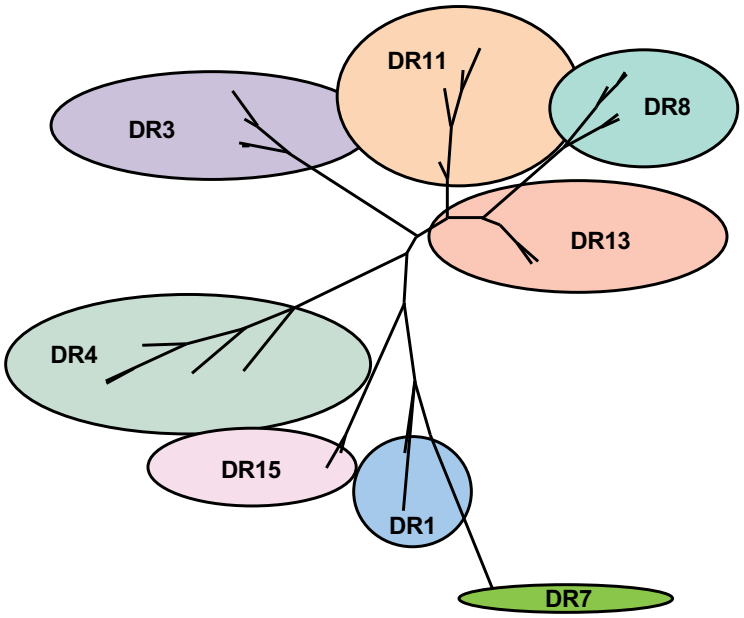


APPROACH:
Many HLA Types Share Peptide Binding Preferences



- Shared Pockets in HLA DR "Super Families
- Shared T cell epitope preferences
- Need to Reduce Redundancy for More accurate Prediction

APPROACH
EpiMatrix HLA "Supertype" Coverage



EpiVax tests for binding potential to the most common HLA molecules within each of the "supertypes"* shown to the left.

This allows us to provide results that are representative of >95% of human populations worldwide without the necessity of testing each haplotype individually.**

*Lund et al. Definition of Supertypes for HLA Molecules Using Clustering of Specificity Matrices. Immunogenetics. 2004; 55(12):797–810.

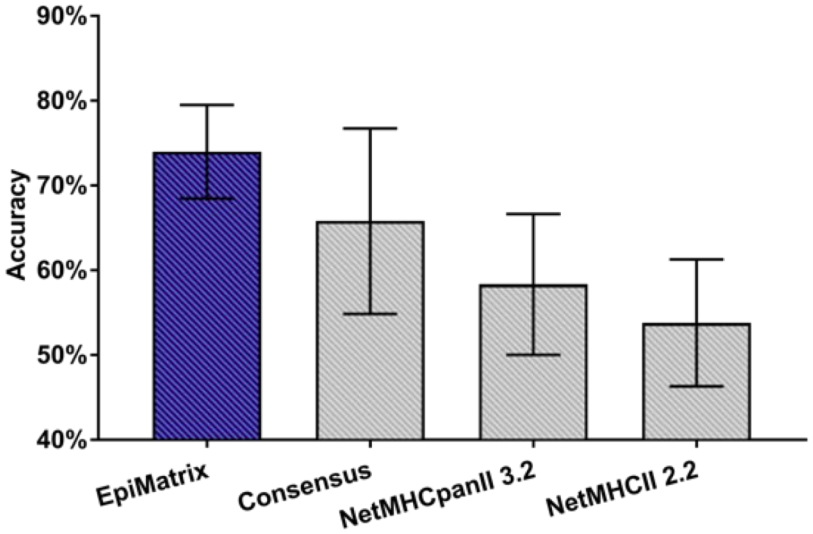
**Southwood et al. Several Common HLA-DR Types Share Largely Overlapping Peptide Binding Repertoires. J Immunol. 1998; 160(7):3363–73.

ACCURACY: Recent study of HLA binding data shows EpiMatrix Class II predictions superior to IEDB tools

Predicting Class II epitopes is more difficult than Class I.

EpiMatrix Class II predictions are **74% accurate** when prospectively tested in *in vitro* HLA binding assays.

IEDB predictions are 54-66% accurate when tested against the same set of peptides.



Mean accuracy (± SD) of DRB1*0101, DRB1*0301, DRB1*0401, DRB1*0701, DRB1*0802, DRB1*1101, DRB1*1302, and DRB1*1501 predictions. Between 175 and 251 peptides were tested per HLA.

Source: peptides prospectively selected by EpiMatrix and tested in *in vitro* HLA binding assays. Peptides were evaluated on IEDB on November 19th 2018.

APPROACH: Break down the protein or peptide Into overlapping frames and scoring each frame

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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
1	APELLGGPS	9	0.1	-0.88	-0.34	-0.84	-0.65	-0.4	-1.72	-0.17	0
2	PELLGGPSV	10	1.07	-0.62	0.33	0.13	-0.09	0.39	-0.28	0.59	0
3	ELLGGPSVF	11	-0.17	0.45	0.26	0.48	-0.28	-0.21	-0.11	-0.32	0
4	LLGGPSVFL	12	1.78	1.73	1.43	1.87	0.69	0.29	1.24	1.93	4
5	LGGPSVFLF	13	-0.21	0.4	-0.13	0.46	-0.32	0.07	0.99	-0.02	0
.	.	.	.	.	.	.	.	.	.	.	.
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87	KEYKCKVSN	95	-0.68	0.07	-1.29	-0.96	1.31	-0.09	0.52	-0.61	0
88	EYKCKVSNK	96	-0.75	-1.04	0.44	-0.78	0.67	-0.64	-0.97	-1.6	0
89	YKCKVSNKA	97	1.85	1.92	1.94	2.58	2.47	2.41	1.56	1.4	6
90	KCKVSNKAL	98	1.15	0.11	0.44	1.59	0.21	0.52	0.53	1	0
91	CKVSNKALP	99	-0.06	1	0.06	-0.47	0.69	1.47	0.86	-0.18	0
92	KVSNKALPA	100	1.6	1.41	1.92	1.26	1.09	1.86	1.54	1.4	2
93	VSNKALPAP	101	-1.29	0.19	-1	-0.98	1.05	0.66	0.74	-0.28	0
94	SNKALPAPI	102	1.28	1.45	0.8	1.05	0.77	0.55	1.62	0.98	0
95	NKALPAPIE	103	0.62	0.3	0.48	-0.19	1.65	0.76	0.62	0.26	1
.	.	.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	.
205	HYTQKSLSL	213	1.44	0.63	1.24	1.46	0.52	0.94	1.49	1.46	0
206	YTQKSLSLS	214	0.68	1.68	0.76	0.86	2.46	2.02	2	0.94	4
207	TQKSLSLSP	215	0.8	0.75	1.4	1.54	0.25	1.09	0.56	0.8	0
208	QKSLSLSPG	216	0.68	0.54	0.67	-0.18	1.64	1.42	0.65	0.95	0
209	KSLSLSPGK	217	0.66	0.57	0.94	0.39	0.47	1.02	0.33	0.8	0

Summarized Results	DRB1*0101	DRB1*0301	DRB1*0401	DRB1*0701	DRB1*0801	DRB1*1101	DRB1*1301	DRB1*1501	Total
Maximum Single Z-score	2.18	2.5	2.42	2.63	2.47	2.41	2.84	2.49	--
Sum of Significant Z-scores	20.14	23.2	22.19	26.64	27.15	20.78	21.88	10.08	172.05
Count of Significant Z-Scores	11	12	11	14	13	11	11	5	88

Total Assessments Performed: 1672	Deviation from Expectation: -13.95	Deviation per 1000 AA: -8.34
Adjusted for Regulatory Epitopes	Deviation from Expectation: -34.27	Deviation per 1000 AA: -20.50

Individual HLA Binding Assessment

Populations

Individuals

EpiMatrix Immunogenicity Score

Tregitope-adjusted Score

APPROACH: Antigen Presenting Cell Math: Immunogenicity = sum of epitopes divided by length

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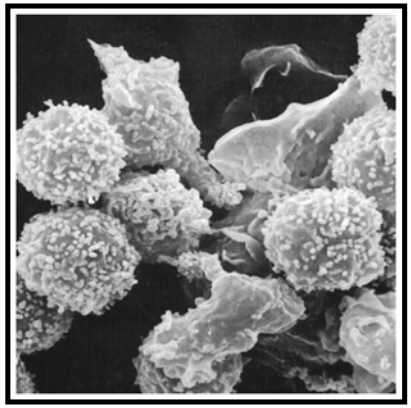
Protein Therapeutic:

1 + 1 + 1 = Response

T cell response is defined by

T cell epitope content + **HLA of subject**

- Protein and peptide immunogenicity can be ranked



Each of these T cells is probably reacting to a different T cell epitope on the surface of the DC: Visual SUM of the immune response

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.

Risk Assessment Scale (Normalized for length)

Adjusted for special cases e.g. antibodies where Tregitopes are present

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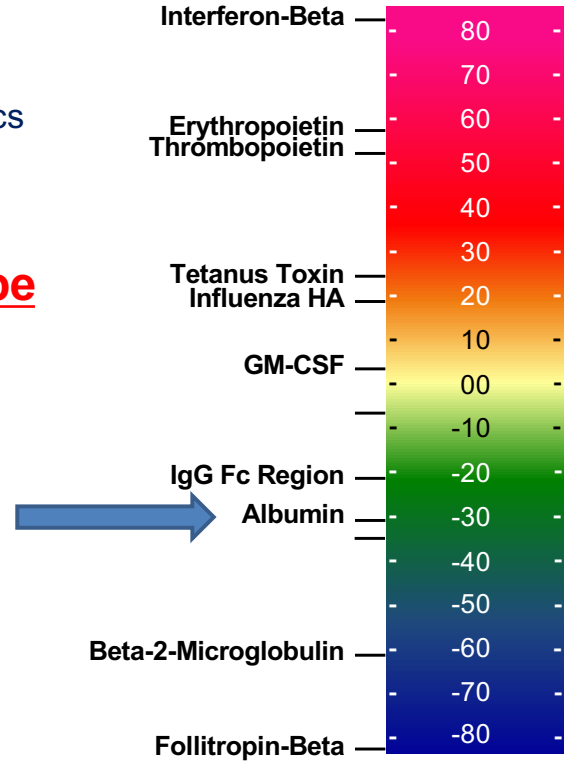
Overall Immunogenicity Risk of Biologics
(not adjusted for Treg epitopes) →

epitope + epitope + epitope
length

Human Proteome –
LOW Potential for Immunogenicity



Candidate Drug Potential for Immunogenicity is High

0 on scale = Expected # T cell epitopes in Random protein

Candidate Drug Potential for Immunogenicity is Low

Potential for Immunogenicity Identified Prior to PRCA Event

The New England Journal of Medicine

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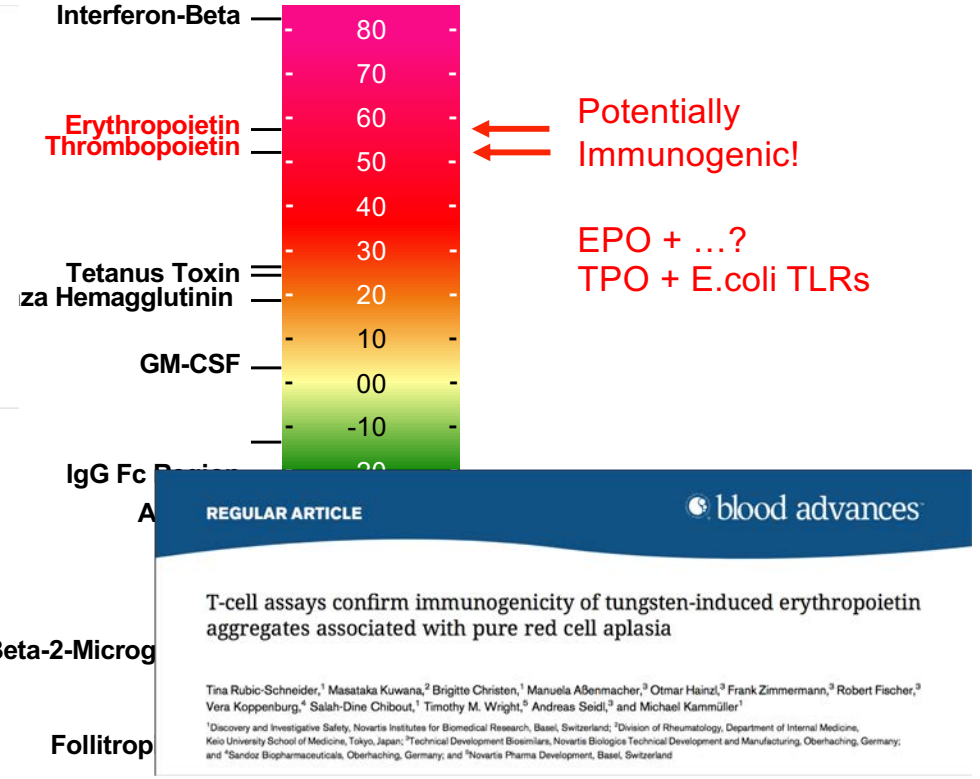
VOLUME 346 FEBRUARY 14, 2002 NUMBER 7

PURE RED-CELL APLASIA AND ANTIERYTHROPOIETIN ANTIBODIES IN PATIENTS TREATED WITH RECOMBINANT ERYTHROPOIETIN

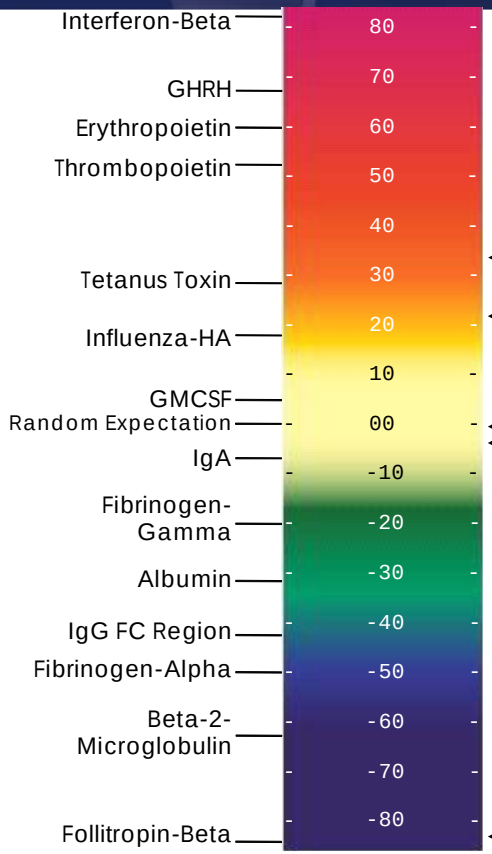
NICOLE CASADEVALL, M.D., JOELLE NATAF, M.D., BÉATRICE VIRON, M.D., AMIR KOLTA, M.D., JEAN-JACQUES KILADJIAN, M.D., PHILIPPE MARTIN-DUPONT, M.D., PATRICK MICHAUD, M.D., THOMAS PAPO, M.D., VALÉRIE UGO, M.D., IRÈNE TEYSSANDIER, B.S., BRUNO VARET, M.D., AND PATRICK MAYEUX, PH.D.

It is clear that innate immune stimulation "broke tolerance". However, if T cell epitopes were not present, Would the protein be immunogenic?

Would purified Recombinant Human Albumin or rFSH + TLR be immunogenic?



In Silico Analysis Demonstrated to be Relevant to Biologic Immunogenicity in the Clinic



FPX 2 Score 34.3
Binding Antibodies **53%**
Neutralizing Antibodies **12%**

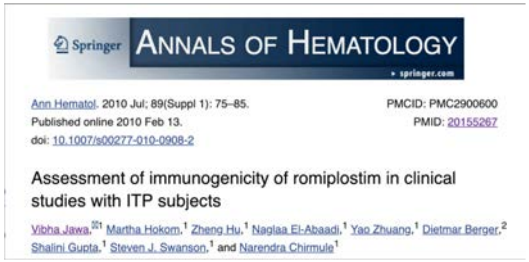
FPX 1 Score 21.9
Binding Antibodies **37%**
Neutralizing Antibodies **40%**

FPX 3 Score 1.62
Binding Antibodies **7.8%**
Neutralizing Antibodies **0.5%**

FPX 4 Score -1.76
Binding Antibodies **5.6%**
Neutralizing Antibodies **N/A**

FPX 5 Score -111.25
Binding Antibodies **9.3%**
Neutralizing Antibodies **0%**

- 2 Amgen Fusion proteins (FPX 1 and 2) tested in clinic. (FPX and GNDF). Blinded retrospective analysis (Koren, Tatarewicz, published). Clinical failures.
- FPX 3-5 analyzed in prospective analysis. Only low scoring proteins went to clinic. One of which - **Romiplastin** = is a demonstrated commercial success)


Springer ANNALS OF HEMATOLOGY
springer.com

Ann Hematol. 2010 Jul; 89(Suppl 1): 75–85.
Published online 2010 Feb 13.
doi: 10.1007/s00277-010-0908-2

PMCID: PMC2900600
PMID: 20155267

Assessment of immunogenicity of romiplostim in clinical studies with ITP subjects

Vibha Jawa,[corr1] Martha Hokom,[1] Zheng Hu,[1] Naglaa El-Abaadi,[1] Yao Zhuang,[1] Dietmar Berger,[2] Shalini Gupta,[1] Steven J. Swanson,[1] and Narendra Chirmule[1]

Koren E, De Groot AS, Jawa V, et al. Clinical validation of the "in silico" prediction of immunogenicity of a human recombinant therapeutic protein Clin Immunol. 2007 Jul.

Tatarewicz SM1, Wei X, Gupta S, Masterman D, Swanson SJ, Moxness MS. Development of a maturing T-cell-mediated immune response in patients with idiopathic Parkinson's disease receiving r-metHuGDNF via continuous intraputaminal infusion. J Clin Immunol. 2007 Nov;27(6):620-7.

More recent study / Presented at PEGS 2018 / Montgomery

bococizumab anti-PCSK9: in silico

```
>bococizumab_H aPCSK9
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGEISPFGGRTNYNEKFKSRVT
RDTSTSTVYMELSSLRSEDTAVYYCARERPLYASDLWGQGTTVTVSSASTKGPSVFPLAPCSRSTSEST
LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTK
KTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVH
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EALHNHYTQKSLSLSPGK
>bococizumab_L aPCSK9
DIQMTQSPSSLSASVGDRVTITCRASQGISSALAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGT
TFTISSLQPEDIATYYCQQRYSLWRTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY
EAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR
C
```
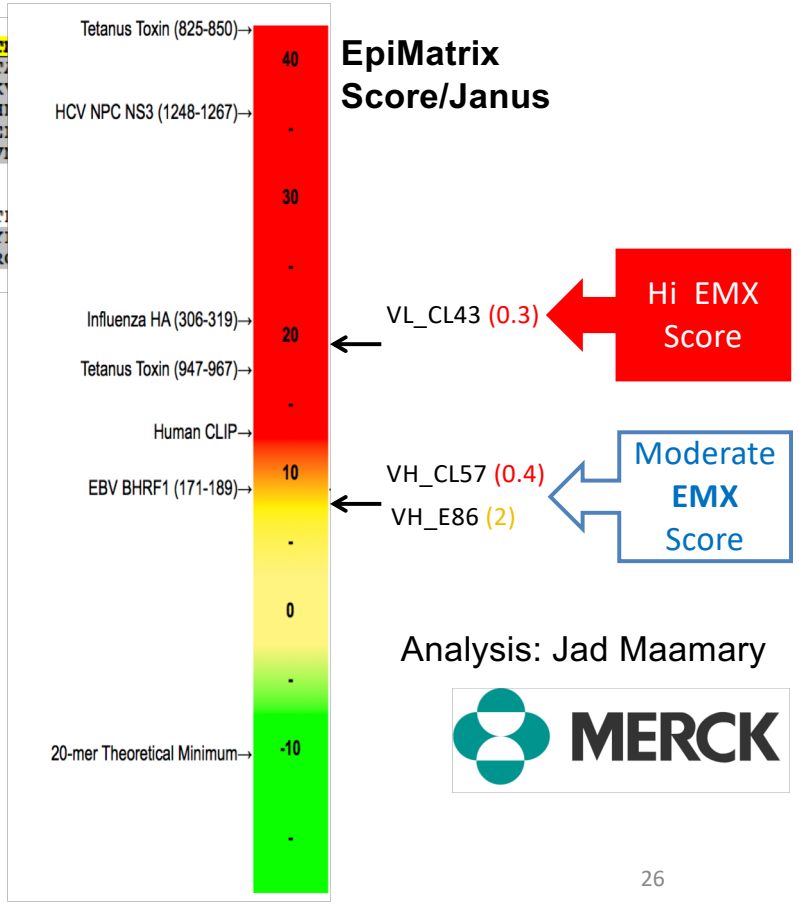

yellow = epitopes able to bind at least four HLA-DR alleles
bold underlined =clusters of HLA DR binding epitopes
red = CDRs (enhanced chothia method)

VL_CL43 **Homology to Human Proteome: Limited**

SEQUENCE	% IDENTITY	% SIMILARITY	FILE	DESCRIPTION
APKLLIYSASYRTGVPSRFSGSG	--	--	A2_CL	--
-------D--NLE----------	83%	88%	P01594	Immunoglobulin kappa variable 1-33 OS=Homo sapiens OX=9606 G...
--R----G--I-A--I-A------	75%	87%	A0A087WSY6	Immunoglobulin kappa variable 3D-15 OS=Homo sapiens OX=9606 ...
--R----D--N-A--I-A------	75%	87%	A0A0A0MRZ8	Immunoglobulin kappa variable 3D-11 OS=Homo sapiens OX=9606 ...
--R----G--T-A--I-A------	75%	87%	P01624	Immunoglobulin kappa variable 3-15 OS=Homo sapiens OX=9606 G...

