De-Risking Protein Therapeutics: Should you Delete that T cell Epitope or Keep it? And Why . . .

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EpiVax 20 YEARS Fearless Science

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EpiVax

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EpiVax

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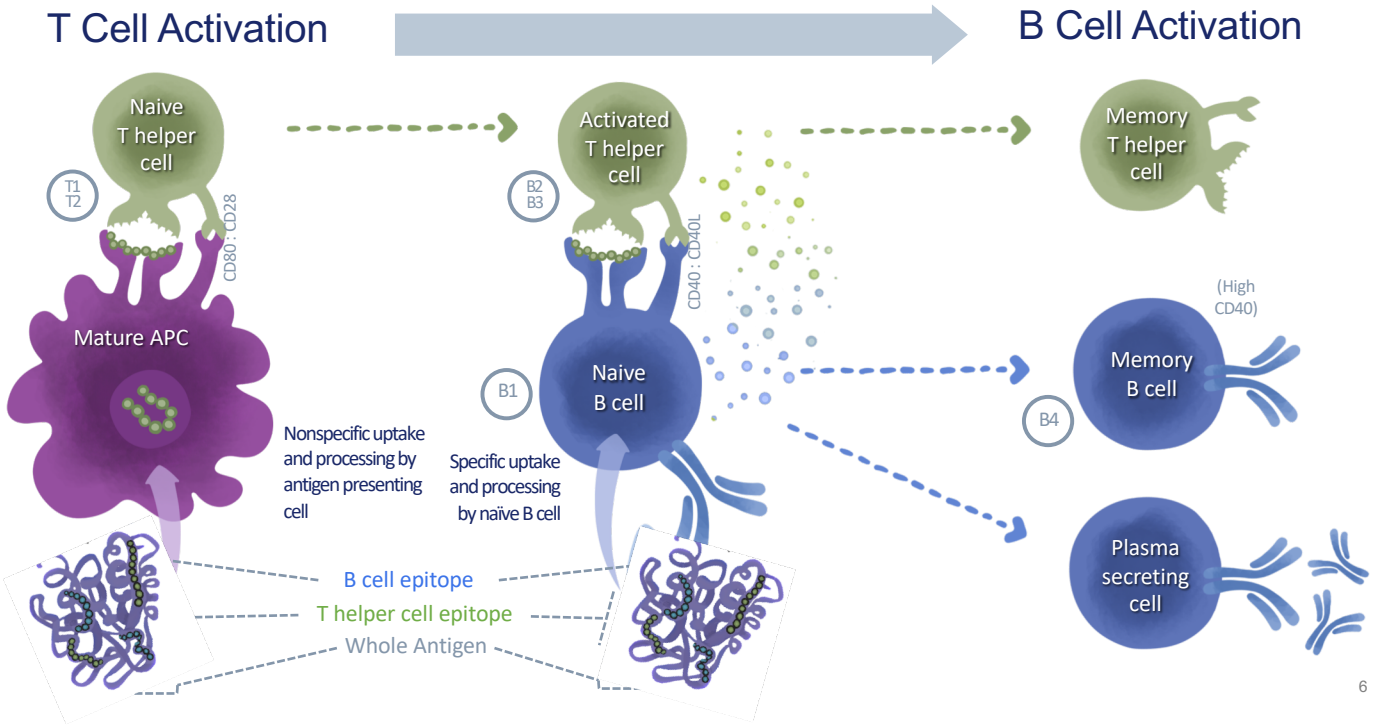
Outline

EpiVax

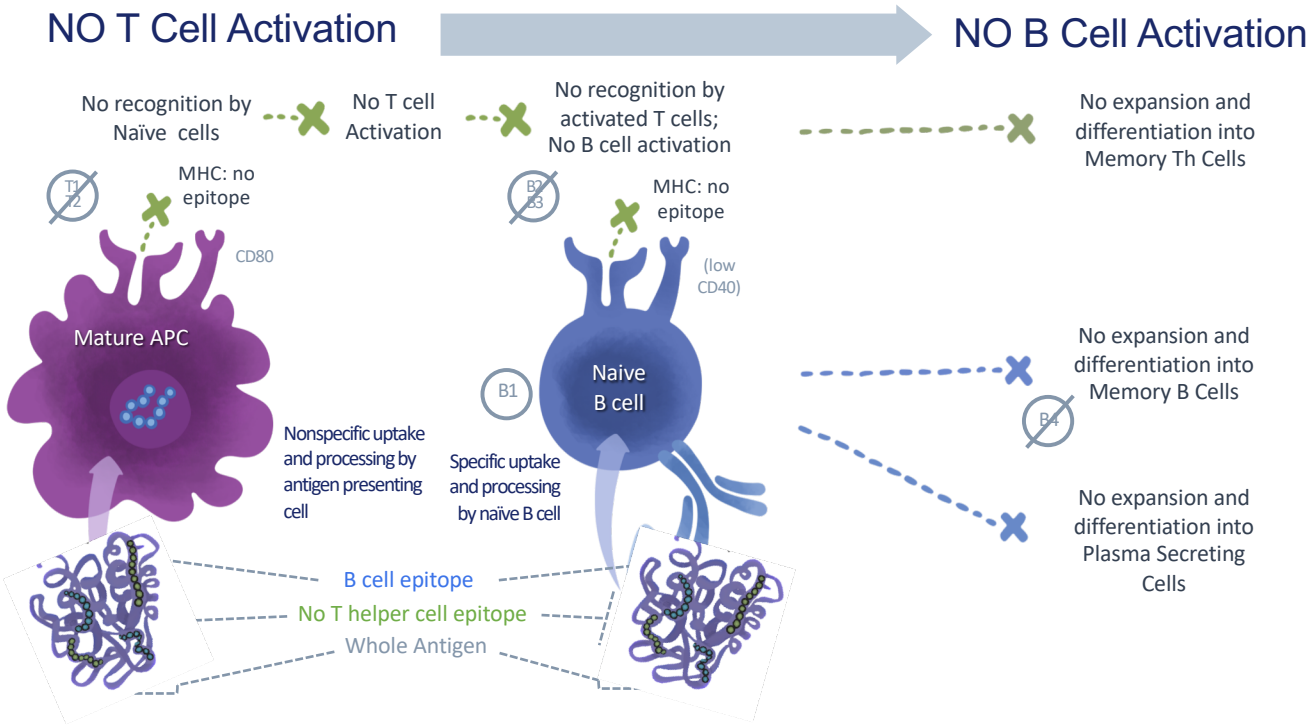
- Defining *Immunogenic/Tolerogenic* Epitopes In Silico – Yes, We Can.
- Comprehensive Immunogenicity Risk Assessment (Includes In Vitro)
- Cutting Edge Tools: JanusMatrix and Tregitope
- Immune Engineering Immunogenicity and Tolerance

Presence of T cell epitopes drives ADA



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

Absence of T cell epitopes reduces ADA

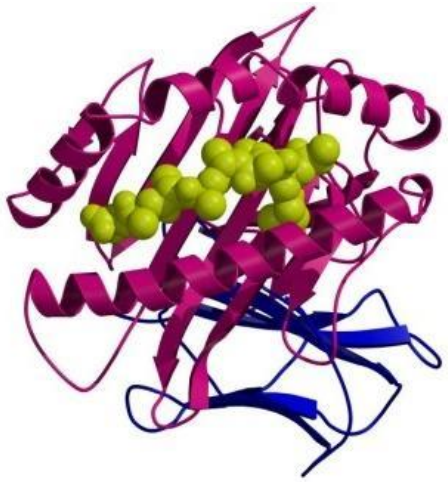


Lack of T cell epitopes abrogates activation of CD4 T cells and T-dependent antibody response

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

EpiVax

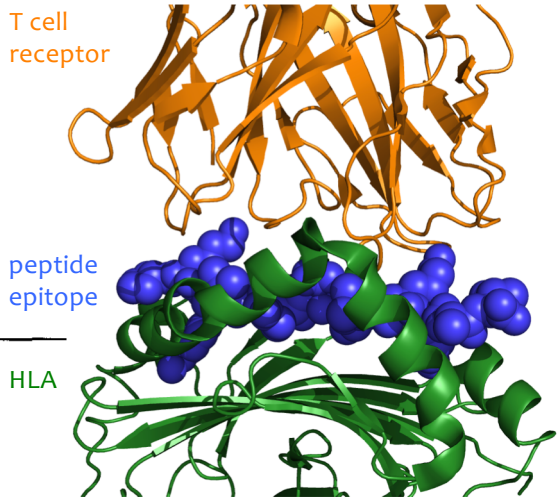
Published July 1, 1993



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

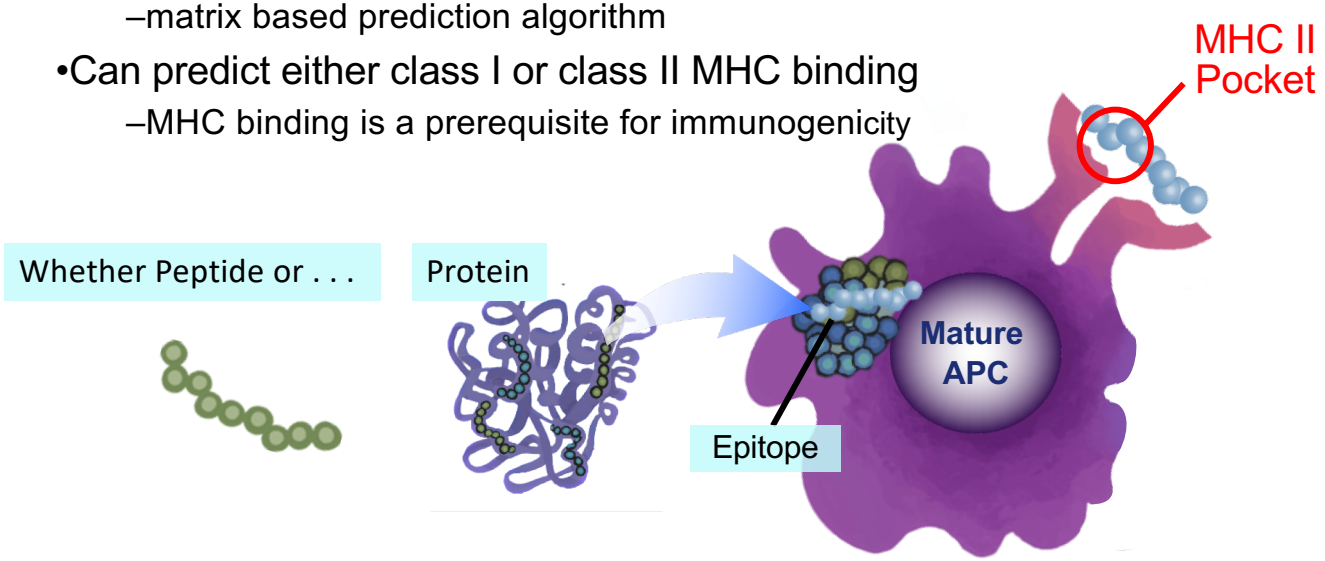
*From the Department of Biochemistry and Molecular Biology and the *Harvard Microchemistry Facility, Harvard University, Cambridge, Massachusetts 02138*