Immunogenicity risk Assessment
CL43 : High
CL57: Intermediate
Bococizumab: High (48% observed)



Analysis: Jad Maamary

MERCK

Presentation by D. Montgomery at PEGS

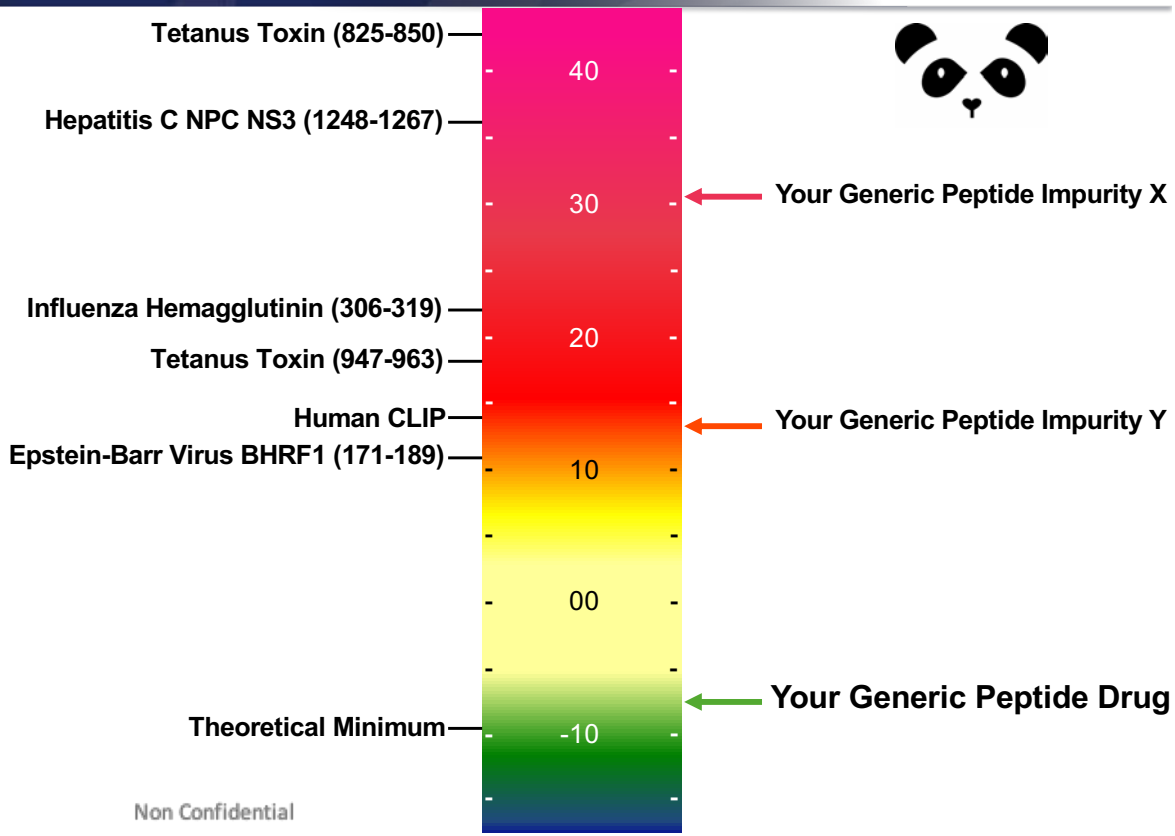
Risk Assessment Scale For Peptides (and Peptide Generic Drug Impurities)

EpiVax

The EpiVax Immunogenicity Scale allows for the comparison of peptides (including peptide generic drugs and their impurities) by T cell epitope content and normalizes for protein length.

Peptides with scores above +10 have a high potential for immunogenicity

$$\frac{1 + 1 + 1}{\text{length}}$$



iTEM : Analyzing Immunogenicity for the Individual
Assess overlapping frames and scoring each frame

EpiVax

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
1	APELLGGPS	9	0.1	-0.88	-0.34	-0.84	-0.65	-0.4	-1.72	-0.17	0
2	PELLGGPSV	10	1.07	-0.62	0.33	0.13	-0.09	0.39	-0.28	0.59	0
3	ELLGGPSVF	11	-0.17	0.45	0.26	0.48	-0.28	-0.21	-0.11	-0.32	0
4	LLGGPSVFL	12	1.78	1.73	1.43	1.87	0.69	0.29	1.24	1.93	4
5	LGGPSVFLF	13	-0.21	0.4	-0.13	0.46	-0.32	0.07	0.99	-0.02	0
.	.	.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	.
87	KEYKCKVSN	95	-0.68	0.07	-1.29	-0.96	1.31	-0.09	0.52	-0.61	0
88	EYKCKVSNK	96	-0.75	-1.04	0.44	-0.78	0.67	-0.64	-0.97	-1.6	0
89	YKCKVSNKA	97	1.85	1.92	1.94	2.58	2.47	2.41	1.56	1.4	6
90	KCKVSNKAL	98	1.15	0.11	0.44	1.59	0.21	0.52	0.53	1	0
91	CKVSNKALP	99	-0.06	1	0.06	-0.47	0.69	1.47	0.86	-0.18	0
92	KVSNKALPA	100	1.6	1.41	1.92	1.26	1.09	1.86	1.54	1.4	2
93	VSNKALPAP	101	-1.29	0.19	-1	-0.98	1.05	0.66	0.74	-0.28	0
94	SNKALPAPI	102	1.28	1.45	0.8	1.05	0.77	0.55	1.62	0.98	0
95	NKALPAPIE	103	0.62	0.3	0.48	-0.19	1.65	0.76	0.62	0.26	1
.	.	.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	.
205	HYTQKSLSL	213	1.44	0.63	1.24	1.46	0.52	0.94	1.49	1.46	0
206	YTQKSLSLS	214	0.68	1.68	0.76	0.86	2.46	2.02	2	0.94	4
207	TQKSLSLSP	215	0.8	0.75	1.4	1.54	0.25	1.09	0.56	0.8	0
208	QKSLSLSPG	216	0.68	0.54	0.67	-0.18	1.64	1.42	0.65	0.95	0
209	KSLSLSPGK	217	0.66	0.57	0.94	0.39	0.47	1.02	0.33	0.8	0

Summarized Results	DRB1*0101	DRB1*0301	DRB1*0401	DRB1*0701	DRB1*0801	DRB1*1101	DRB1*1301	DRB1*1501	Total
Maximum Single Z-score	2.18	2.5	2.42	2.63	2.47	2.41	2.84	2.49	--
Sum of Significant Z-scores	20.14	23.2	22.19	26.64	27.15	20.78	21.88	10.08	172.05
Count of Significant Z-Scores	11	12	11	14	13	11	11	5	88
Total Assessments Performed: 1672		Deviation from Expectation: -13.95				Deviation per 1000 AA: -8.34			
Adjusted for Regulatory Epitopes		Deviation from Expectation: -34.27				Deviation per 1000 AA: -20.50			

Individual HLA Bindiing Assessment

Populations

Individuals

EpiMatrix Immunogenicity Score

Tregitope-adjusted Score

HLA Restricts Immune Response (Personalizing Risk Assessment) / iTEM

Protein Therapeutic:

1 + 1 + 1 = Response

T cell response depends on:

T cell epitope content + **HLA of subject**

➢ protein immunogenicity can be ranked

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.

Different HLA, Different Binding Pockets

HLA-DR B*0101

HLA-DR B*0301

iTEM Analysis – Individualized T cell Epitope Measure
HLA Background Defines Personalized Immunogenicity

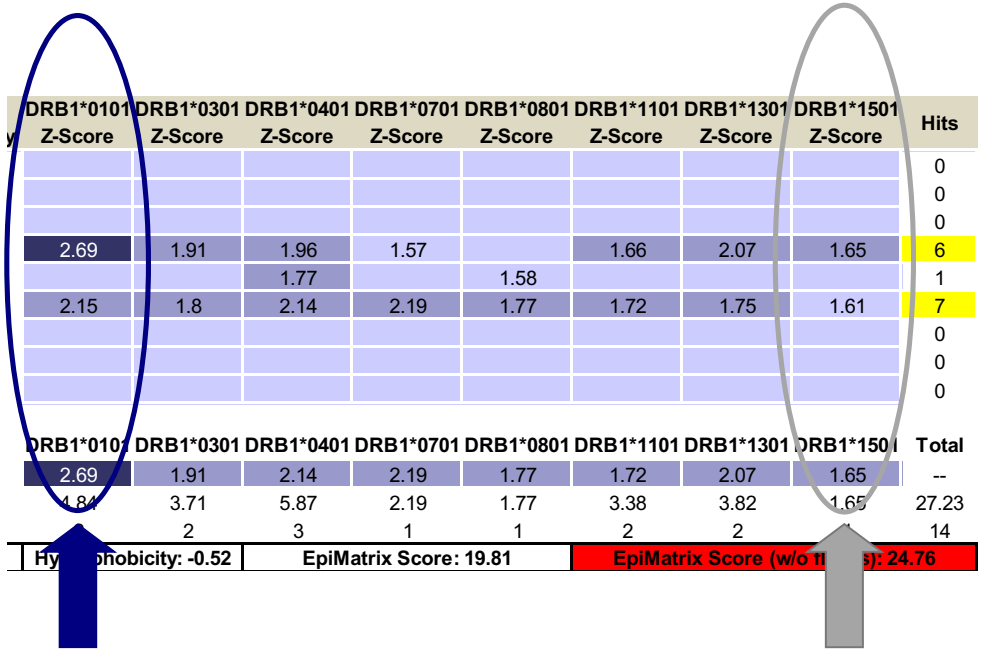
Immunogenicity is

HLA Restricted

DRB1*0101 is predicted

to present this peptide more effectively

than DRB1*1501



	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
									0
									0
									0
	2.69	1.91	1.96	1.57		1.66	2.07	1.65	6
			1.77		1.58				1
	2.15	1.8	2.14	2.19	1.77	1.72	1.75	1.61	7
									0
									0
									0

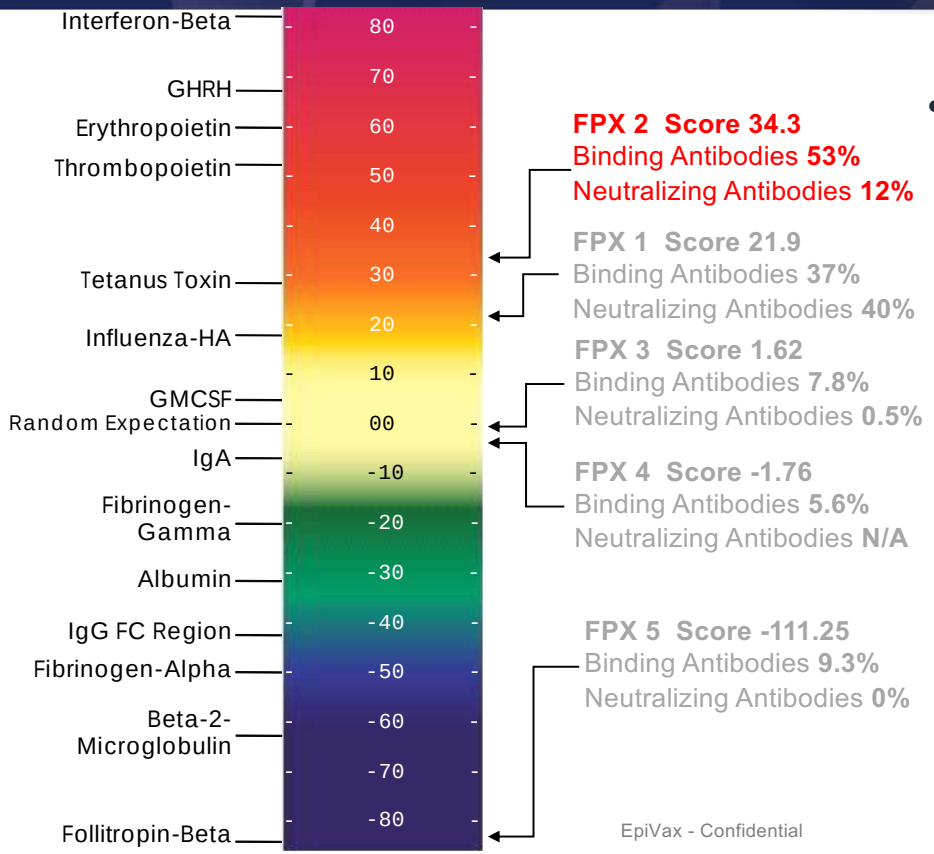
	DRB1*0101	DRB1*0301	DRB1*0401	DRB1*0701	DRB1*0801	DRB1*1101	DRB1*1301	DRB1*150	Total
	2.69	1.91	2.14	2.19	1.77	1.72	2.07	1.65	--
	4.84	3.71	5.87	2.19	1.77	3.38	3.82	1.65	27.23
		2	3	1	1	2	2	1	14

Hy phobicity: -0.52 | EpiMatrix Score: 19.81 | EpiMatrix Score (w/o 1 s): 24.76

Different Immune Response Expected

Highly Relevant to Enzyme and Factor Replacement Therapy

Prospective Study: *Correlation of EpiMatrix Scores and Immunogenicity in human studies*



- Amgen FC Fusion peptide (FPX 2) in clinic. **Blind EpiMatrix retrospective analysis of "PEPTIBODY"**

Koren E, De Groot AS, Jawa V, Beck KD, Boone T, Rivera D, Li L, Mytych D, Koscec M, Weeraratne D, Swanson S, Martin W. Clinical validation of the "in silico" prediction of immunogenicity of a human recombinant therapeutic protein Clin Immunol. 2007 Jul.

Correlation between Haplotype, iTEM , Antibody Concentration and Immune Response is Excellent

EpiVax

HLA	iTEM	Ab conc (mg/ml)	IFN-g ratio	IL-4 ratio
0701/1501	6.25	20.20	26.0	89.0
0301/0701	4.75	5.60	1.74	2.60
0101/0103	2.83	2.80	2.00	3.34
0301	1.67	NA	1.04	1.30

iTEM Heat Maps: for estimating HLA-specific risk

From global population…

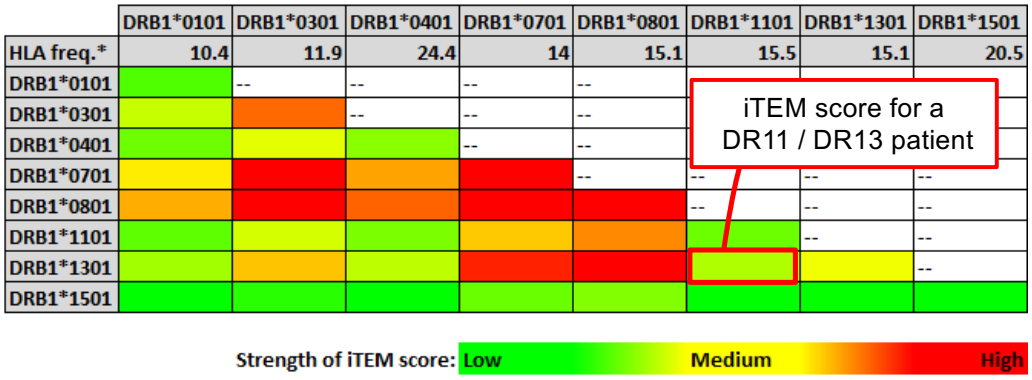
EpiMatrix protein score of **-20.5**

→ **low** immunogenic potential

… to individuals

iTEM scores ranging from **-62.99** to **8.63**

→ **low or high** immunogenic potential



* Southwood et al., J. Immunol. 1998;160;3363-3373

iTEM Heat Maps: for estimating HLA-specific risk

From global population…

EpiMatrix protein score of **-20.5**

→ **low** immunogenic potential **"non-immunogenic"**

… to individuals

iTEM scores ranging from **-62.99** to **8.63**

→ **low or high** immunogenic potential

Immunogenic in some patients!

	DRB1*0101	DRB1*0301	DRB1*0401	DRB1*0701	DRB1*0801	DRB1*1101	DRB1*1301	DRB1*1501
HLA freq.*	10.4	11.9	24.4	14	15.1	15.5	15.1	20.5
DRB1*0101		--	--	--	--	--		
DRB1*0301			--	--	--	--		
DRB1*0401				--		--		
DRB1*0701					--			
DRB1*0801								
DRB1*1101								
DRB1*1301								
DRB1*1501								

Strength of iTEM score: Low Medium High

iTEM has the potential to identify patients within human cohorts that have a **high potential to develop an immune response to a biologic/vaccine candidate…**

* Southwood et al., J. Immunol. 1998;160;3363-3373

iTEM Heat Maps: for estimating HLA-specific risk

From global population…

EpiMatrix protein score of **-20.5**

→ **low** immunogenic potential **"non-immunogenic"**

… to individuals

iTEM scores ranging from **-62.99** to **8.63**

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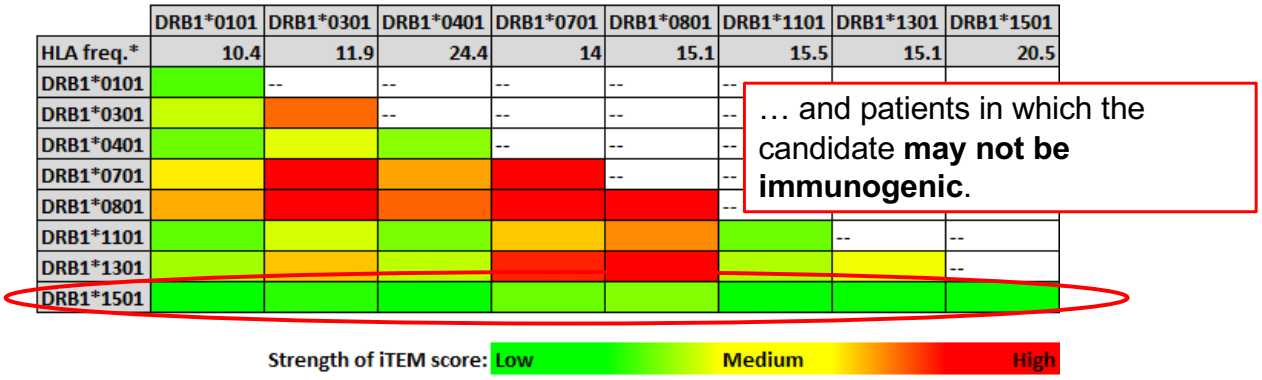
Immunogenic in some patients!



	DRB1*0101	DRB1*0301	DRB1*0401	DRB1*0701	DRB1*0801	DRB1*1101	DRB1*1301	DRB1*1501
HLA freq.*	10.4	11.9	24.4	14	15.1	15.5	15.1	20.5
DRB1*0101		--	--	--	--	--		
DRB1*0301			--	--	--	--		
DRB1*0401				--	--	--		
DRB1*0701					--	--		
DRB1*0801						--		
DRB1*1101							--	--
DRB1*1301								--
DRB1*1501								

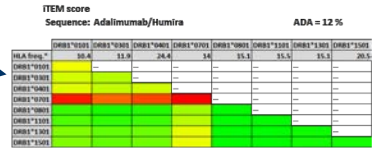
Strength of iTEM score: Low Medium High

… and patients in which the candidate **may not be immunogenic**.