Identifying T cell epitopes Is key to assessing Immunogenicity Risk

EpiVax

- 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



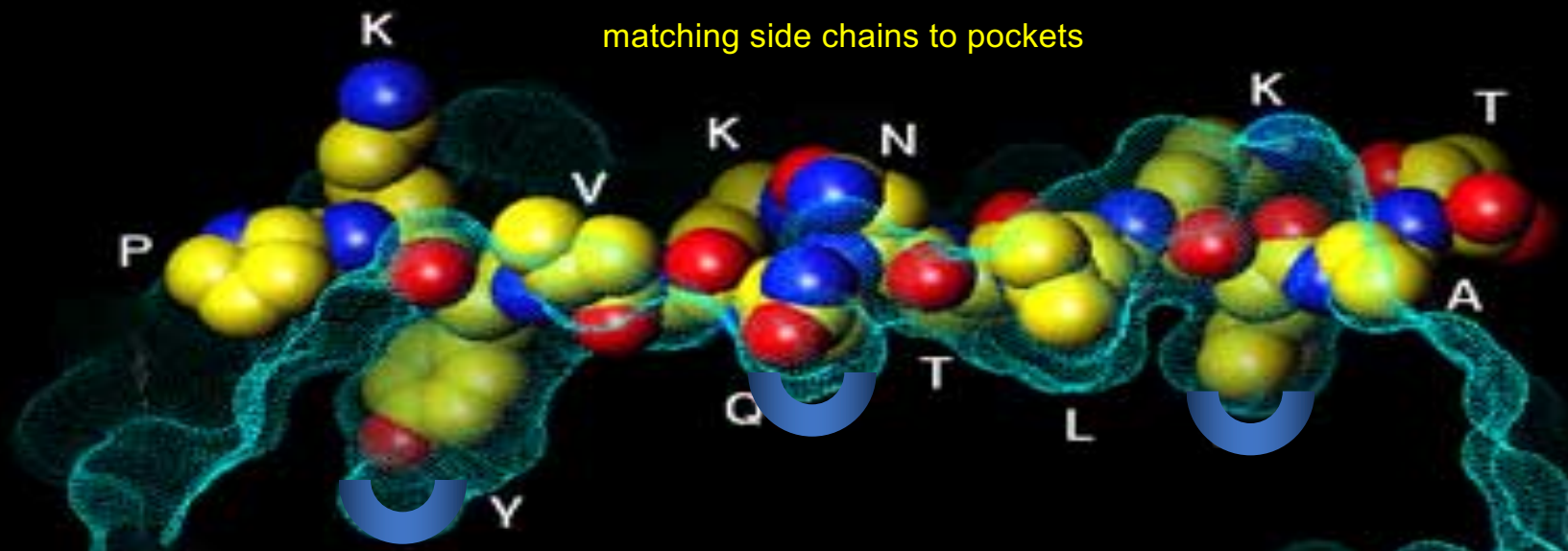
- –Full suite of HLA-based predictions; Class II usually used for biologics.
- –Cloud-based tool used by most **large Biotech companies: ISPRI**
- –Separate website available for **vaccine design: iVAX**

Outline

- Defining T cell Epitopes In Silico – Yes, We Can.
- Comprehensive Immunogenicity Risk Assessment (Includes In Vitro)
- Cutting Edge Tools: The two-faced T cell epitope and Tregitopes
- Immune Engineering Immunogenicity and Tolerance
- Personalizing immunogenicity Risk

Epitope binding to HLA involves

matching side chains to pockets



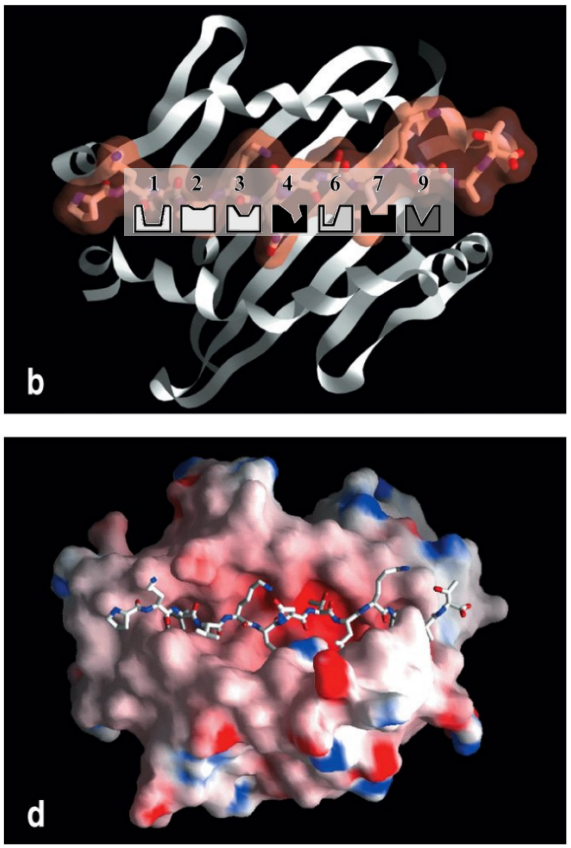
Side chains of amino acids (R group) anchor the peptide in place.

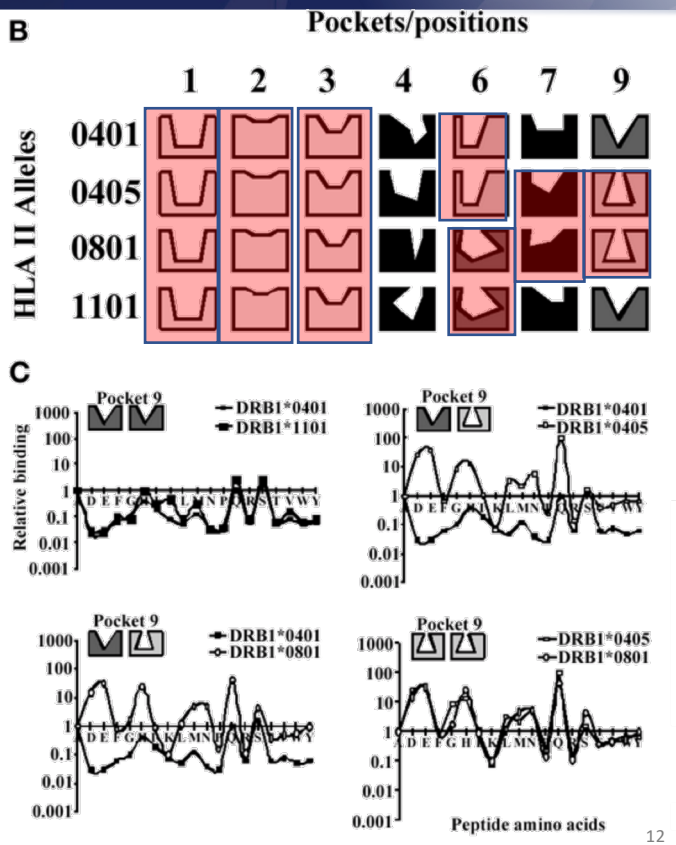
The side chains are anchored into specific pockets

Pockets are conserved in evolution - - -

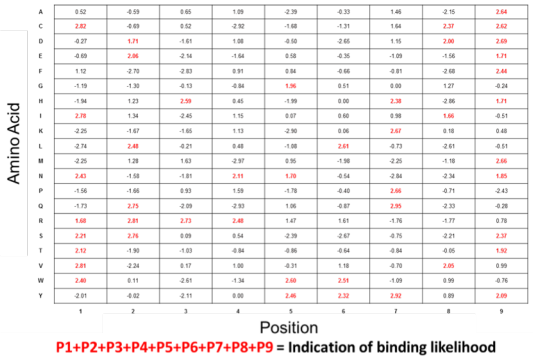
http://www.umassmed.edu/pathology/graphics/sternfig1.jpg *(malaria epitope in DRB1*0101)*

HLA Pocket Profiles – Are Redundant
Sturiolo and Hammer 1999

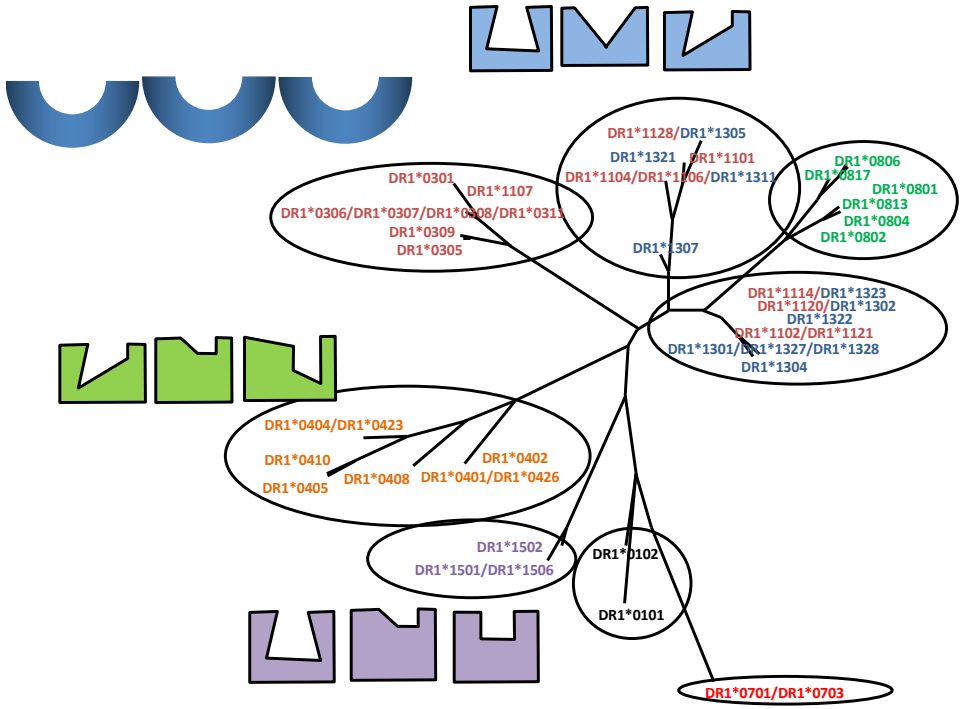




- We maintain a set of allele specific models of MHC-ligand binding.
- We refer to these models collectively as the EpiMatrix System.
- "Matrix" - models are driven by a 20x9 set of coefficients (one for each binding position and amino acid).
- Matrices can be combined with pocket profiles to develop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