* Southwood et al., J. Immunol. 1998;160;3363-3373

Case study:
Adalimumab

1. iTEM scores are generated for each patients

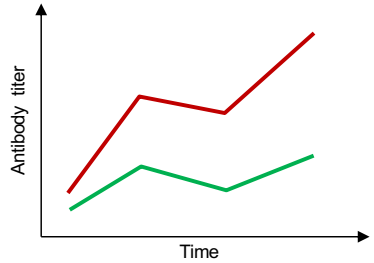


Adalimumab Heatmap

2. Patients are separated based on their associated immunogenic risk

High-risk patients (based on iTEM)

Low-risk patients (based on iTEM)

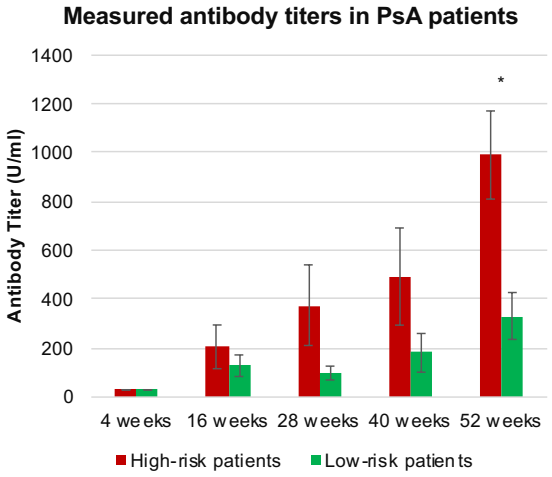


3. Average antibody titers are plotted through time

iTEM Analysis: Adalimumab study

High titers in Psoriatic Arthritis patients w/ high iTEM

Psoriatic Arthritis (PsA) patient group: N = 44



Findings:

(1) PsA patients with higher iTEM scores tend to have higher antibody titers.

(2) PsA patients who had low iTEM scores had lower antibody titers over time.

(3) This difference reached statistical significance at the later time point.

(4) High-risk patients developed ADA titers even while being on methotrexate.

iTEM / “PIMA” for Pompe Disease

EpiVax

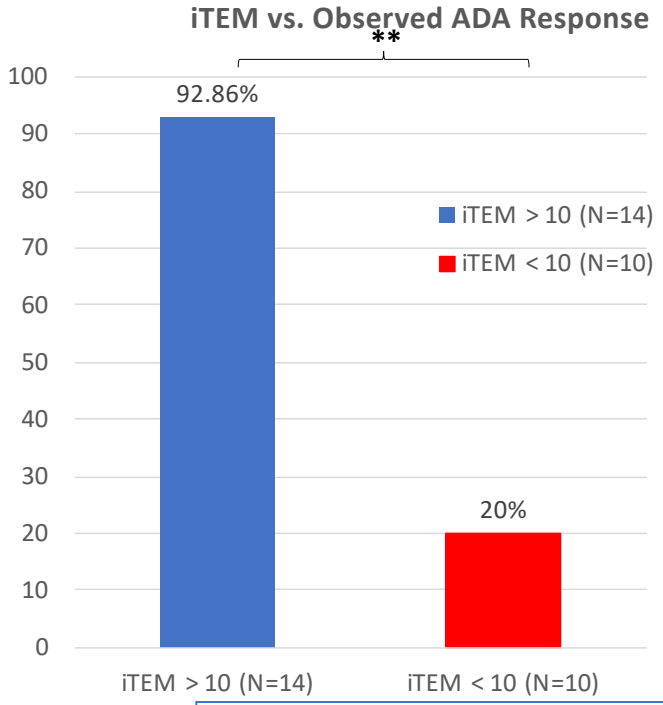
Immunogenicity is Personal

HLA- and Genotype-Based Risk Assessment Model to Identify Infantile Onset Pompe Disease Patients at High-Risk of Developing Significant Anti-Drug Antibodies (ADA)

De Groot AS[1*], Kazi ZB[2], Martin RF[1], Terry FE[1], Desai AK[2], Martin WD[1], Kishnani PS[2*]

PIMA: Personalized Immunogenicity Risk Assessment (Pompe, other “replacement” proteins)

Results of iTEM Analysis
Complete Cohort – CRIM-Positive & CRIM-Negative



For All Patients (n=24)	
Odds Ratio (Diff iTEM >10 vs Diff iTEM <10)	52

Odds of developing high ADA are 52 times higher in iTEM>10 patients compared to those with iTEM<10

p value = 0.0005
(Fisher's exact test)

Incorrect Low ADA predictions have dropped from roughly 1 in 2 (using only CRIM Status) to 1 in 5.

Immunogenicity is Personal

In Silico Identification of Putative Treg epitopes

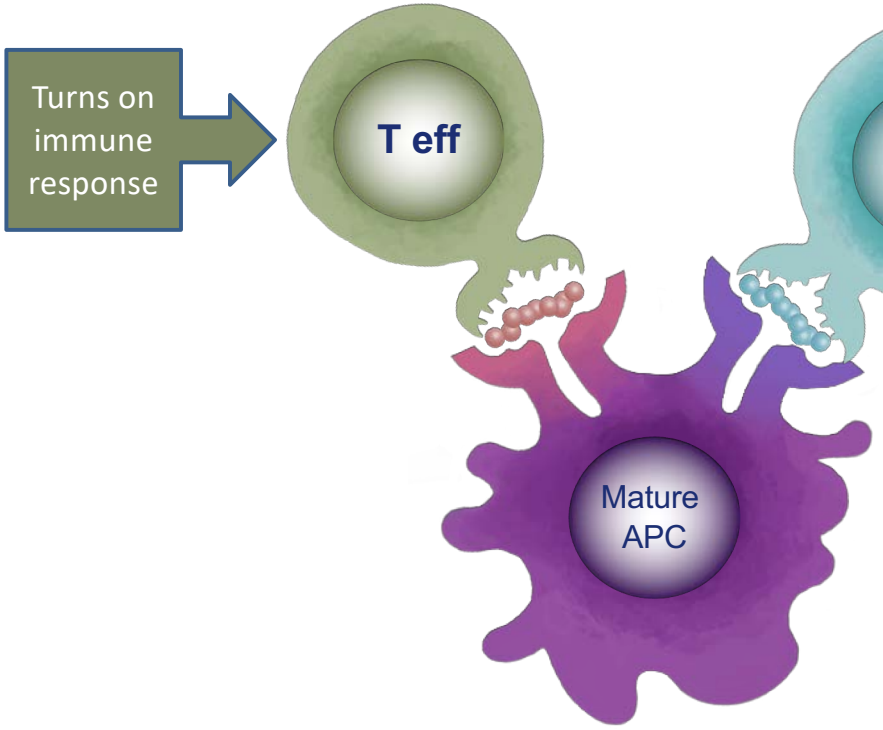
EpiVax

So, you say that immunogenic potential increases with increasing T cell epitope content,

What is the impact of Treg epitopes?

In Silico Tools for Characterizing Putative T Reg Epitopes

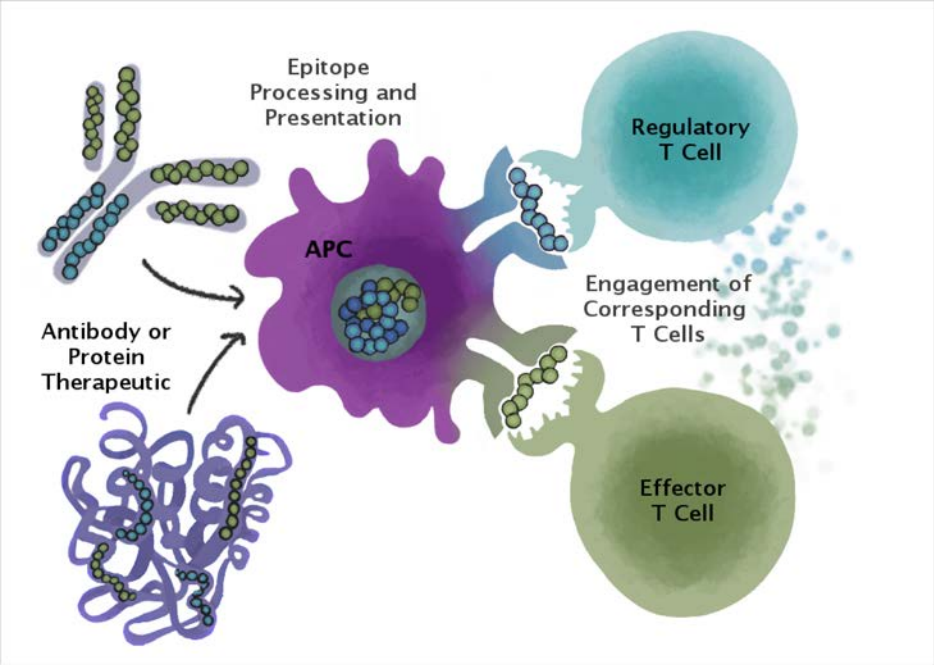
EpiVax



Epitopes can be *either* effector or regulatory

Most in silico algorithms cannot differentiate between these two

Published Treg epitopes in IgG: Tregitopes Also highly conserved by JanusMatrix



De Groot A.S., et al., Activation of Natural Regulatory T cells by IgG Fc-derived Peptide "Tregitopes". Blood, 2008,112: 3303. http://tinyurl.com/ASDeGroot-Blood-2008

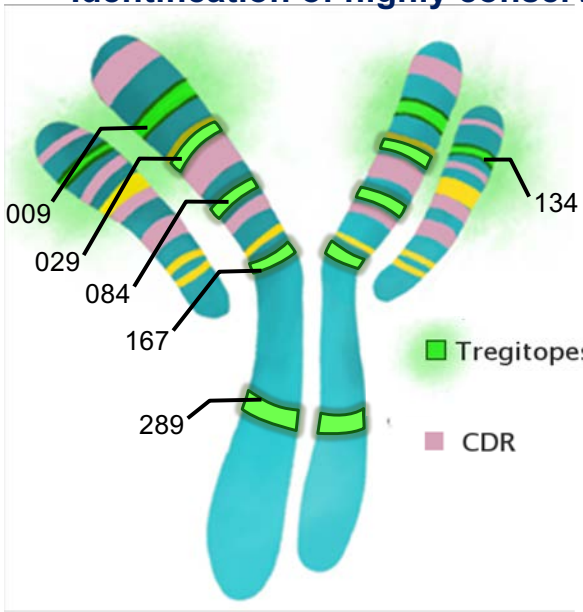
- Discovered & patented by EpiVax
- Highly conserved peptide sequences in Fc and Fab regions of antibodies
- High affinity, promiscuous binders across HLA alleles
- **One mechanism of action of IVIG?**
- **Activate antigen-specific regulatory T cells**
- Can be co-formulated or synthesized with therapeutic proteins or carriers

http://bit.ly/Treg1

Discovery of Treg + epitopes = Tregitopes In an Abundant Protein: IgG – Tolerizing Epitopes

EpiVax

Identification of highly conserved epitopes while screening Mabs



- **15-20 mer peptides in conserved regions**
- **Strong signals for T cells ("EpiBars")**
- **Highly conserved among IgG molecules**
- **Conserved across species (mouse…)**
- **One mechanism of action of IVIg?**
- **Induce natural Tregs to modify immune response … and expand iTregs in vitro and in vitro**

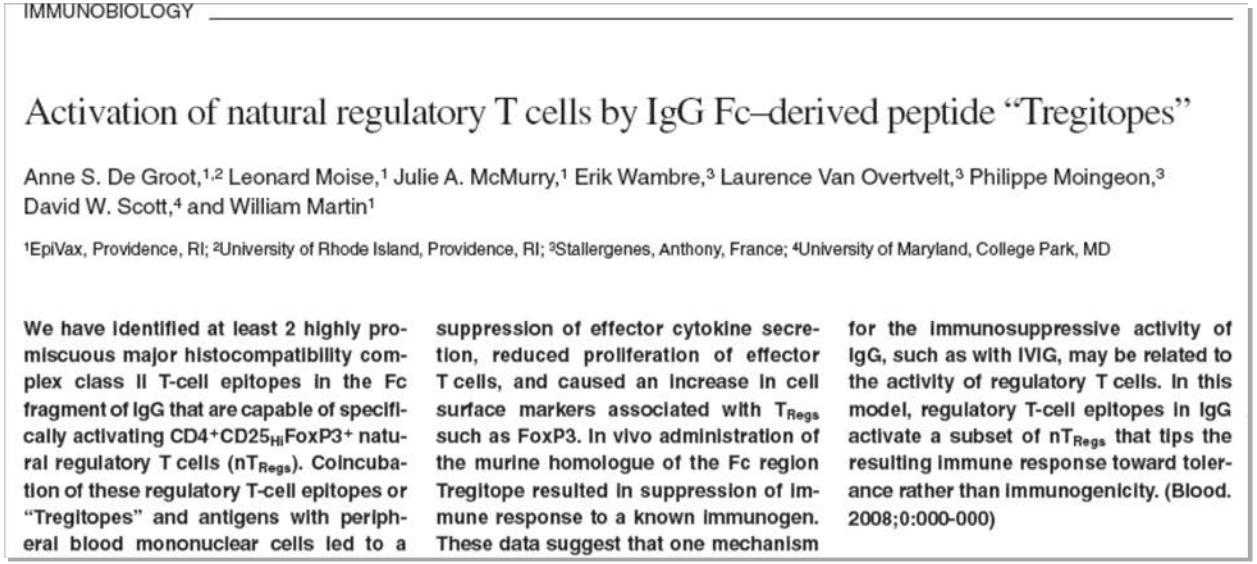
De Groot A.S., et al., Activation of Natural Regulatory T cells by IgG Fc-derived Peptide "Tregitopes". Blood, 2008,112: 3303. http://tinyurl.com/ASDeGroot-Blood-2008

Original Tregitope Publication

EpiVax

Published in Blood, 25 July 2008

Reprints available on request



IMMUNOBIOLOGY

Activation of natural regulatory T cells by IgG Fc–derived peptide "Tregitopes"

Anne S. De Groot,[1,2] Leonard Moise,[1] Julie A. McMurry,[1] Erik Wambre,[3] Laurence Van Overtvelt,[3] Philippe Moingeon,[3] David W. Scott,[4] and William Martin[1]

[1]EpiVax, Providence, RI; [2]University of Rhode Island, Providence, RI; [3]Stallergenes, Anthony, France; [4]University of Maryland, College Park, MD

We have identified at least 2 highly promiscuous major histocompatibility complex class II T-cell epitopes in the Fc fragment of IgG that are capable of specifically activating $CD4^+CD25_{Hi}FoxP3^+$ natural regulatory T cells (nT_{Regs}). Coincubation of these regulatory T-cell epitopes or "Tregitopes" and antigens with peripheral blood mononuclear cells led to a suppression of effector cytokine secretion, reduced proliferation of effector T cells, and caused an increase in cell surface markers associated with T_{Regs} such as FoxP3. In vivo administration of the murine homologue of the Fc region Tregitope resulted in suppression of immune response to a known immunogen. These data suggest that one mechanism for the immunosuppressive activity of IgG, such as with IVIG, may be related to the activity of regulatory T cells. In this model, regulatory T-cell epitopes in IgG activate a subset of nT_{Regs} that tips the resulting immune response toward tolerance rather than immunogenicity. (Blood. 2008;0:000-000)

http://bit.ly/Tregitope_API

Re-discovery of Tregitopes …*Thanks Alex Sette !*

EpiVax

Autoimmunity

http://informahealthcare.com/aut
ISSN: 0891-6934 (print), 1607-842X (electronic)

Autoimmunity, Early Online: 1–8

DOI: 10.3109/08916934.2015.1027817

informa
healthcare

ORIGINAL ARTICLE

Fine specificities of natural regulatory T cells after IVIG therapy in patients with Kawasaki disease

Jane C. Burns[1], Ranim Touma[1], Yali Song[1], Robert L. Padilla[1], Adriana H. Tremoulet[1], John Sidney[2], Alessandro Sette[2], and Alessandra Franco[1]

[1]Department of Pediatrics, Rady Children's Hospital, School of Medicine, University of California San Diego, La Jolla, CA, USA and
[2]Division of Vaccine Discovery, La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA

***Abstract**: The activation of natural regulatory T cells (nTreg) recognizing the heavy constant region (Fc) of IgG is an important mechanism of action of intravenous immunoglobulin (IVIG) therapy in Kawasaki disease (KD). Lack of circulating Fc-specific nTreg in the sub-acute phase of KD is correlated with the development of coronary artery abnormalities (CAA). Here, we characterize the fine specificity of nTreg in sub-acute (2- to 8-week post-IVIG) and convalescent (1- to 10-year post-IVIG) KD subjects by testing the immunogenicity of 64 peptides, 15 amino acids in length with a 10 amino acid-overlap spanning the entire Fc protein. About 12 Fc peptides (6 pools of 2 consecutive peptides) were recognized by nTreg in the cohorts studied, including two patients with CAA. To test whether IVIG expands the same nTreg populations that maintain vascular homeostasis in healthy subjects, we compared these results with results obtained in healthy adult controls. Similar nTreg fine specificities were observed in KD patients after IVIG and in healthy donors. These results suggest that T cell fitness rather than T cell clonal deletion or anergy is responsible for the lack of Fc-specific nTreg in KD patients who develop CAA. Furthermore, we found that adolescents and adults who had KD during childhood without developing CAA did not respond to the Fc protein in vitro, suggesting that the nTreg response induced by IVIG in KD patients is short-lived. Our results support the concept that peptide epitopes may be a viable therapeutic approach to expand Fc-specific nTreg and more effectively prevent CAA in KD patients.*

IVIG-derived Peptide Pool Reactions - Tregitopes

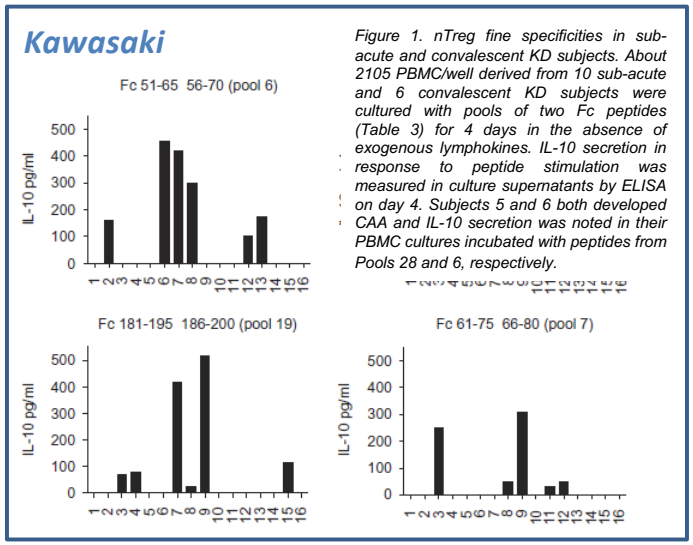


Figure 1. nTreg fine specificities in sub-acute and convalescent KD subjects. About 2105 PBMC/well derived from 10 sub-acute and 6 convalescent KD subjects were cultured with pools of two Fc peptides (Table 3) for 4 days in the absence of exogenous lymphokines. IL-10 secretion in response to peptide stimulation was measured in culture supernatants by ELISA on day 4. Subjects 5 and 6 both developed CAA and IL-10 secretion was noted in their PBMC cultures incubated with peptides from Pools 28 and 6, respectively.

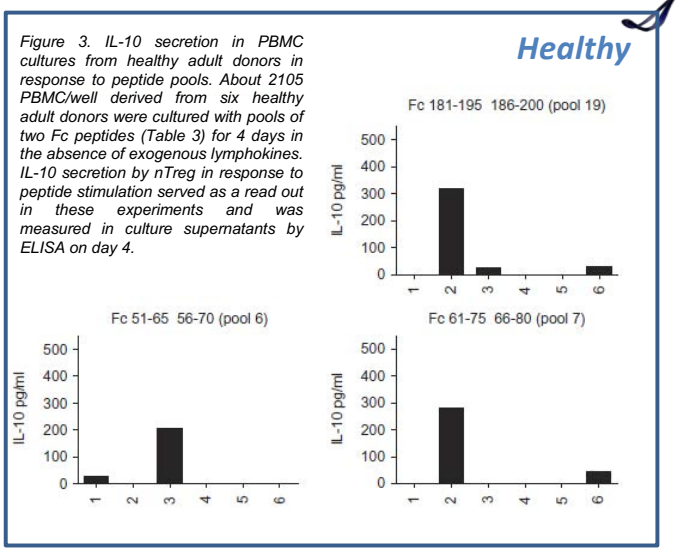


Figure 3. IL-10 secretion in PBMC cultures from healthy adult donors in response to peptide pools. About 2105 PBMC/well derived from six healthy adult donors were cultured with pools of two Fc peptides (Table 3) for 4 days in the absence of exogenous lymphokines. IL-10 secretion by nTreg in response to peptide stimulation served as a read out in these experiments and was measured in culture supernatants by ELISA on day 4.