HLA "Supertype" Families – Pockets are Similar



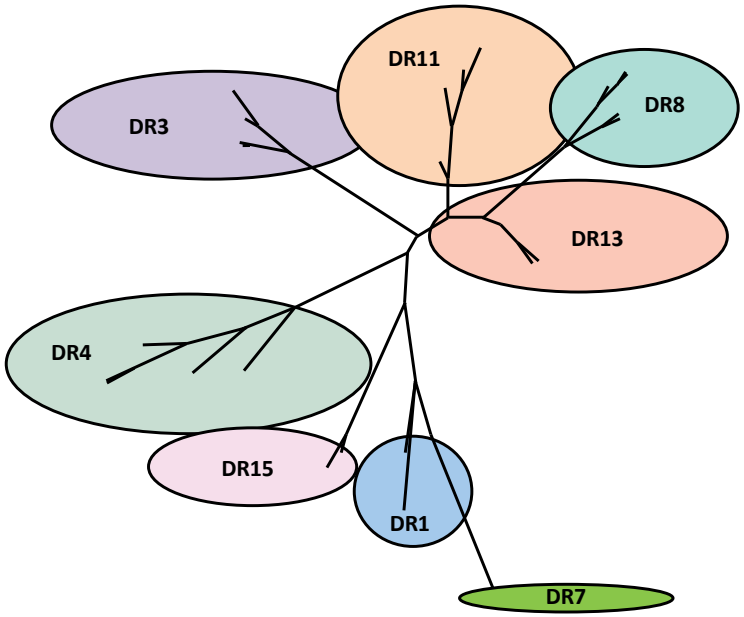
EpiVax tests for binding potential to the most common HLA molecules within each of the "supertypes"* shown to the left.

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.

HLA “Supertype” Families – Pockets are Similar



We assess for binding potential to the most common HLA molecules within each of the “supertypes”* shown to the left.

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

EpiVax

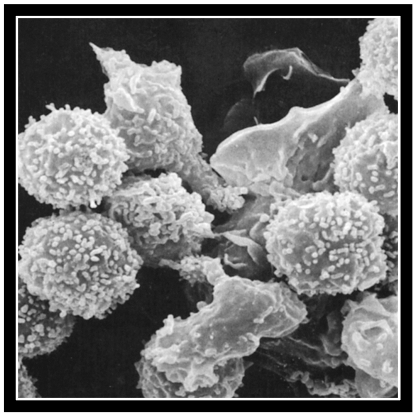
Protein Therapeutic:

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.

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

bococizumab anti-PCSK9: in silico

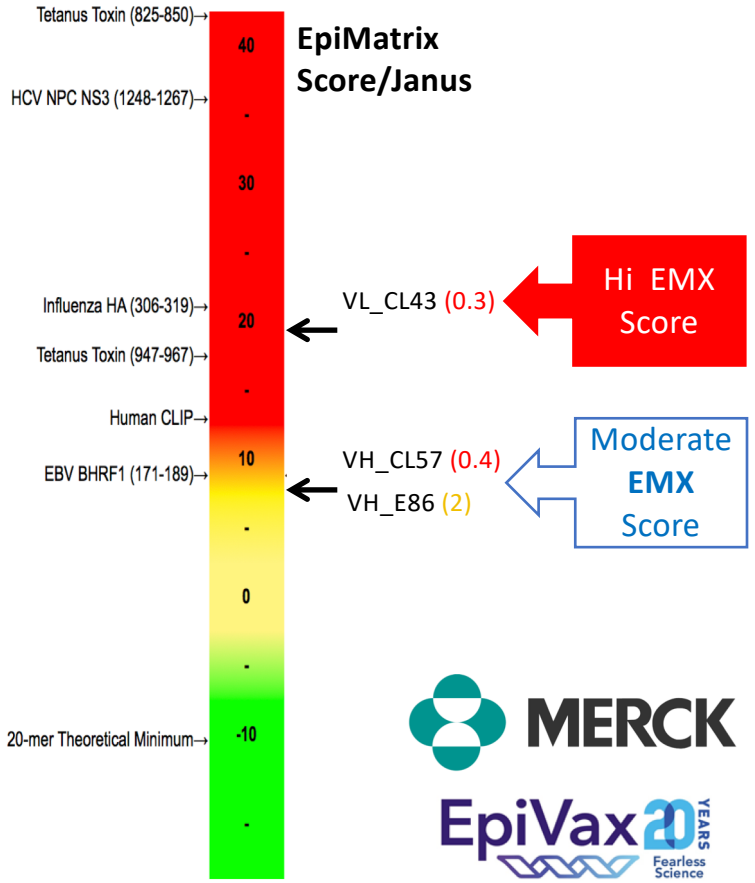
>bococizumab_H aPCSK9
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGEISPFGGRTNYNEKFKSRVT
RDTSTSTVYMELSSLRSEDTAVYYCARERPLYASDLWGQGTTVTVSSASTKGPSVFPLAPCSRSTSEST
LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTK
KTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVH
KTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPSSIEKTISKTKGQPREPQVYTLPPSRE
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV
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**

SEQUENCE	% IDENTITY	% SIMILARITY	FILE	DESCRIPTION
APKLLIYSASYRYTGVPSRFSGSG	--	--	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)



Presentation by D. Montgomery at PEGS Analysis: Jad Maamary

Breaking down the protein or peptide
Into 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

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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*1501	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
[illegible]	2	3	1	1	2	2	[illegible]	14

Hy[illegible]phobicity: -0.52 | EpiMatrix Score: 19.81 | EpiMatrix Score (w/o f[illegible]s): 24.76

Different Immune Response Expected

Highly Relevant to Enzyme and Factor Replacement Therapy

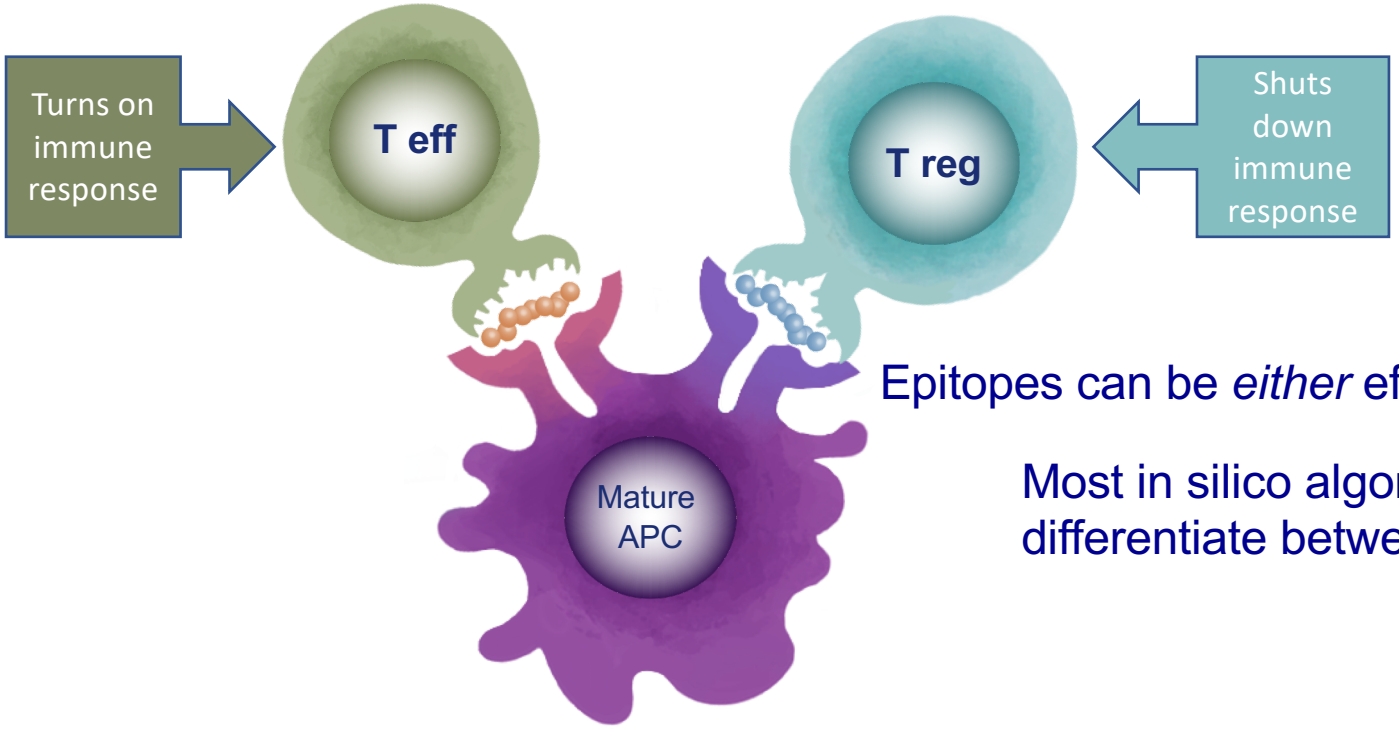
Immunogenicity and Tolerance: Role of Tregs

EpiVax

If, immunogenic potential increases with increasing T cell epitope content,

What is the impact of Treg epitopes?

Characterizing Putative T cell Epitopes



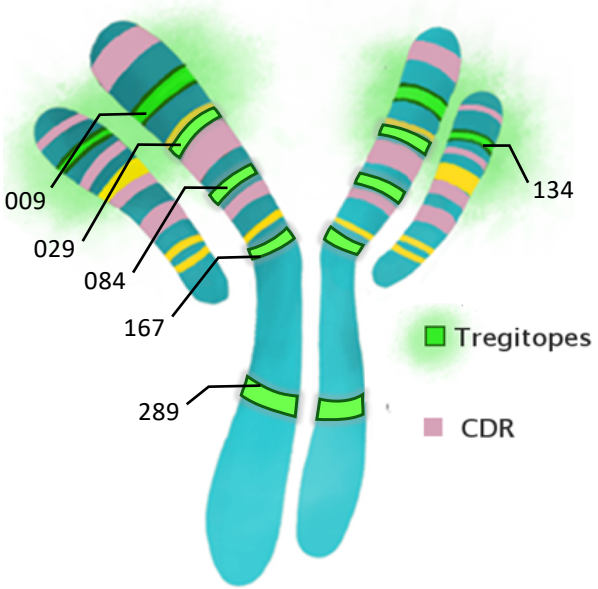
Epitopes can be *either* effector or regulatory

Most in silico algorithms cannot differentiate between these two

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"