Pool 5	41–55	NSGALTSGVHTFPAV
	46–60	TSGVHTFPAVLQSSG
Pool 6	51–65	TFPAVLQSSGLYSLS
	56–70	LQSSGLYSLSSVVTV
Pool 7	61–75	LYSLSSVVTVPSSSL
	66–80	SVVTVPSSSLGTQTY
Pool 8	71–85	PSSSLGTQTYICNVN
	76–90	GTQTYICNVNHKPSN
Pool 17	161–175	VDGVEVHNAKTKPRE
	166–180	VHNAKTKPREEQYNS
Pool 18	171–185	TKPREEQYNSTYRVV
	176–190	EQYNSTYRVVSVLTV
Pool 19	181–195	TYRVVSVLTVLHQDW
	186–200	SVLTVLHQDWLNGKE

Green underline indicates Tregitope 9-mer that was split
VVTVPSSSL (Pool 7) also has 3 hits but is not a green Tregitope on ISPRI

Peptide Pool Reactions – “Non”-Tregitope*

***not included in ISPRI / nor patents because HLA-restricted**

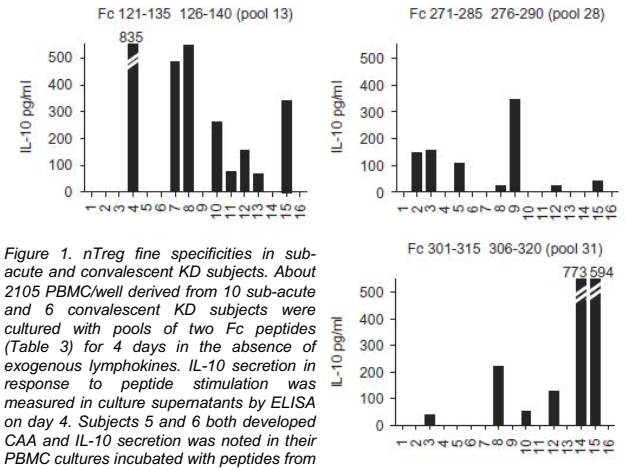


Figure 1. nTreg fine specificities in sub-acute and convalescent KD subjects. About 2105 PBMC/well derived from 10 sub-acute and 6 convalescent KD subjects were cultured with pools of two Fc peptides (Table 3) for 4 days in the absence of exogenous lymphokines. IL-10 secretion in response to peptide stimulation was measured in culture supernatants by ELISA on day 4. Subjects 5 and 6 both developed CAA and IL-10 secretion was noted in their PBMC cultures incubated with peptides from Pools 28 and 6, respectively.

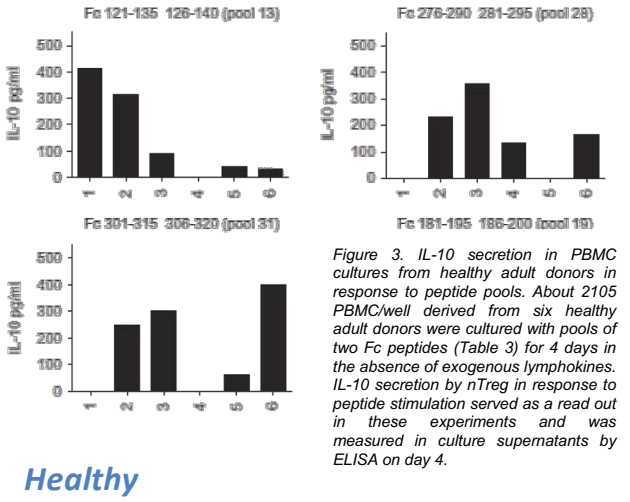


Figure 3. IL-10 secretion in PBMC cultures from healthy adult donors in response to peptide pools. About 2105 PBMC/well derived from six healthy adult donors were cultured with pools of two Fc peptides (Table 3) for 4 days in the absence of exogenous lymphokines. IL-10 secretion by nTreg in response to peptide stimulation served as a read out in these experiments and was measured in culture supernatants by ELISA on day 4.

These peptides each have between 1 and 3 EpiMatrix hits for HLA-DR alleles
None of these peptides have any predicted binding motifs (at top 5% cutoff) for HLA-DP and -DQ alleles on IEDB

Most are highly HLA restricted – and thus while, in principle, they are Treg epitopes, they are not Tregitopes (druggable Treg epitopes).

"We didn't know what we didn't' know" …
… But what we know now . . .

- ✓ Tregitope sequences are **highly conserved** in similar autologous proteins
- ✓ Almost all Tregitopes exhibit single 9-mer frames predicted by our EpiMatrix epitope prediction algorithm to bind to at least four different HLA DR alleles
  →likely to be **broadly recognized in the human population**
- ✓ Other possible Tregitopes exist in IgG (see Franco and Sette publication – these are more HLA restricted.
- ✓ In response to incubation with Tregitopes, (in vitro and in vivo) T cells exhibit a **T regulatory phenotype** ($CD4^+CD25^+FoxP3^+$)
- ✓ **Perhaps most important:** Co-incubation of Human T cells with Tregitopes and immunogenic peptides **inhibits effector T cell** (Teff) response, **suppresses antigen-specific secretion of effector cytokines and induces** **antigen-specific tolerance**.

Adjust for Treg epitopes when Measuring Immunogenic Potential

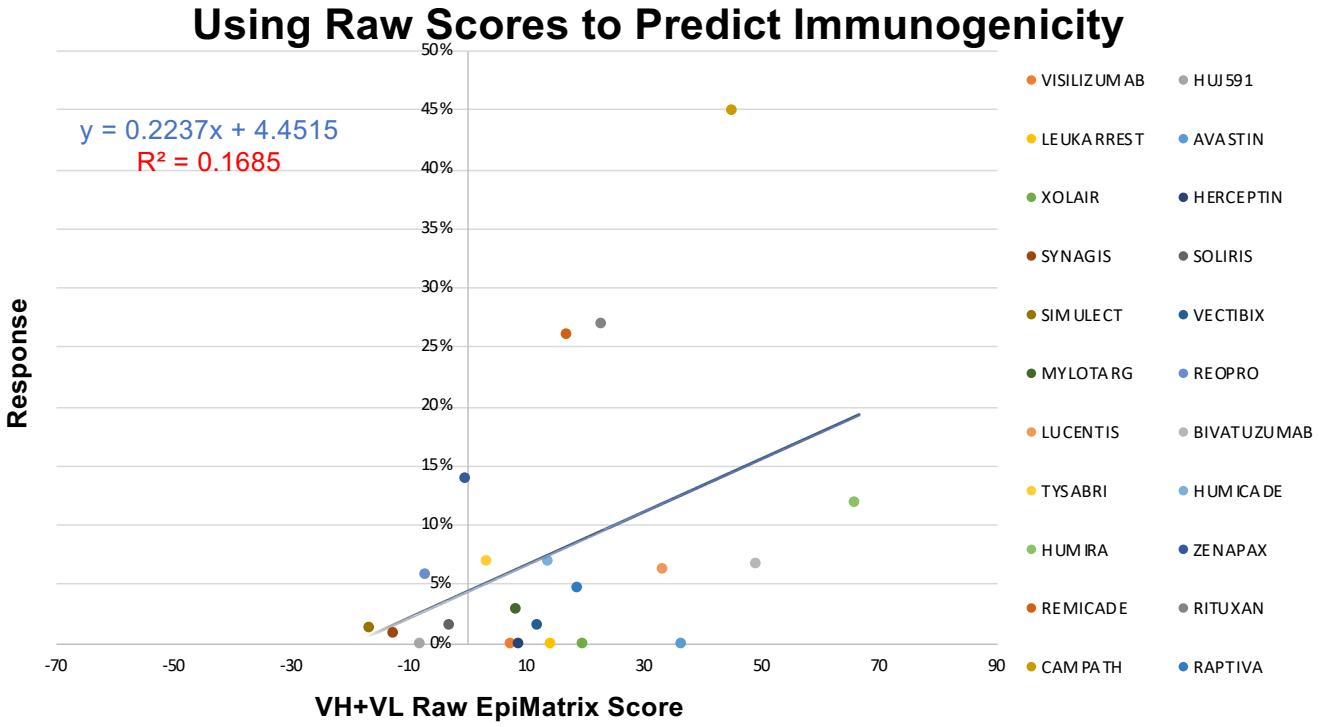
Peptides OR Antibodies:

1 + 1 - 1 = Response

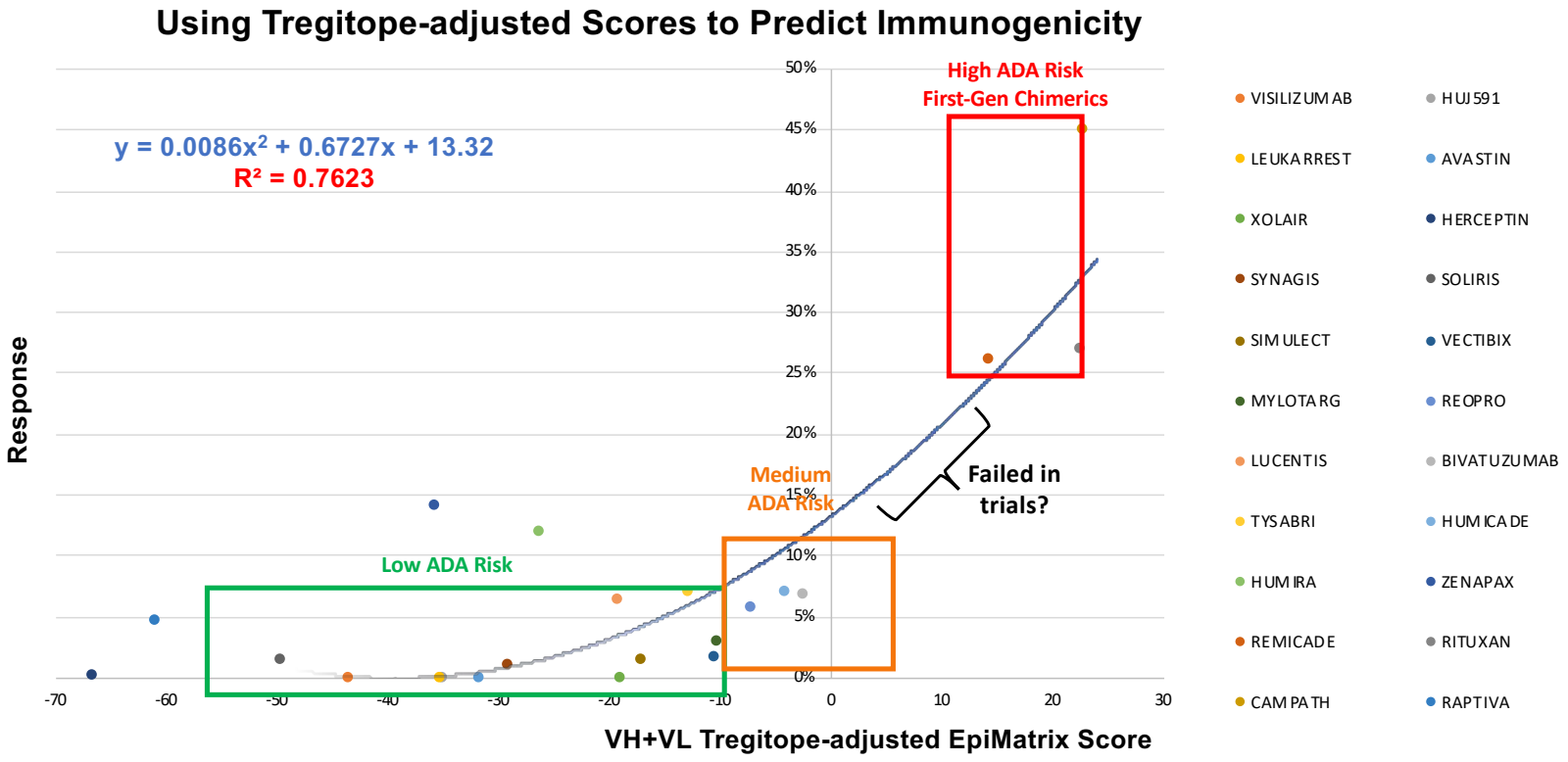
T cell response depends on:

T cell epitope content – Tregitope content + HLA of subject

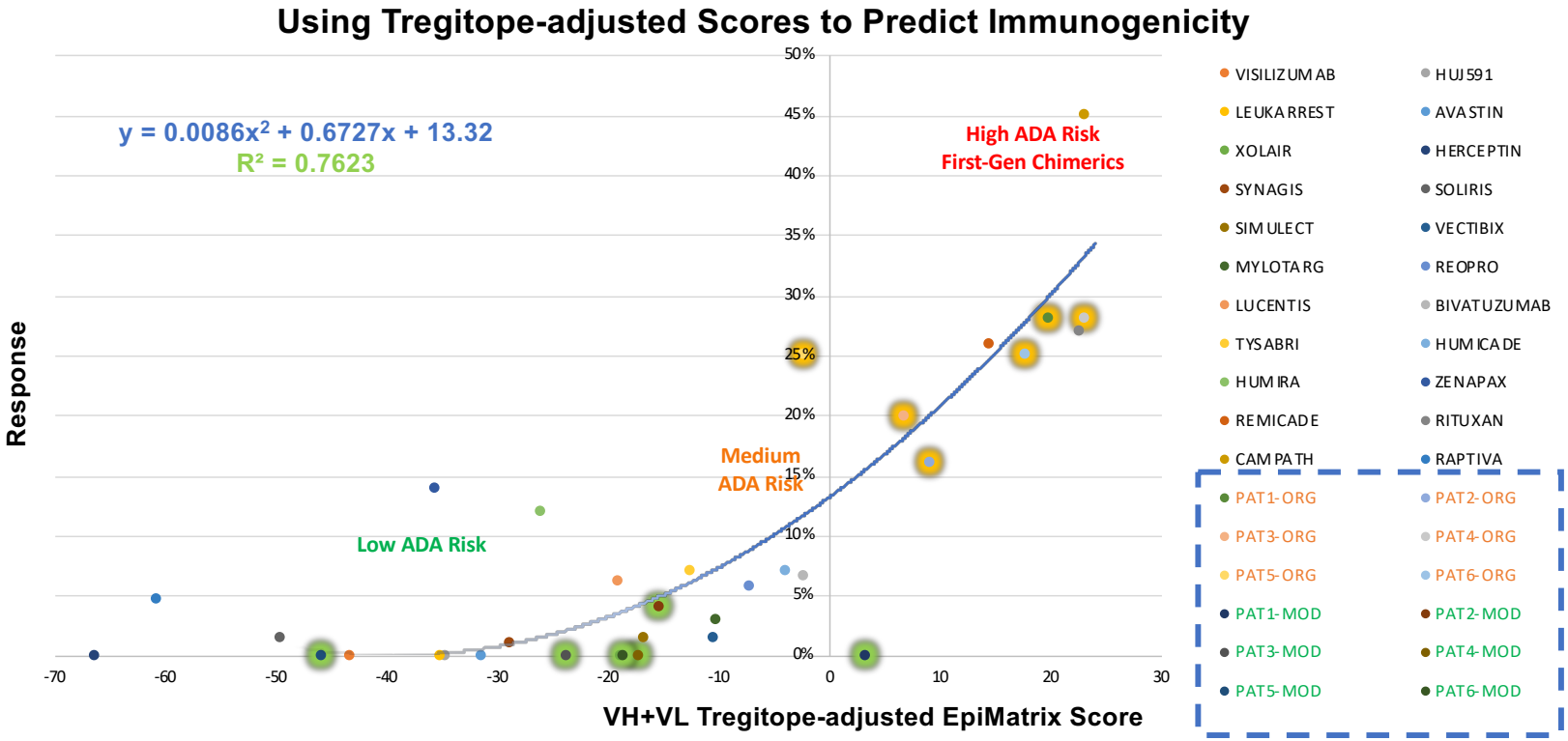
Can we assess antibody immunogenicity in silico? Without Tregitope Adjustment



Can we assess antibody immunogenicity in silico?
With Tregitope Adjustment Yes!

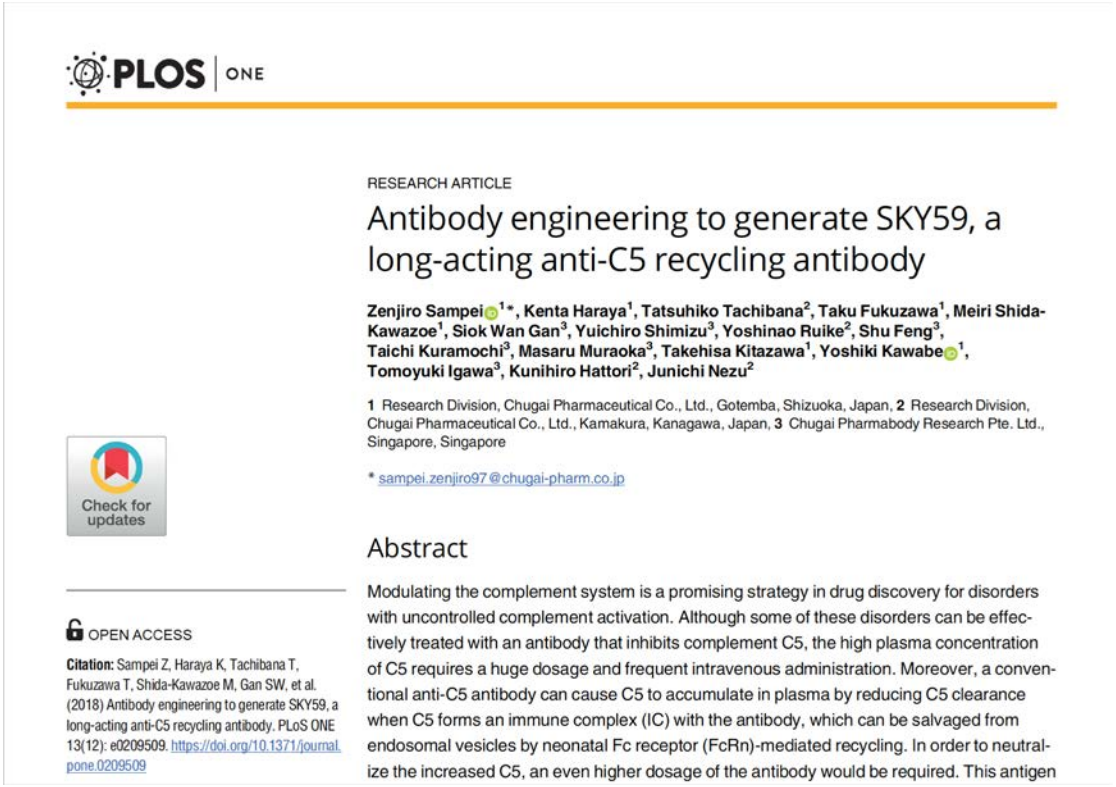


Can we predict antibody immunogenicity? Prospectively? Yes!



What happens when you don't correct for Tregitopes? Recent publication showing EpiBase vs. EpiMatrix (ISPRI)

EpiVax



PLOS ONE

RESEARCH ARTICLE

Antibody engineering to generate SKY59, a long-acting anti-C5 recycling antibody

Zenjiro Sampei[1]*, Kenta Haraya[1], Tatsuhiko Tachibana[2], Taku Fukuzawa[1], Meiri Shida-Kawazoe[1], Siok Wan Gan[3], Yuichiro Shimizu[3], Yoshinao Ruike[2], Shu Feng[3], Taichi Kuramochi[3], Masaru Muraoka[3], Takehisa Kitazawa[1], Yoshiki Kawabe[1], Tomoyuki Igawa[3], Kunihiro Hattori[2], Junichi Nezu[2]

1 Research Division, Chugai Pharmaceutical Co., Ltd., Gotemba, Shizuoka, Japan, **2** Research Division, Chugai Pharmaceutical Co., Ltd., Kamakura, Kanagawa, Japan, **3** Chugai Pharmabody Research Pte. Ltd., Singapore, Singapore

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Check for updates

OPEN ACCESS

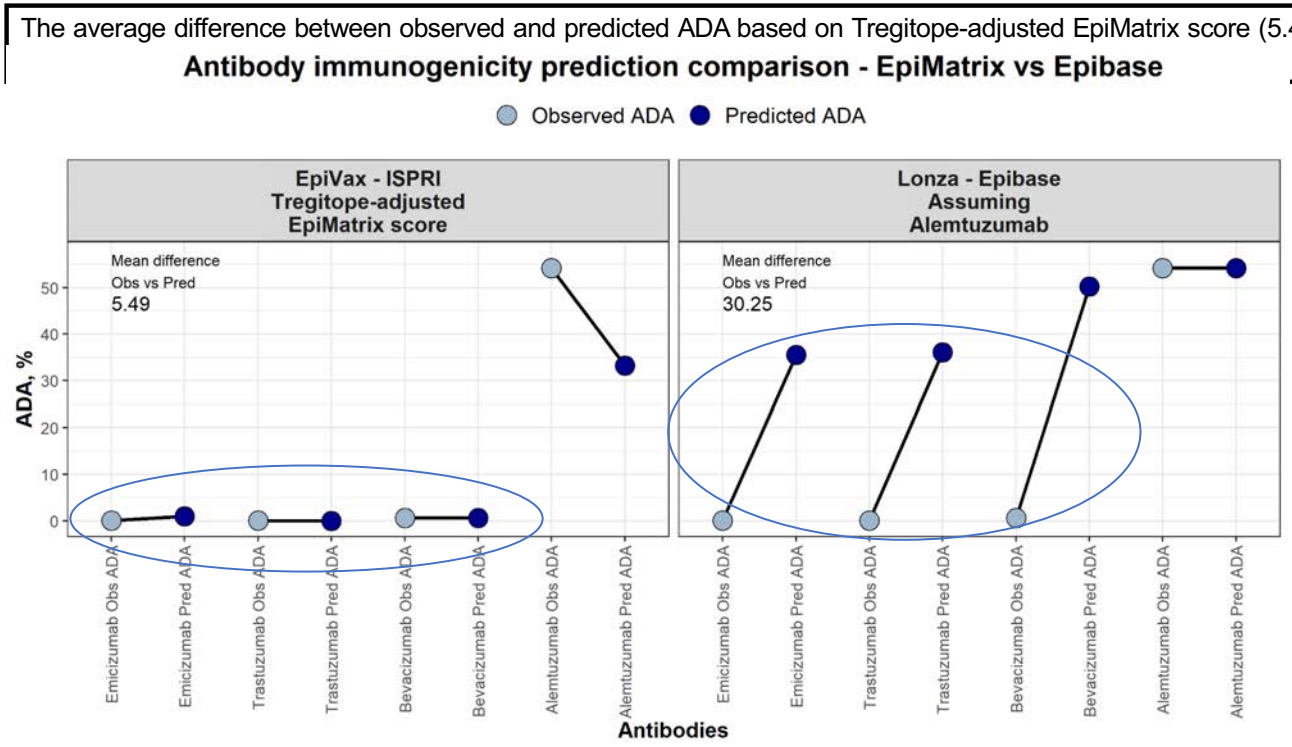
Citation: Sampei Z, Haraya K, Tachibana T, Fukuzawa T, Shida-Kawazoe M, Gan SW, et al. (2018) Antibody engineering to generate SKY59, a long-acting anti-C5 recycling antibody. PLoS ONE 13(12): e0209509. https://doi.org/10.1371/journal.pone.0209509

Abstract

Modulating the complement system is a promising strategy in drug discovery for disorders with uncontrolled complement activation. Although some of these disorders can be effectively treated with an antibody that inhibits complement C5, the high plasma concentration of C5 requires a huge dosage and frequent intravenous administration. Moreover, a conventional anti-C5 antibody can cause C5 to accumulate in plasma by reducing C5 clearance when C5 forms an immune complex (IC) with the antibody, which can be salvaged from endosomal vesicles by neonatal Fc receptor (FcRn)-mediated recycling. In order to neutralize the increased C5, an even higher dosage of the antibody would be required. This antigen

EpiVax antibody immunogenicity prediction is correct for the 4 mAbs (EpiBase prediction incorrect)

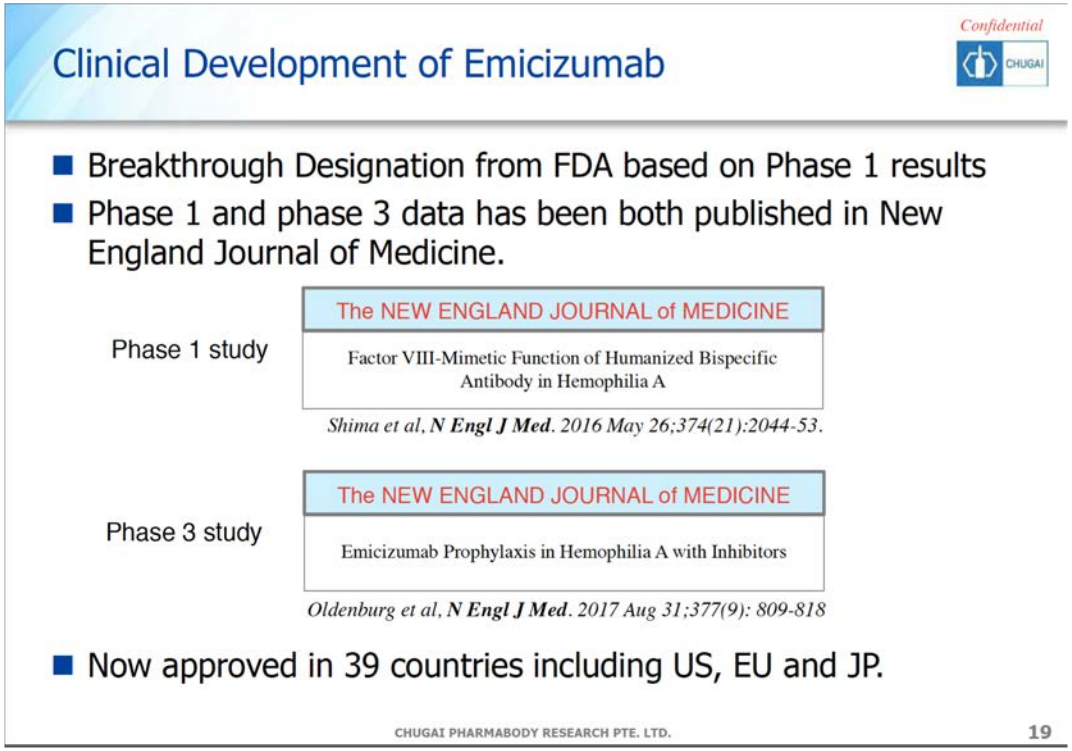
The average difference between observed and predicted ADA based on Tregitope-adjusted EpiMatrix score (5.49) is



Tregitope-adjusted EpiMatrix score more accurately predicted immunogenicity than EpiBase

Emicizumab – low immunogenicity antibody produced by Chugai using ISPRI

EpiVax



Tregitope-adjusted EpiMatrix score was used to advance this drug to the clinic: non immunogenic

After thorough comparison ISPRI vs. EpiBase Chugai chooses ISPRI: Press Release from 2014

EpiVax

Chugai Licenses ISPRI and OptiMatrix Platform from EpiVax for De-Risking Biologics

by Annie De Groot | Oct 29, 2014 | Blog, News |

Providence, Rhode Island. October 30, 2014

http://epivax.com/blog/chugai-licenses-ispri-and-optimatrix-platform-from-epivax-for-de-risking-biologics

Take away: Adjust for Treg epitopes when Measuring Immunogenic Potential

Peptides OR Antibodies:

1 + 1 - 1 = Response

T cell response depends on:

T cell epitope content – Tregitope content + HLA of subject

Relevance of Tregitope to monoclonal antibodies

EpiVax

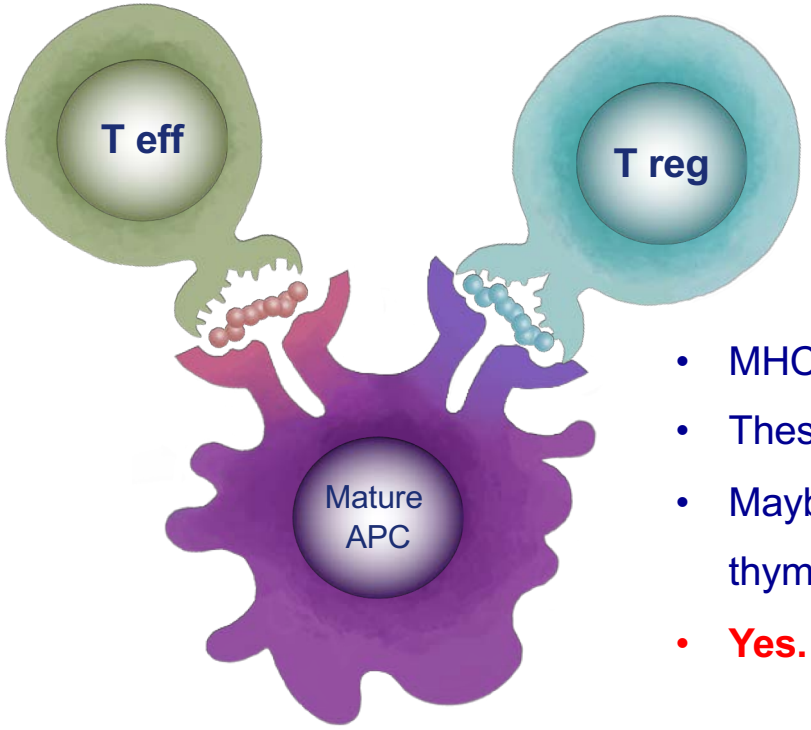
- Tregitopes can be found in mAb sequences
- Correcting “predicted immunogenicity” for Tregitopes improves predictions
- Impact of HLA-restriction on T eff and T reg response is still important
- Retrospective and prospective correlations are published.

2014 FDA Guideline:
. . . Tregitopes tolerize - do not remove Treg epitopes

Additional advanced analyses of primary sequence are also likely to detect HLA class II binding epitopes in nonpolymorphic human proteins. Such epitopes may elicit and activate regulatory T-cells, which enforce self-tolerance, or, opposingly, could activate T-helper (Th) cells when immune tolerance to the endogenous protein is not robust (Barbosa and Celis 2007; Tatarewicz et al. 2007; De Groot et al. 2008; Weber et al. 2009). However, if considered appropriate, engineering of changes to the primary sequence to eliminate immunogenic Th cell epitopes or addition of tolerogenic T-cell epitopes should be done cautiously, because these modifications may alter critical product quality attributes such as aggregation, deamidation, and oxidation and thus alter product stability and immunogenicity. Therefore, extensive evaluation and testing of

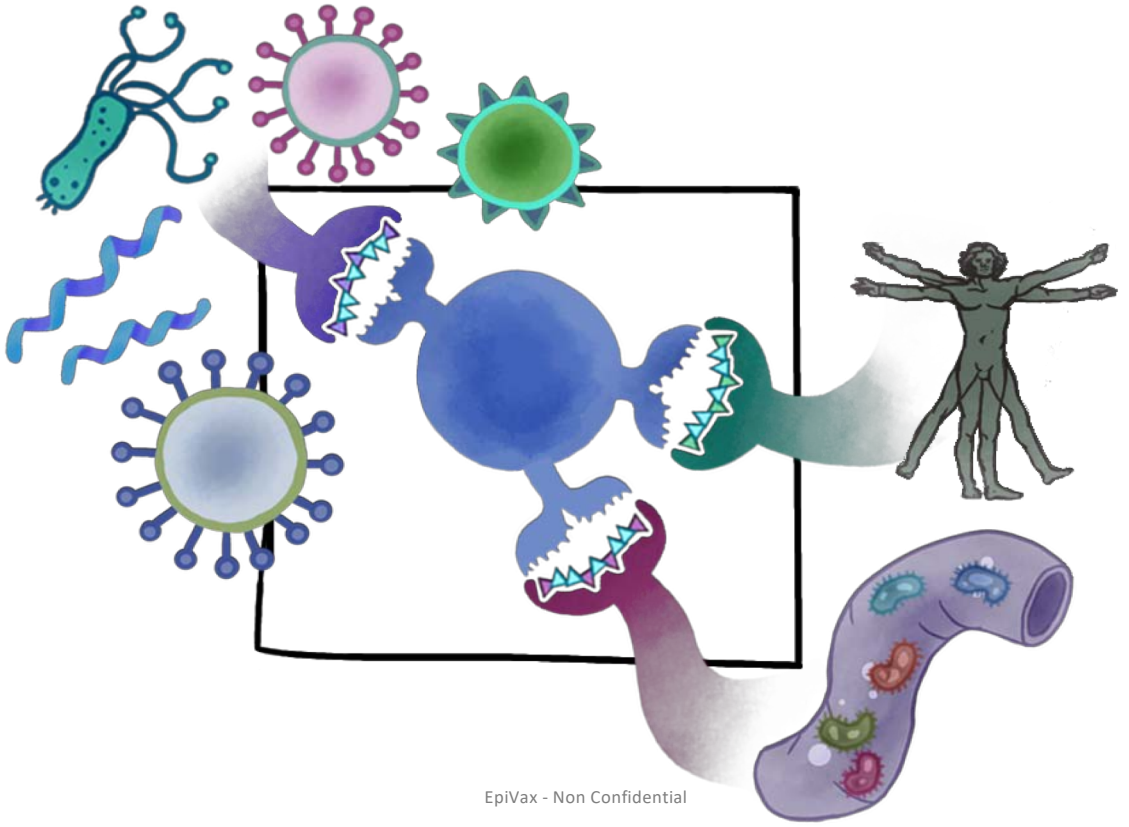
References are to work done by EpiVax Group

You asked: “Why are they Treg epitopes?” We answered . . .



- MHC binding is the same. Not weaker or stronger.
- These epitopes are present in prevalent proteins.
- Maybe there are ‘natural’ T regs trained in the thymus that are reinforced in the periphery?
- **Yes.**

Novel Discovery – Treg Epitopes in Pathogens Immune Camouflage

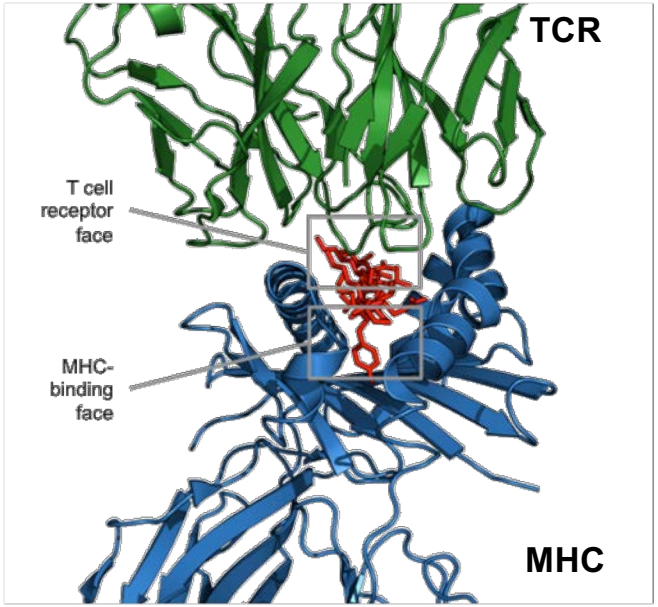


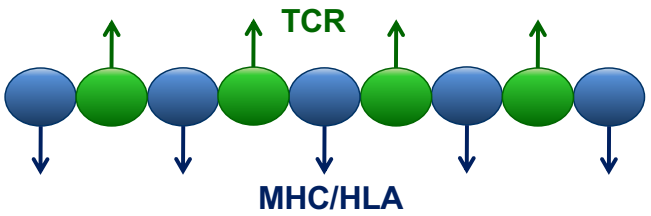
JanusMatrix 2013
A New Way to Search for Homology with Self

Each MHC ligand has two faces:

1. The MHC-binding face (agretope) and
2. The TCR-interacting face (epitope)

JanusMatrix is designed to predict the potential for cross-reactivity between epitope clusters and the human genome, based on conservation of TCR-facing residues in their putative HLA ligands.





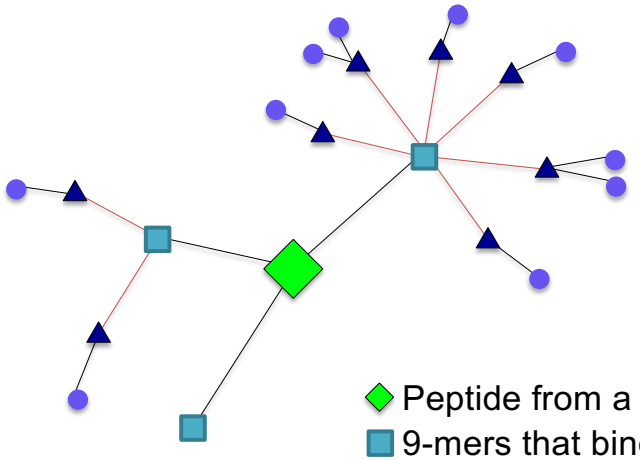
JanusMatrix

Find predicted 9-mer ligands with:

- **Identical T cell-facing residues**
- **Same HLA allele and minimally different MHC-facing residues**

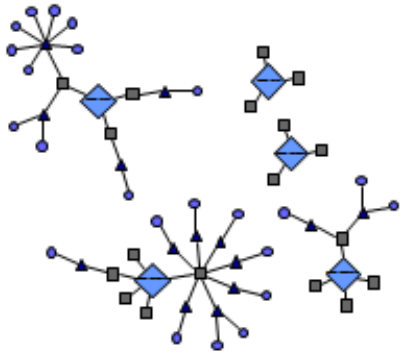
Moise L et al. Hum Vaccin Immunother. 2013 Jul;9(7):1577-86

Networks used to provide visual map of epitope cross-conservation

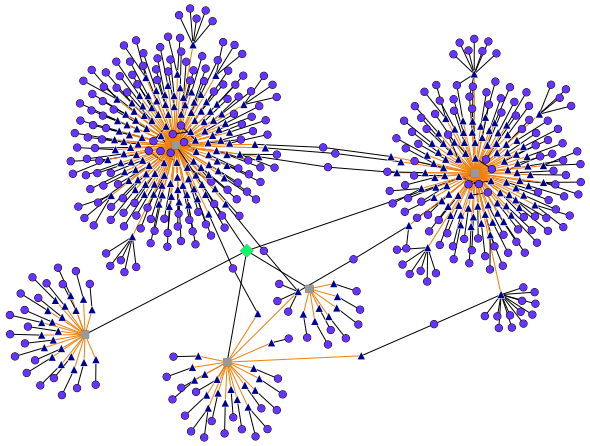


- Peptide from a drug, antigen, etc.
- 9-mers that bind HLA
- 9-mers from human genome that present same TCR face
- Source proteins of the human 9-mers

Published example from HCV
Teff vs. Treg epitopes identified by JanusMatrix



HCV Vaccine epitopes example
All Induce T effector response



HCV epitope
Induced Treg response

Source (pathogen) protein

Losikoff PT, Mishra S, Terry F, Gutierrez A, Ardito MT, Fast L, Nevola M, Martin WD, Bailey-Kellogg C, De Groot AS, Gregory SH. **HCV Epitope, Homologous to Multiple Human Protein Sequences, Induces a Regulatory T Cell Response in Infected Patients.** J Hepatol. 2014 Aug 22. pii: S0168-8278(14)00613-8. doi: 10.1016/j.jhep.2014.08.026.

Relevance to Biologics – EIP – AbiRisk Study
Non-IgG Tolerated Epitope in Infliximab

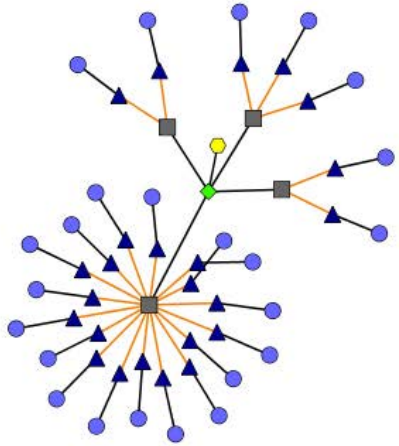
IL-10–Producing Infliximab-Specific T Cells Regulate the Antidrug T Cell Response in Exposed Patients

Alessandra Vultaggio,* Francesca Nencini,† Sara Pratesi,† Daniele Cammelli,*
Maria Totaro,* Sergio Romagnani,† Enrico Maggi,† and Andrea Matucci*
on behalf of the ABIRISK Consortium

Infliximab (IFX) is a chimeric mAb that can lead to the appearance of anti-drug Abs. Recent research has identified the presence of circulating IFX-specific T cells in treated patients. The aim of the study was to analyze the functional characteristics of IFX-specific T cells, in particular their capability to produce biologically active regulatory cytokines. Drug-stimulated PBMCs or coculture systems were used to detect memory T cells in treated patients. The cytokines produced by IFX-specific T cells, T cell lines, and T cell clones were evaluated at the mRNA and protein levels. Drug infusion induced an increase in IL-10 serum levels

AIM: "Analyze the functional characteristics of IFX-specific T cells, in particular their capability to **produce biologically active regulatory cytokines"**
METHODS: "Drug-stimulated PBMCs or coculture systems were used to detect memory T cells in treated patients. The cytokines produced by IFX-specific T cells, T cell lines, and T cell clones were evaluated at the mRNA and protein levels"
CONCLUSIONS: "Drug infusion induced an increase in IL-10 serum levels in vivo, whereas other cytokines were unchanged…IFX-specific T cells as a source of biologically active IL-10 and suggest interference by IL-10–producing cells in the detection of drug-specific T cells

JanusMatrix Human Homology Score*: 2.94



*in the context of eight HLA-DR alleles

EpiVax - confidential

MAPPS vs. ISPRI -Predicted Epitopes
MAPPS does not define PHENOTYPE of response

Annette Karle – Months of hard work!