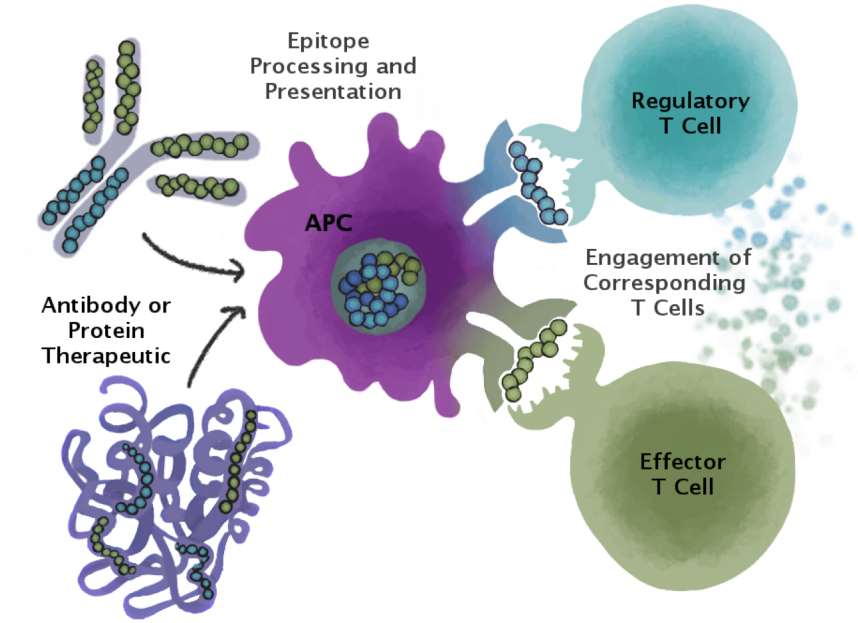
Anne S. De Groot,[1,2] Leonard Moise,[1] Julie A. McMurry,[1] Erik Wambre,[3] Laurence Van Overtvelt,[3] Philippe Moingeon,[3] David W. Scott,[4] and William Martin[1]

[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

Tregitopes Actively Suppress Immune Response and induce Antigen-Specific Tolerance



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

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

Outline

- Defining T cell Epitopes In Silico – Yes, We Can.
- Comprehensive Immunogenicity Risk Assessment (Includes In Vitro)
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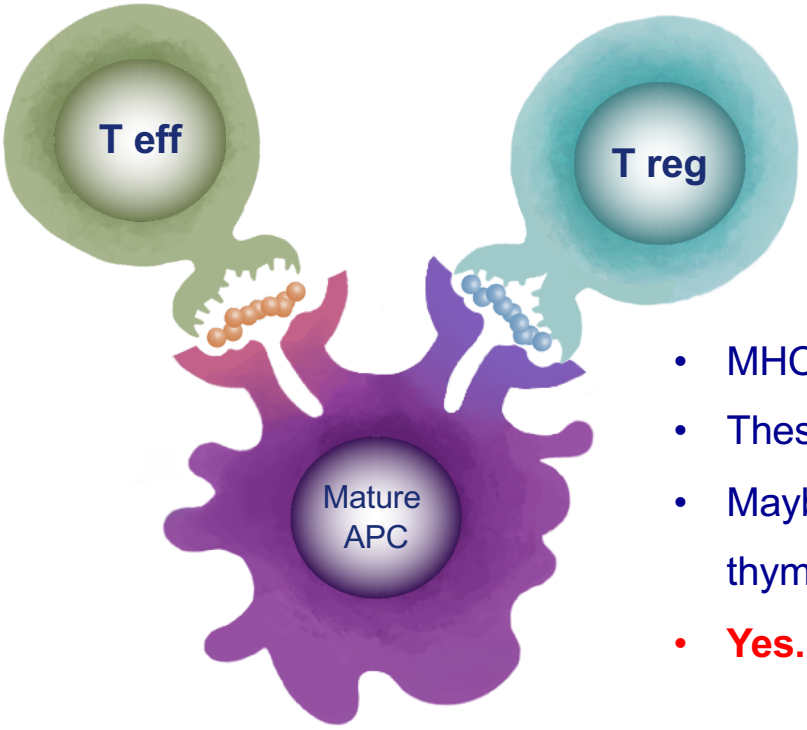
- Immune Engineering Immunogenicity and Tolerance

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.**

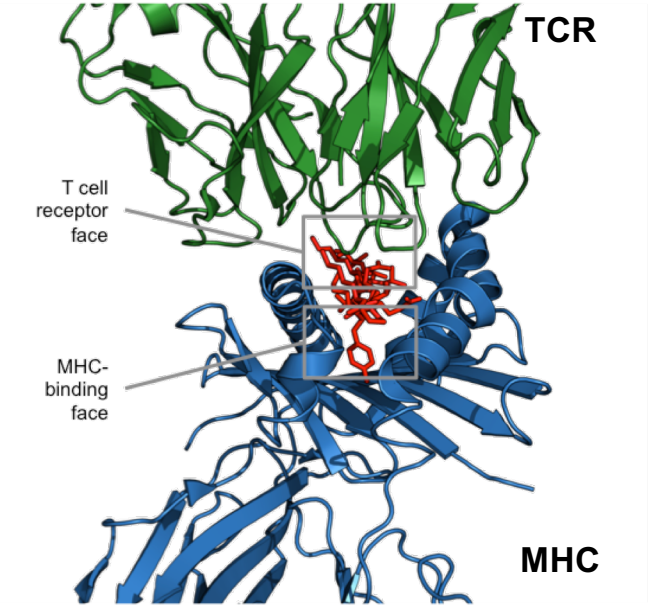
Tool for defining Tregs using "Epitope Networks"
JanusMatrix

EpiVax

Each MHC ligand has two faces:

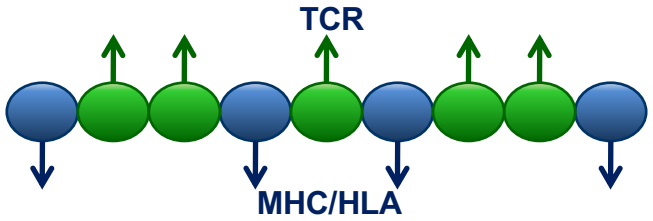
The MHC-binding face: **agretope**

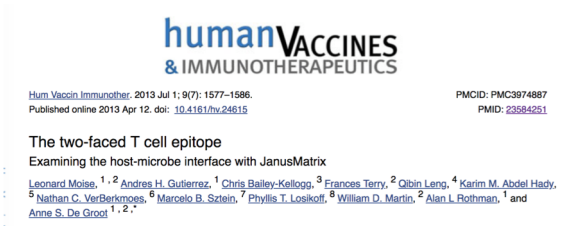
and the TCR-interacting face: **epitope**