MAPPS assays give patient-level data.

In silico analysis is fast and gives a very good assessment of immunogenicity risk.

In silico data can provide putative phenotype and population-level risk.

MAbs. 2016 Apr; 8(3): 536–550.
Published online 2016 Jan 28.
doi: 10.1080/19420862.2015.1136761
PMCID: PMC4966846

Secukinumab, a novel anti–IL-17A antibody, shows low immunogenicity potential in human in vitro assays comparable to other marketed biotherapeutics with low clinical immunogenicity

Anette Karle, Sebastian Spindeldreher, and Frank Kolbinger

Author information ▶ Article notes ▶ Copyright and License information ▶

EpiMatrix, ClustiMer and JanusMatrix put to use in a recent study by Diane Montgomery of Merck

secukinumab (COSENTYX) anti-IL17A: in silico

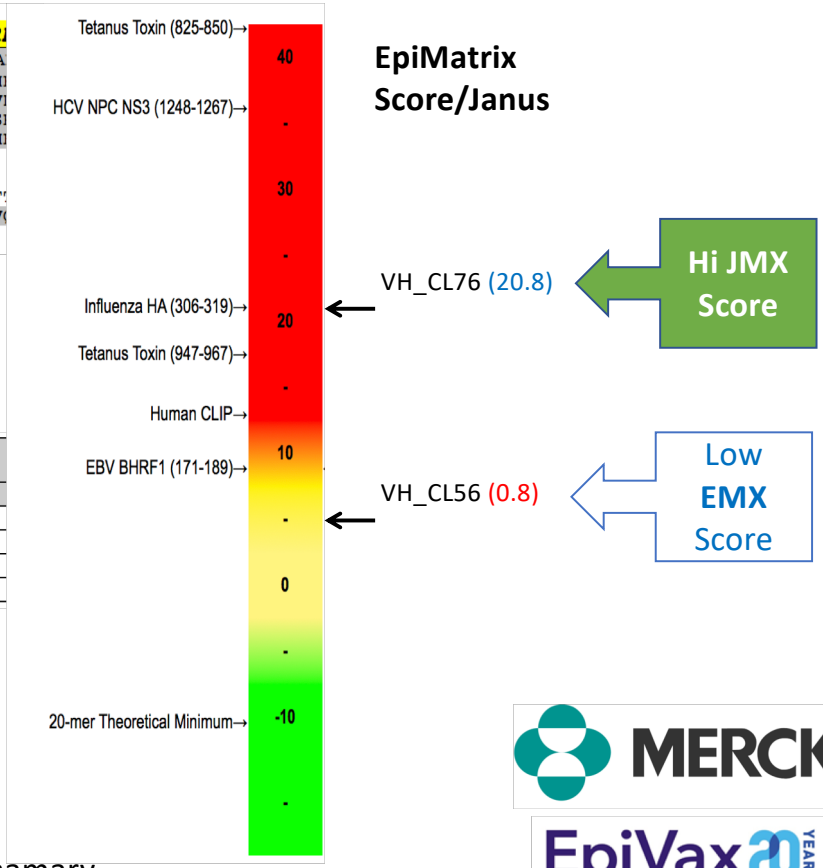
```
>secukinumab_H COSENTYX_aIL17a
EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMNWVRQAPGKGLEWVAAINQDGSEKYYVGSVKGRF
SRDNAKNSLYLQMNSLRVEDTAVYYCVRDYYDILTDYYIHYWYFDLWGRGTLVTVSSASTKGPSVFPLA
KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH
NTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
HNHYTQKSLSLSPGK
>secukinumab_L COSENTYX_aIL17a
EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDF
ISRLEPEDFAVYYCQQYGSSPCTFGQGTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
```

yellow = epitopes able to bind at least four HLA-DR alleles
bold underlined =clusters of HLA DR binding epitopes
red = CDRs (enhanced chothia method)

VH_CL76 **Homology to Human**

SEQUENCE	% IDENTITY	% SIMILARITY	FILE	DESCRIPTION
KNSLYLQMNSLRVEDT	--	--	A2_CL	--
----------A---	94%	97%	P01762	Immunoglobulin heavy variable 3-11 OS=Homo sapiens OX=9606 G...
----------T---	94%	97%	A0A0B4J1X8	Immunoglobulin heavy variable 3-43 OS=Homo sapiens OX=9606 G...
--T---------A---	88%	95%	P01767	Immunoglobulin heavy variable 3-53 OS=Homo sapiens OX=9606 G...
----------NT---	88%	95%	A0A0B4J1Y9	Immunoglobulin heavy variable 3-72 OS=Homo sapiens OX=9606 G...

Immunogenicity risk
CL_76 : low **(High janus score)**
CL56: low **(low immunogenicity score)**
Secukinumab: low (observed <1%)



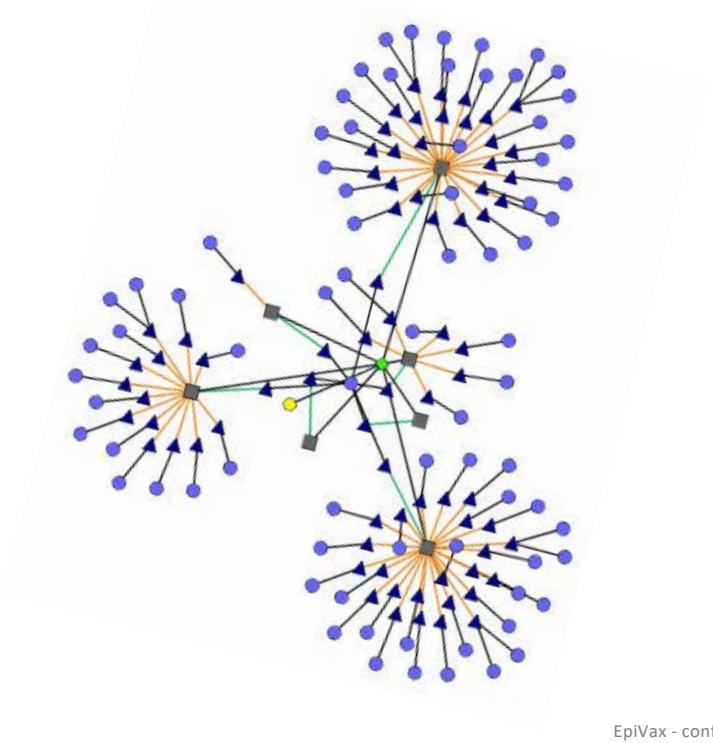
Presentation by D. Montgomery at PEGS Analysis: Jad Maamary

MERCK

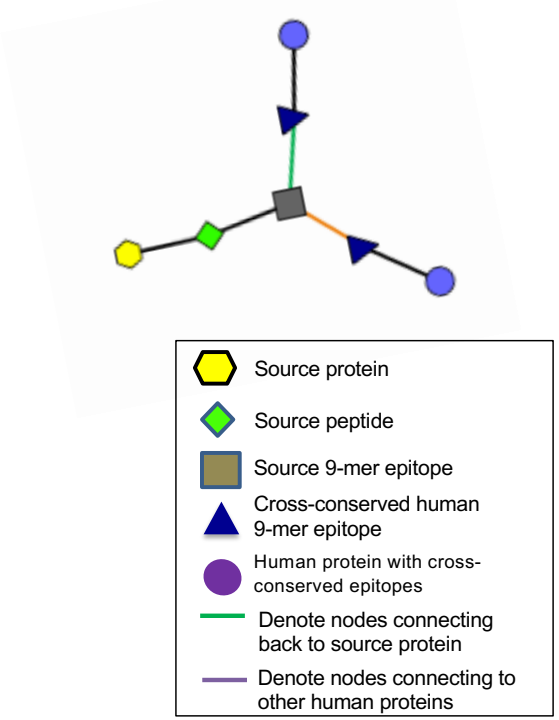
EpiVax 20 YEARS Fearless Science

Also Relevant to Cancer
Melan A (MAR1, Uniprot ID: Q16655)

Peptide 1; JanusMatrix Score: 9.37

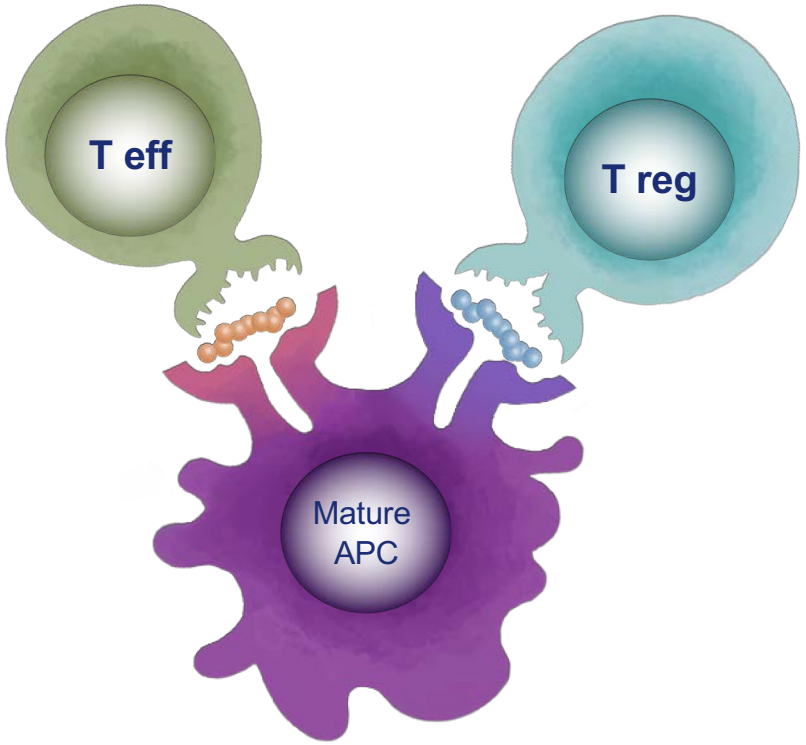


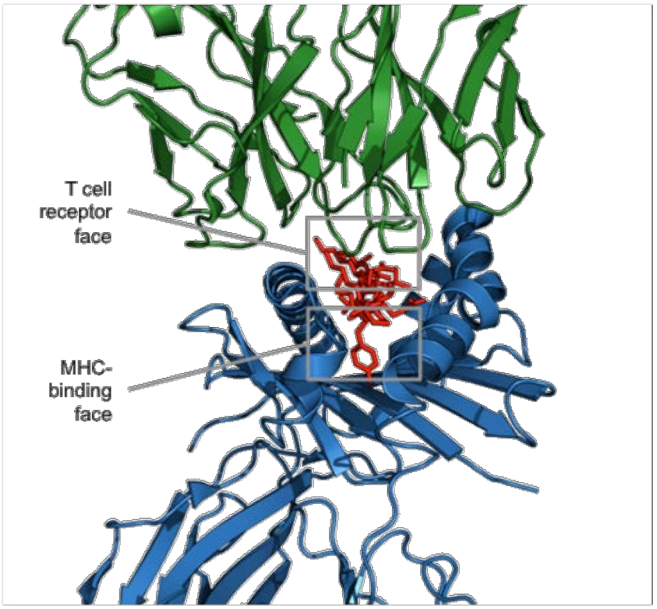
Peptide 2; JanusMatrix Score: 1.14



iTEM to find HLA restricted tolerance
+ JanusMatrix to find Treg/Tolerated epitopes = J-iTEM

EpiVax





J-iTEM
Janus adjusted Individualized T cell epitope Measure

EpiVax

For each volunteer, we calculated a J-iTEM score for that peptide.
Example shown for volunteer XXX for peptide 48

Protein ID	Protein D					DRB1 0101	DRB1 *0301	DRB1 *0401	DRB1 *0701	DRB1 *0801	DRB1 *0901	DRB1 *1101	DRB1 *1301	DRB1 *1501
RH5_305-326 (Peptide 48)						DB Ver: September 03, 2017					EpiMatrix Ver: 1.2			
						DRB1 0101	DRB1 *0301	DRB1 *0401	DRB1 *0701	DRB1 *0801	DRB1 *0901	DRB1 *1101	DRB1 *1301	DRB1 *1501
						-0.61	-0.24	-0.78	-1.15	0.58	-1.02	0.67	-0.01	-0.6
		306	EYNTKKKKL		0	0.46	-0.18	-0.64	0.15	-0.87	0.65	0.22	0.85	0.54
		307	YNTKKKKLI		7	1.19	1.75	-0.11	1.65	2.8	1.66	.89	2.38	0.82
sp|P56559|ARL4C_HUMAN	ADP-ribosylation factor-like protei					1.53	1.21	1.94	1.81	0.93	1.11	.72	1.18	0.38
sp|A2A2Z9|AN18B_HUMAN	Ankyrin repeat dom					2.65	1.27	1.76	1.89	2.26	1.34	.72	1.69	1.57
sp|Q8IVF6|AN18A_HUMAN	Ankyrin repeat don					2.65	1.27	1.76	1.89	2.26	1.34	.72	1.69	1.57
sp|Q9P2D7|DYH1_HUMAN	Dynein heavy chai					1.07	1.42	0.7	1.87	1.3	1.9	.24	1.59	1.9
sp|Q6ZWJ1|STXB4_HUMAN	Syntaxin-binding p					1.48	0.79	0.6	2.18	1.35	0.83	.34	1.21	1.37
sp|P62487|RPB7_HUMAN	DNA-directed RNA					0.83	0.78	0.32	1.95	0.82	1.68	.11	1.18	0.24
sp|Q15911|ZFHX3_HUMAN	Zinc finger homeob					0.92	1.22	-0.1	0.87	2.42	0.11	.36	1.64	0.67
						-0.56	0.64	-0.44	-0.65	1.59	-0.79	1.32	1.23	-0.08
						-0.49	-0.28	-0.94	0.11	1.26	-0.65	0.26	0.15	-0.02
						-0.12	0.33	-0.68	1.7	0.97	-0.09	0.1	0.99	-0.39
		311	KKKLIKCIK		0	0.67	0.11	-0.2	0.03	0.92	-0.43	1.98	0.21	0.59
		312	KKLIKCIKN		0	0.58	-0.3	1.5	0.74	0.41	0.14	0.76	0.03	0.6
						-1	-0.47	-0.5	0.44	1.27	0.25	0.3	0.2	-1.24

DRB1*0701 iTEM = 1.7 + (1.65 / 2) = 2.52

DRB1*0901 iTEM = 1.66

0701/0901 iTEM = 2.52+1.66= 4.18

These two hits each have 2 or more cross-conserved hits with the human genome i.e. JMX=2; could be tolerated/actively tolerogenic

To calculate J-iTEM, we remove these hits from the calculation, but deductions are maintained.

DRB1*0701 J-iTEM = 1.7 + ~~(1.65 / 2)~~ = 1.7

DRB1*0901 J-iTEM = ~~1.66~~

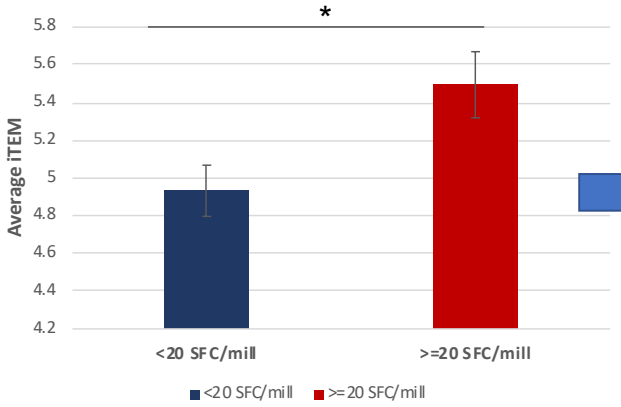
0701/0901 J-iTEM = 1.7

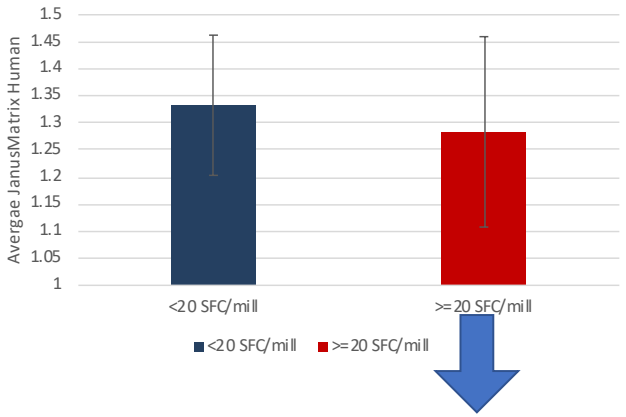
USAID analysis for Malaria Study (Leidos/Oxford

J-iTEM Clinical Results –Assay Data T cells / Oxford (Malaria Vaccine)

EpiVax

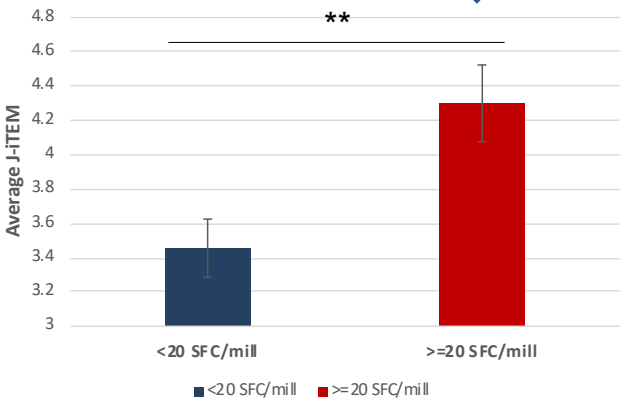
Standard iTEM





JMX

Without iTEM



J-iTEM

iTEM + JMX

- **288 T cell assays. # <20SFC = 168; # ≥20SFC = 120**
- iTEM scores of **negative responses** <20 are lower than iTEM scores of **positive responses** ≥ 20. p<0.05
- **JanusMatrix** scores of **negative responses** <20 **are higher** than JMX scores of positive responses ≥ 20. (ns)
- **Combined J-iTEM scores of responses <20 are significantly lower than J-iTEM scores of responses ≥ 20. p<0.01**
- **Simon Draper, Oxford**

UNIVERSITY OF OXFORD

Take home message from last section: Individual Tolerance modifies Personal Immunogenicity Risk

EpiVax

- In silico tools now predict T effector and T reg epitopes
- Each person's HLA may define whether Teff or Treg response dominates.
- Personalized Immunogenicity Risk Assessment is Feasible.
- In Vitro Assays (Treg/Teff) can be used to validate predictions.

Last section of this talk

EpiVax

- Improving “Quality by Design” using Immune Engineering

Immune Engineering – for biologics

EpiVax

Reduce immunogenicity by engineering proteins that

- ***Remove T cell epitopes*** – reduce epitopes that induce CD4+ T cell epitopes that augment antibody responses.

  → *Engineer **out** effector T cell epitopes*

- ***Induce Treg response*** – retain or introduce epitopes that induce CD4+ Treg responses that suppress protective antibody and cellular responses.

  → ***Engineer in regulatory T cell epitopes***

Immune Engineering – for vaccines

EpiVax

Enhance immunogenicity by engineering proteins that

- ***Induce good (T) memories*** – add epitopes that induce CD4+ T cell memory responses to augment antibody and cellular responses.

  → *Engineer **in** effector T cell epitopes*

- ***Recall no bad (Treg) memories*** – remove epitopes that induce CD4+ Treg responses that suppress protective antibody and cellular responses.

  → ***Engineer out regulatory T cell epitopes***

Immune Engineering Vaccines – Avian Flu
Treg epitope discovered – 3 Amino Acids Modified

EpiVax

	297	309	
H7-HA	P R Y V K Q **R S** L **L** L A T		Treg Epitope
	306	318	
H3-HA	P R Y V K Q **N T** L **K** L A T		T Eff Epitope
	297	309	
H7-HA-Opt1	P R Y V K Q **N T** L **K** L A T		T Eff Epitope

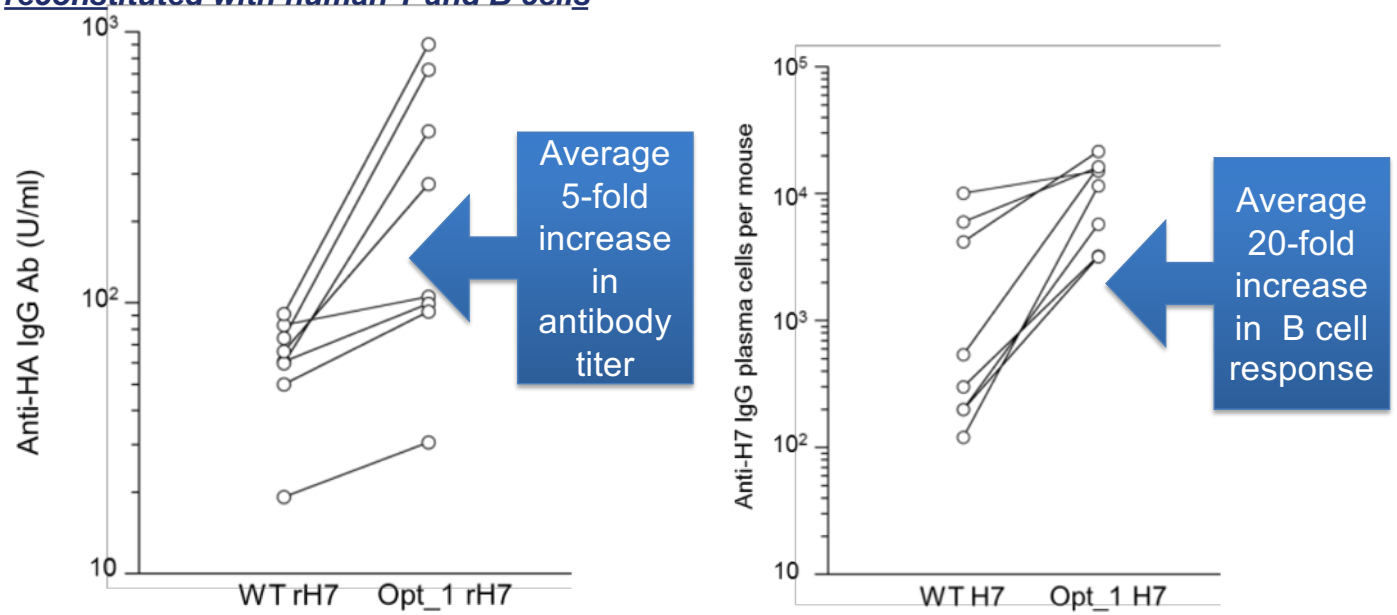
simultaneous Treg epitope knock-out and Teff epitope knock-in

Wada *et al. Sci Rep.* 2017; 7(1):1283

Epitope-Enhanced H7 HA Antigenicity

"Opt_1 rH7 HA" Optimized with 3 AA changes – Tested in mice by NIID

Opt_1 rH7-HA is better at boosting anti-H7 B cell responses than WT rH7-HA in SCID mice reconstituted with human T and B cells



(Study performed in collaboration with NIID Japan)

Wada *et al. Sci Rep.* 2017; 7(1):1283

Remove Treg Epitopes and Make Better Vaccines
H7N9 (Avian Flu) example

NIID 国立感染症研究所 NATIONAL INSTITUTE OF INFECTIOUS DISEASES

EpiVax

RESEARCH PAPER

Human Vaccines & Immunotherapeutics 11:9, 2241–2252; September 2015; Published with license by Taylor & Francis Group, LLC

H7N9 T-cell epitopes that mimic human sequences are less immunogenic and may induce Treg-mediated tolerance

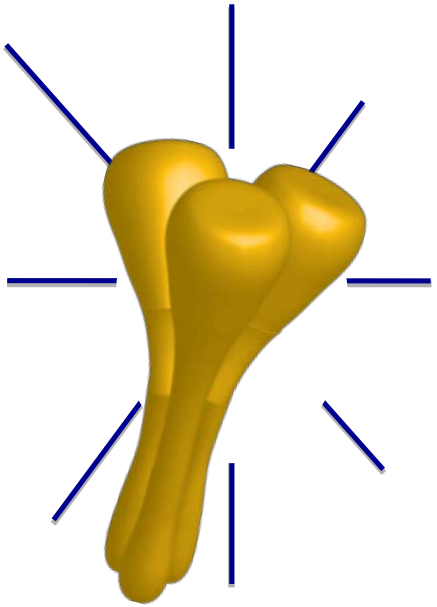
Rui Liu[1], Leonard Moise[1,2], Ryan Tassone[1], Andres H Gutierrez[1], Frances E Terry[2], Kotou Sangare[3], Matthew T Ardito[2], William D Martin[2], and Anne S De Groot[1,2,*]

[1]Institute for Immunology and Informatics; University of Rhode Island; Providence, RI USA; [2]EpiVax Inc.; Providence, RI USA; [3]Laboratory of Applied Molecular Biology (LBMA); University of Bamako; Bamako, Mali

Identify potential regions where epitopes can be improved

Remove Treg Epitopes

Result: **20-Fold More Immunogenic**



SCIENTIFIC REPORTS

OPEN

A humanized mouse model identifies key amino acids for low immunogenicity of H7N9 vaccines

Received: 17 November 2016
Accepted: 29 March 2017

Yamato Wada[1,2], Arnone Nithichanon[1,3], Eri Nobusawa[4], Leonard Moise[5,6], William D. Martin[6], Norio Yamamoto[4,7], Kazutaka Terahara[1], Haruhisa Hagiwara[8], Takato Odagiri[9], Masato Tashiro[4], Ganjana Lertmemongkolchai[3], Haruko Takeyama[2], Anne S. De Groot[5,6], Manabu Ato[1] & Yoshimasa Takahashi[1]

Wada *et al. Sci Rep.* 2017; 7(1):1283

Engineering Vaccines as an example

EpiVax

- Treg epitopes in vaccine antigens can be discovered using JanusMatrix
- Pathogens use Tregitopes to suppress immune response to themselves
- Modifying the antigen to reduce ‘human-like’ T cell epitopes improves response
- Data is published (please ask for USB drive)

OptiMatrix = Tool for Improving "Quality by Design"

- *In silico* screening tools, if applied correctly, are a quick and efficient way of identifying and modifying:
  - **Teff epitopes**, which promote effector responses to therapeutics
  - **Treg epitopes**, which promote tolerance to therapeutics

- **The Immune Engineering concept**, drugs can be modified:
  - Teff epitopes can be removed → **deimmunization**
  - Treg epitopes can be introduced → **tolerization**

OptiMatrix – In Silico Immune Engineering

Use OptiMatrix to redesign potentially immunogenic clusters

EpiVax

Accession: FLU-HA - Sequence: BOSTON-2025 - Cluster: 254

September 25, 2009 (Epx Ver. 1.2)

Click to Print Save Deimmunized Sequence Back to Summary Report

ORIGINAL SEQUENCE

254	255	256		258	259	260	261	262	263	264	265	266	267	268	269
P	R	G		F	K	I	R	T	G	K	T	T	I	M	R
0	4.72	0		15.79	15.94	12.99	16.69	3.71	2.98	12.23	3.32	3.32	4.63	1.78	1.72

MODIFIED SEQUENCE

254	255	256		258	259	260	261	262	263	264	265	266	267	268	269
P	R	G		F	K	I	R	T	G	K	T	T	I	M	R
0	4.72	0		15.79	15.94	12.99	16.69	3.71	2.98	12.23	3.32	3.32	4.63	1.78	1.72
P	R	G	Y	F	K	I	R	T	G	K	T	T	I	M	R

A C D E F G H I K L M N P Q R S T V W Y

The number below each amino acid indicates that residue's relative impact on EpiMatrix scores averaged across all alleles and frames. In this Logo Report the size and color of each amino acid is keyed to its EpiMatrix score. Higher scoring amino acids are represented larger, indicating that they are more "sensitive" than lower scoring amino acids.

Show Suggested Substitutions Show ISPRI Cluster Report Show ISPRI Blast Summary Best Single Change

Frame Start	AA Sequence	Frame Stop	Hydro-phobicity	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
254	PRGYFKIRT	262	-0.23									0
255	RGYFKIRTG	263	-0.2									0
256	GYFKIRTGK	264	-0.19									
257	YFKIRTGKT	265	-0.9	2.38		2.41	2.51	1.4	2.2			
258	FKIRTGKTT	266	-0.83	2.41			2.13	1.69	1.32			
259	KIRTGKTTI	267	-0.14							1.4		0
260	IRTGKTTIM	268	0		1.97	1.42				1.48		1
261	RTGKTTIMR	269	-0.21							1.33		0

Summarized Results (25-SEP-2009)	DRB1*0101	DRB1*0301	DRB1*0401	DRB1*0701	DRB1*0801	DRB1*1101	DRB1*1301	DRB1*1501	Total
Maximum Single Z score	2.41	1.97	2.41	2.51	1.69	2.2	1.48	1.98	--
Sum of Significant Z scores	4.79	1.97	2.41	4.64	1.69	2.2	0	1.98	19.68
Count of Significant Z Scores	2	1	1	2	1	1	0	1	9
Total Assessments Performed: 64	Hydrophobicity: -0.84		EpiMatrix Score: 13.08			EpiMatrix Score (w/o flanks): 16.05			
Scores Adjusted for Tregitope:	--		EpiMatrix Score: 13.08			EpiMatrix Score (w/o flanks): 16.05			

DEIMMUNIZE

See Deimmunization Effects on Epitopes in Real Time

EpiVax

T effector Epitopes can be Taken out – and Treg epitopes can be Introduced

254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269
P	R	G	Y	F	K	I	R	T	G	K	T	T	I	M	R
0	4.72	0	11.92	15.79	15.94	12.99	16.69	3.71	2.98	12.23	3.32	3.32	4.63	1.78	1.72

MODIFIED SEQUENCE

254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269
P	R	G	A	F	K	I	R	T	G	K	T	T	I	M	R
0	4.72	0	3.22	15.79	15.94	12.99	16.69	3.71	2.98	12.23	3.32	3.32	4.63	1.78	1.72
P	R	G	A	F	K	I	R	T	G	K	T	T	I	M	R

The number below each amino acid indicates that residue's relative impact on EpiMatrix scores averaged across all alleles and frames. In this Logo Report the size and color of each amino acid is keyed to its EpiMatrix score. Higher scoring amino acids are represented larger, indicating that they are more "sensitive" than lower scoring amino acids.

Show Suggested Substitutions Show ISPRI Cluster Report Show ISPRI Blast Summary Best Single Change

Frame Start	AA Sequence	Frame Stop	Hydro-phobicity	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
254	PRGAFKIRT	262	-0.15									0
255	RGAFKIRTG	263	-0.13									0
256	GAFKIRTGK	264	-0.11									0
257	AFKIRTGKT	265	-0.56									
258	FKIRTGKTT	266	-0.83	2.41			2.13	1.69	1.32			
259	KIRTGKTTI	267	-0.14							1.44		
260	IRTGKTTIM	268	0		1.97	1.42				1.48		1
261	RTGKTTIMR	269	-0.21							1.33		0

Summarized Results (25-SEP-2009)	DRB1*0101	DRB1*0301	DRB1*0401	DRB1*0701	DRB1*0801	DRB1*1101	DRB1*1301	DRB1*1501	Total
Maximum Single Z score	2.41	1.97	1.42	2.13	1.69	1.32	1.48	1.53	--
Sum of Significant Z scores	2.41	1.97	0	2.13	1.69	0	0	0	8.2
Count of Significant Z Scores	1	1	0	1	1	0	0	0	4

Total Assessments Performed: 64	Hydrophobicity: -0.64	EpiMatrix Score: 1.6	EpiMatrix Score (w/o flanks): 4.57
Scores Adjusted for Tregitope:	--	EpiMatrix Score: 1.6	EpiMatrix Score (w/o flanks): 4.57

DEIMMUNIZE

Application of OptiMatrix: Alpha Interferon Remove Epitopes But Preserve Funcation

Clinical Immunology 176 (2017) 31–41

Contents lists available at ScienceDirect

Clinical Immunology

journal homepage: www.elsevier.com/locate/yclim

De-immunized and Functional Therapeutic (DeFT) versions of a long lasting recombinant alpha interferon for antiviral therapy

Eduardo F. Mufarrege [a,*], Sofía Giorgetti [a], Marina Etcheverrigaray [a], Frances Terry [b], William Martin [b], Anne S. De Groot [b,c]

[a] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Laboratorio de Cultivos Celulares, FBCB, UNL, Santa Fe, Argentina
[b] EpiVax, Inc., Providence, RI, USA
[c] Institute for Immunology and Informatics, University of Rhode Island, RI, USA

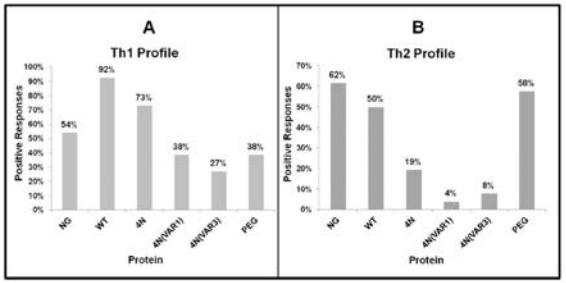
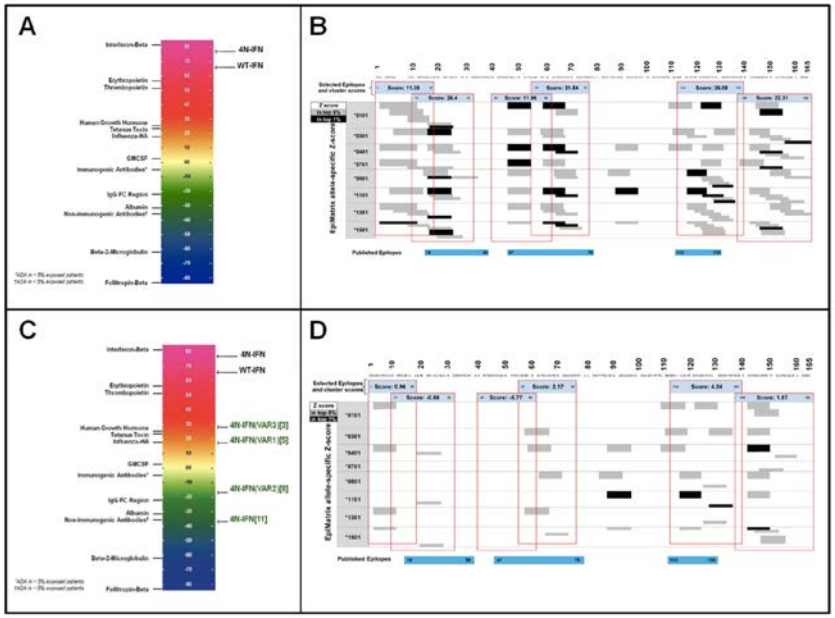
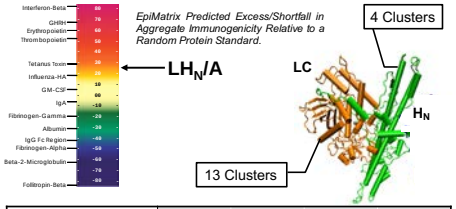


Fig. 6. 4N-IFN de-immunized variants showed a marked reduced immunogenicity in comparison with other IFN versions. *Ex vivo* cytokine secretion by T-cells after incubation with IFN-pulsed dendritic cells. Data were obtained from 26 donors. A Stimulation Index (SI) was defined as a ratio of the cytokine concentration (IFN-γ (A) and IL-4 (B)) from protein challenged samples divided by cytokine concentration from excipient treated samples. A geometric mean (GM) of the SI was then calculated and a positive donor was defined when SI > GM.



The modified alpha interferon is not only less immunogenic, it is also still functional.

Another Example of OptiMatrix Deimmunization of Botulinum Toxin



BoNT/A epitopes identified with EpiMatrix		BT-H1_WT	BT_H2_WT	BT_H3_WT	BT_H4_WT
HLA binding	DRB*0101				
	DRB*0401				
	DRB*0701				
	DRB*1501				
Human T cell response (donors exposed to BoNT/A) N=10	B_1400_N				
	B_1401_N	N/T	N/T	N/T	N/T
	B_1402_N				
	B_1403_N				
	B_1404_N	N/T			
	B_1405_N				
	B_1406_N				
	B_1407_N				
	B_1408_N				
	B_1408_N				
De-immunization candidate?		N	Y	Y	Y

- Transport and enzymatic domains from botulinum toxins (BoNTs) are platforms for development of targeted secretion inhibitors.
- The domains derived from BoNT serotype A form the LH_N/A molecule.
- Immunoinformatic analysis using EpiMatrix and ClustiMer showed **LH_N/A bears significant immunogenicity potential.**

H_N Domain In Vitro Immunogenicity Screen:

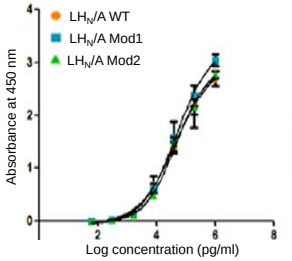
- HLA binding assays validated predictions and demonstrated promiscuous binding to HLA types in the large majority of the human population.
- Predicted epitope clusters are immunoreactive in BoNT/A-exposed human donors.

➢ **BT_H2_WT elicits strongest and most frequent immune responses.**

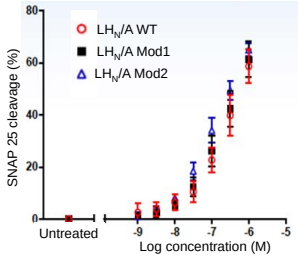
➢ **BT_H2_WT selected for targeted de-immunization.**

Variant Protein Assessment: Preserved Function

Cell-free light chain activity assay



Western blot SNAP-25 cleavage assay



Cleavage at maximal concentration

Sample	Mean % cleavage at 1000 nM	SD
LH_N/A WT	58.70%	± 3.8
LH_N/A Mod1	61.40%	± 3.9
LH_N/A Mod2	65.00%	± 1.4

- Two variant LH_N/A proteins that contain mutation in BT_H2_WT epitope were designed and produced (Mod 1, Mod2).
- No apparent differences in SNAP-25 cleavage function and potency between LH_N/A WT and Mod proteins.

➢ **Epitope modifications do not perturb variant LH_N/A function.**

Immunogenicity - Recent Data Also Relevant to Checkpoint Inhibitors!

EpiVax

Combination Therapy // Increased ADA // Reduced Efficacy

- Incidence of anti-drug antibodies to single agent check point monoclonal antibodies is low, considering that immune inhibitory "brake is released": but higher when "more ***brakes"*** released
  - Pembrolizumab (anti-PD-1): 2% ADA;1 of 4 tested for NABs positive
  - *Nivolumab (anti-PD-1): 11%;* ***combined with Ipi-38%; ~5% NABs***
  - *Ipilimumab (anti-CTLA4): 1.1%-4.9% ADA:* ***combined with nivo -8.4%***
  - Avelumab (anti-PDL-1): 4.1%

FDA U.S. FOOD & DRUG ADMINISTRATION

Presentation by Amy Rosenberg, CHI, 2017

Many Tools are Available

Table 2 Summary of T cell epitope mapping tools, in alphabetical order.

NAME	DEVELOPERS/INSTITUTION	TYPE	WEBSITE
Epibase	I. Lasters and P. Stas Algonomics NV/Lonza, Inc.	Commercial	www.lonza.com
ISPRI	A.S. De Groot and W.D. Martin EpiVax, Inc.	Collaborative/Commercial	www.epivax.com
IEDB	Vita R, Zarebski L, Greenbaum JA, Emami H, Hoof I, Salimi N, Damle R, Sette A, Peters B. The immune epitope database 2.0. Nucleic Acids Res. 2010 Jan;38:D854-62.	Mixed collection of tools of assorted derivation	www.iedb.com
MHC2PRED	G.P.S. Raghava Bioinformatics Center, Institute of Microbial Technology, Chandigarh, India	Public	www.imtech.res.in/raghava/mhc2pred/
MHCPRED	D.R. Flower The Jenner Institute	Public	www.ddg-pharmfac.net/mhcpred/MHCPred/
PROPRED/ TEPITOPE	G.P.S. Raghava and H. Singh Bioinformatics Center, Institute of Microbial Technology, Chandigarh, India	Public	www.imtech.res.in/raghava/propred/
RANKPEP	P.A. Reche Harvard Medical School	Public	http://bio.dfci.harvard.edu/RANKPEP/
SVRMHC	P. Donnes, A. Elofsson Division for Simulation of Biological Systems, University of Tubingen, Germany	Public	http://svrmhc.biolead.org/
SYFPEITHI	H.G. Rammensee Department of Immunology, Tubingen, Germany	Public	http://www.syfpeithi.de
SMM-Align/ NetMHCII-2.2	M. Nielsen, C.Lundegaard, and O. Lund Center for Biological Sequence Analysis, Department of Systems Biology, Technical University of Denmark	Public	www.cbs.dtu.dk/services/NetMHCII-2.2/
TCED/iTope	M. Baker and F. Carr Antitope, Ltd.	Commercial	www.antitope.co.uk/

But only **ISPRI** is

- Comprehensive
- Commercial Grade
- Includes Unique Tools

Comprehensive In Silico Immunogenicity Risk Assessment – ISPRI vs IEDB

EpiVax

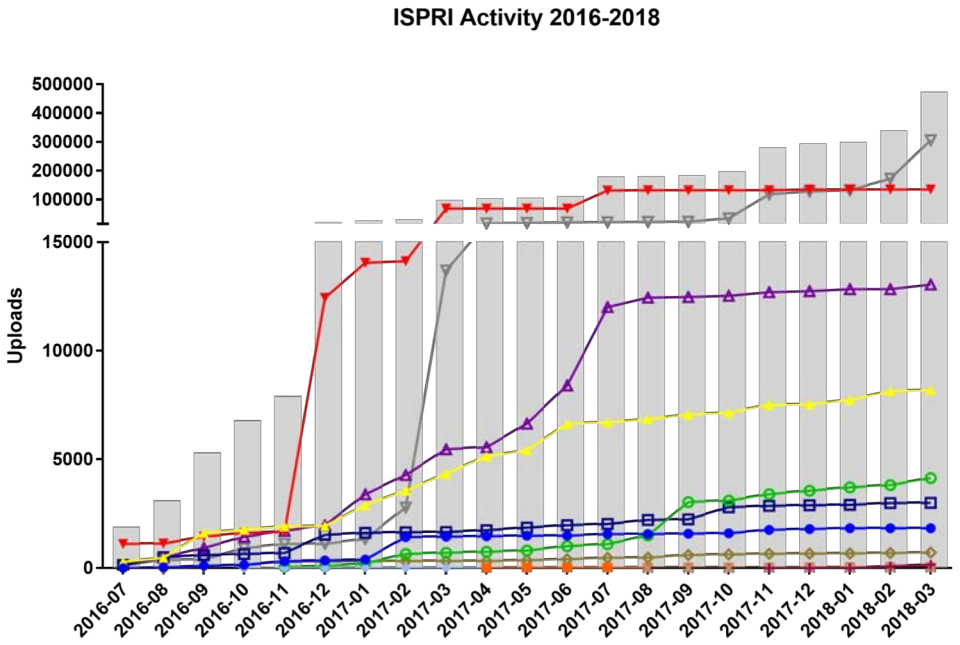
Features	ISPRI (EpiVax)	NetMHC/IEDB
Epitope Prediction	✓	✓[1]
Promiscuous T cell epitope discovery	✓	✓[2]
Immunogenicity Scale (normalized)	✓*	X
Personalized Immunogenicity Risk Analysis	✓*	X
Tregitope Adjustment	✓*	X
JanusMatrix (TCR facing comparison)	✓	X
Human/Other Proteome Comparison	✓	X
High-Throughput Antibody Analysis	✓	X
Published Validation	✓	✓[3]
Expert Consulting Services	✓	X

ISPRI: Developed for Biologics

- **ISPRI** is EpiVax's integrated **in silico toolkit** for prediction, analysis and reduction of T cell immunogenicity of protein therapeutics
- Predictions **reduce laboratory work** (typically at least **20-fold**) and **focus** development on **critical protein regions**
- In silico immunogenicity screening helps researchers **save time, money and effort** by providing **actionable data** on protein immunogenicity

Most Large Pharma Use ISPRI
Cumulative Website Use last 12 months

Biologics developers are incorporating in silico immunogenicity risk assessment at an accelerating rate



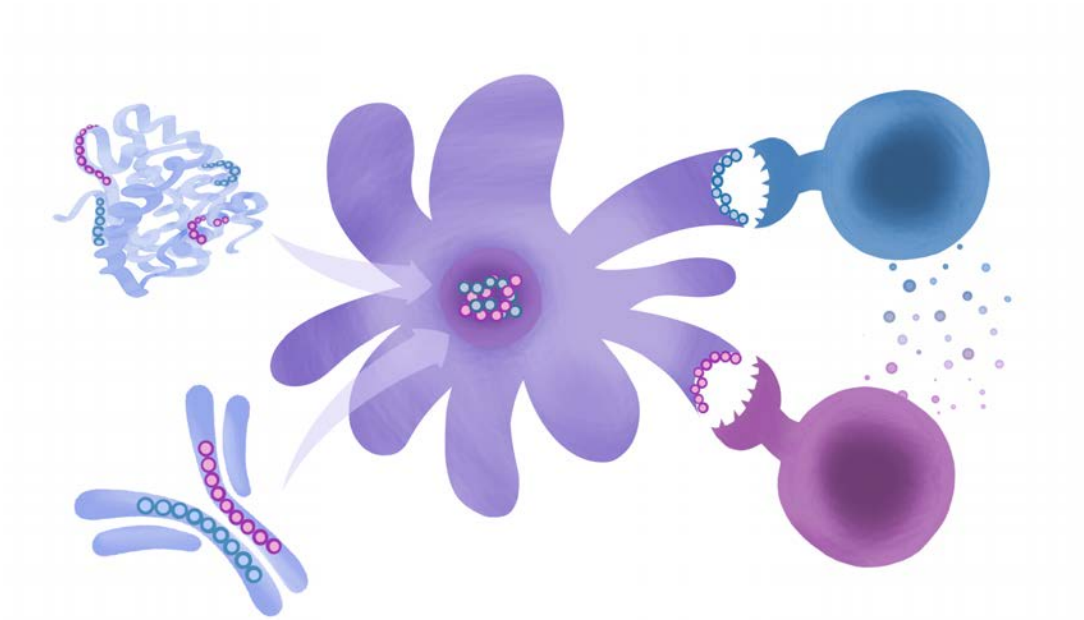
Summary – In Silico Tools for Immunogenicity

EpiVax

- Defining T cell Epitopes In Silico – *Yes, we can.*
- Comprehensive Immunogenicity Risk Assessment ***includes In Vitro***
- Defining Tregs In Silico? – *Yes, we can.*
- Immune Engineering Immunogenicity and Tolerance? – *Yes, we can.*
- Peptides (and their impurities) play by the same rules. 
- Personalizing Immunogenicity Risk ? – *Yes, we can.*
- . . . Can we **immune-engineer**? – *Yes, we can.*
- *Be attentive to potential Treg epitopes!*

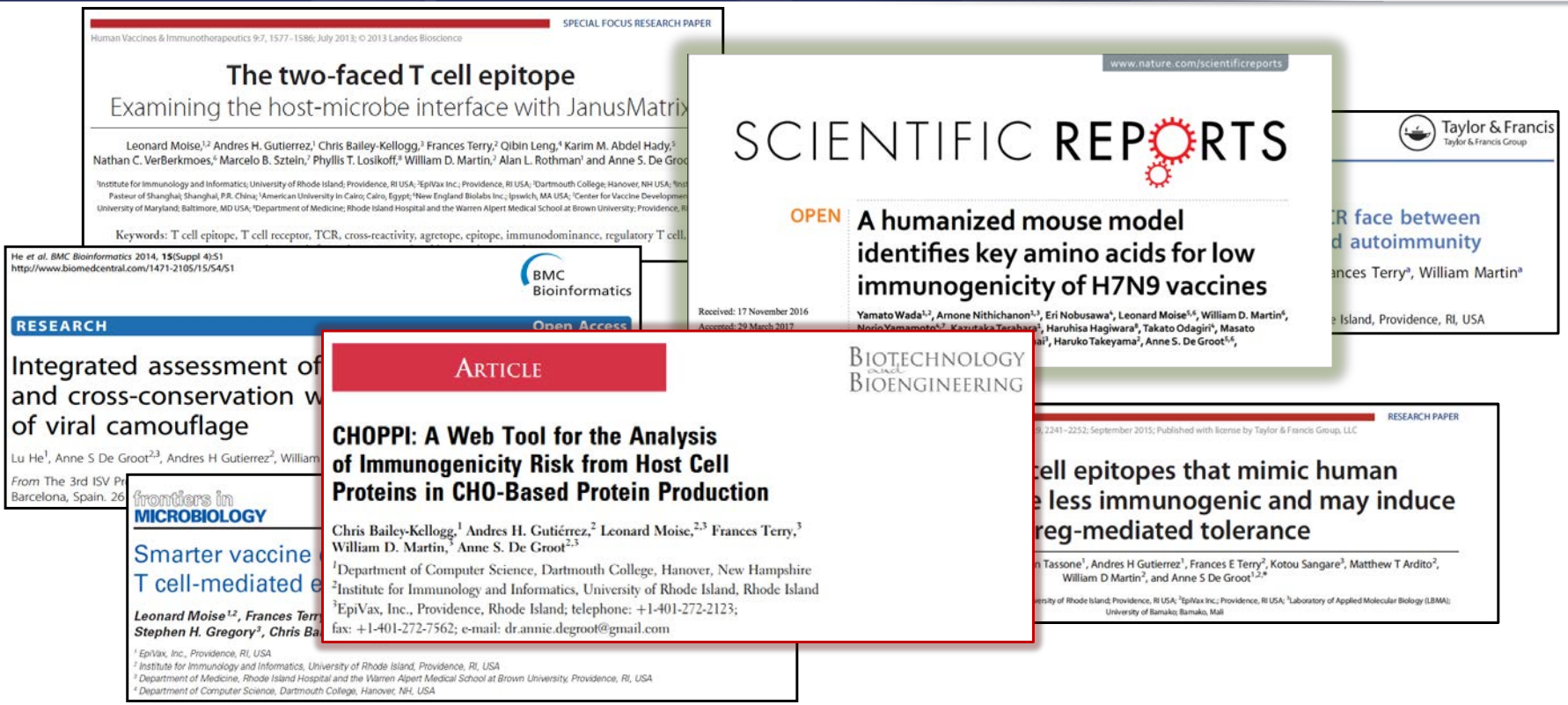
Thank you! Questions?

EpiVax

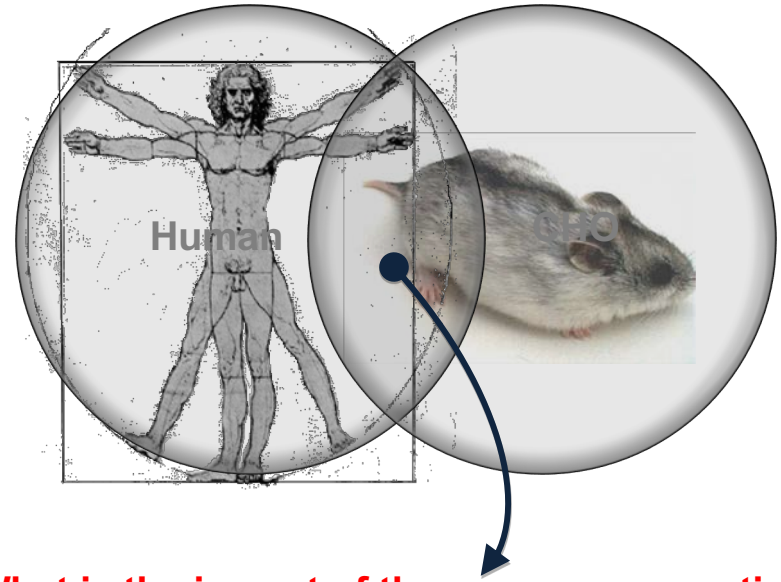


EpiVax 20 YEARS Fearless Science

Treg epitopes in vaccines and host cell proteins

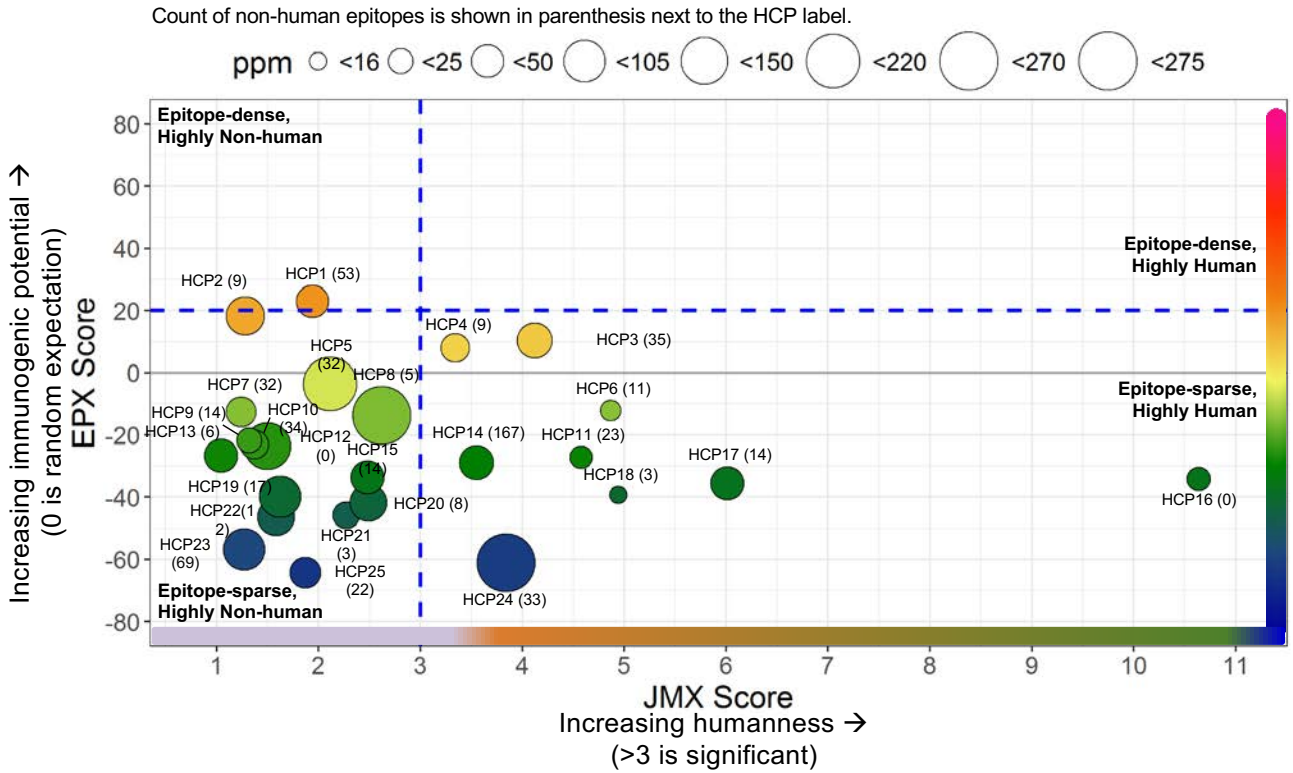


Host Cell Proteins:
High Degree of Conservation with Human Epitopes



What is the impact of the cross-conservation between CHO and Human?

Example Data Representation
Epitope content vs. humanness



Recent ISPRI-HCP Application

EpiVax

Hamster Phospholipase B-Like 2 (PLBL2): A Host-Cell Protein Impurity in Therapeutic Monoclonal Antibodies Derived from Chinese Hamster Ovary Cells

by Martin Vanderlaan, Wendy Sandoval, Peter Liu, Julie Nishihara, George Tsui, Margaret Lin, Feny Gunawan, Sara Parker, Robert Ming Wong, Justin Low, Xiangdan Wang, Jihong Yang, Karthik Veeravalli, Patrick McKay, Chris Yu, Lori O'Connell, Benjamin Tran, Rajesh Vij, Chris Fong, Kathleen Francissen, Judith Zhu-Shimoni, Valerie Quarmby and Denise Krawitz

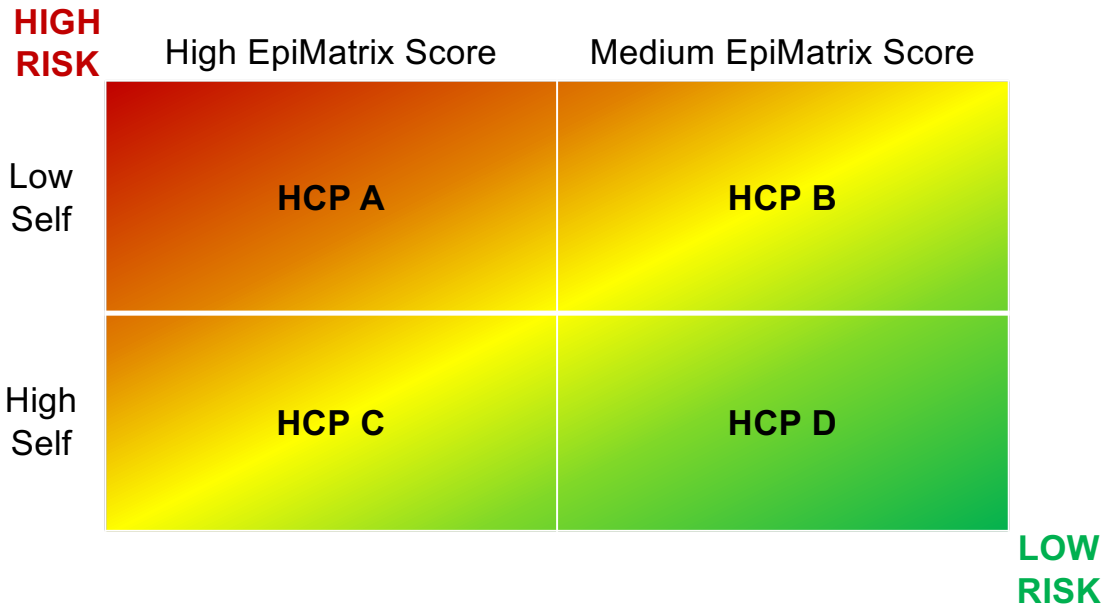
Tuesday, April 14, 2015 5:50 pm

BioProcess International (Vol. 13 Issue 4)

Report that the same HCP (phospholipase B-like 2, PLBL2) co-purifies with multiple Chinese hamster ovary (CHO)-produced antibody therapeutics.

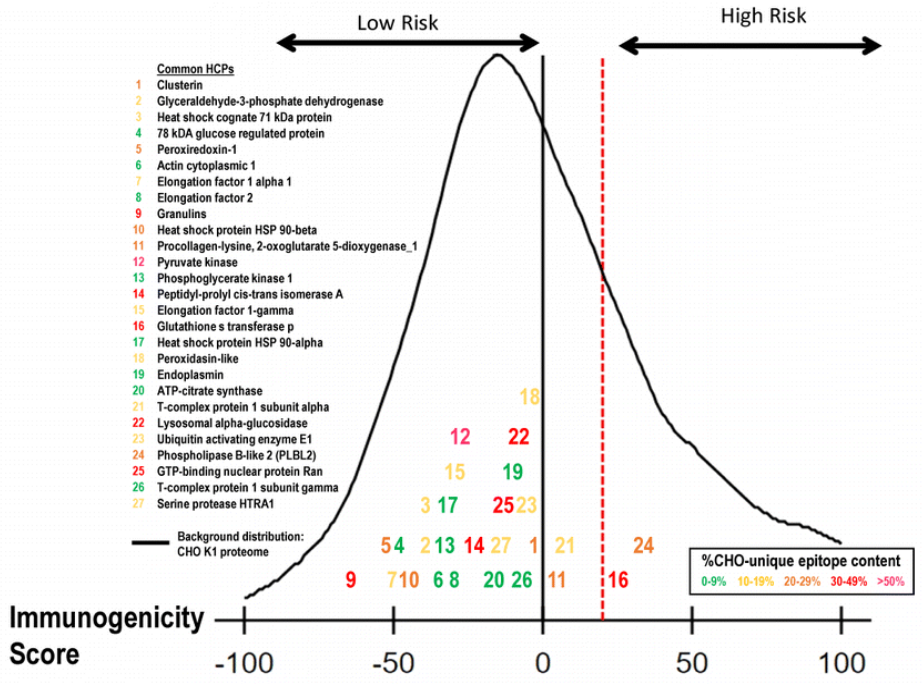
Foreign vs. Self

How can we assess the risk of HCP contaminants?



Protein scores are adjusted for “self” T cell epitope content

Published Example of Host Cell Protein Immunogenicity Analysis



Supplemental Figure 1. Each protein in the figure is represented with 3 characteristics. (1) The numerical number identifies the protein from the list (2) Each protein is placed on X-axis based on their value on EpiMatrix Immunogenicity Score (3) the color represents the %CHO-unique epitope content and their similarity to the CHO K1 proteome; green=0-9% ; yellow=10-19%;orange=20-29%;red=30-49% and pink=>50%.

Jawa V et al. AAPS J 2016