Find predicted 9-mer ligands with:

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





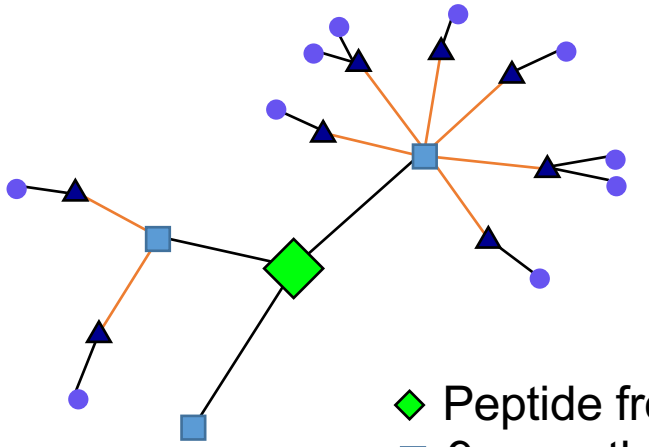
humanVACCINES & IMMUNOTHERAPEUTICS

Hum Vaccin Immunother. 2013 Jul 1; 9(7): 1577–1586. PMCID: PMC3974887
Published online 2013 Apr 12. doi: 10.4161/hv.24615 PMID: 23584251

The two-faced T cell epitope
Examining the host-microbe interface with JanusMatrix

Leonard Moise, [1,2] Andres H. Gutierrez, [1] Chris Bailey-Kellogg, [3] Frances Terry, [2] Qibin Leng, [4] Karim M. Abdel Hady, [5] Nathan C. VerBerkmoes, [6] Marcelo B. Sztein, [7] Phyllis T. Losikoff, [8] William D. Martin, [2] Alan L. Rothman, [1] and Anne S. De Groot [1,2,*]

Epitope Networks: A 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

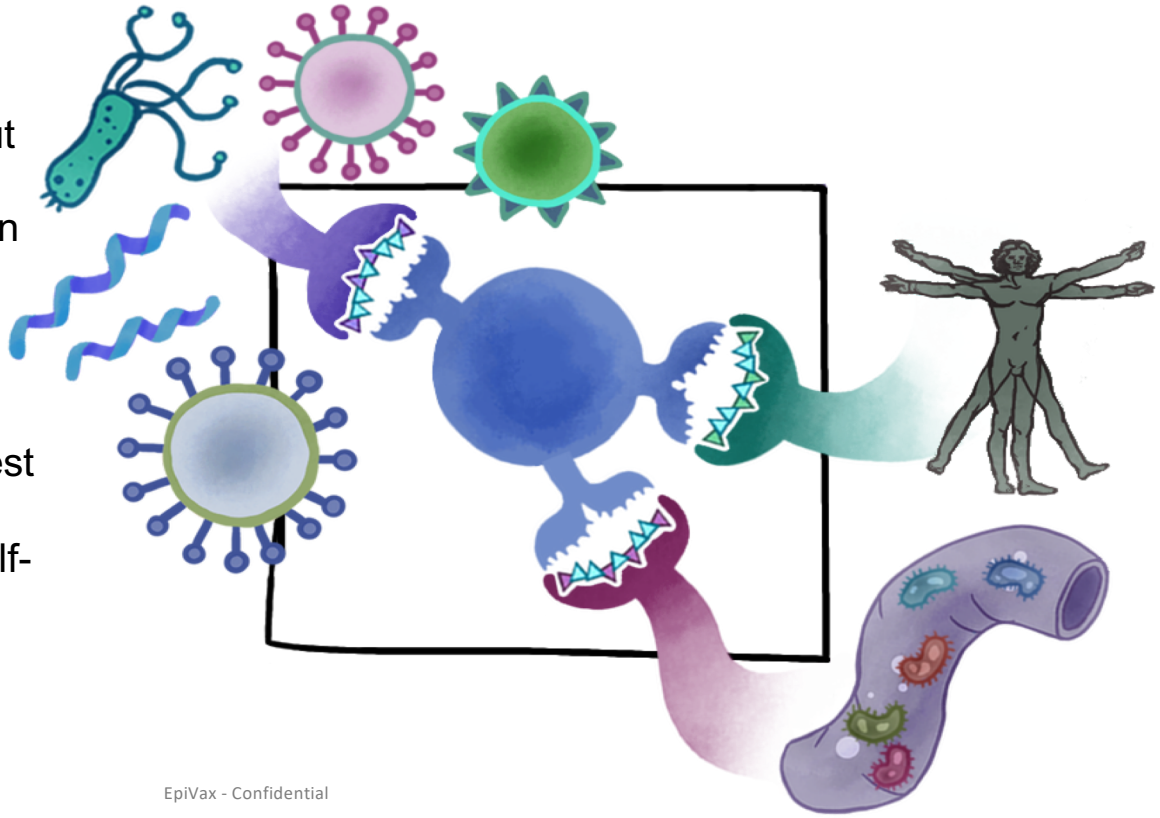
The Two Faced T cell epitopes – Immune Camouflage
Commensal pathogens self/non-self relationships

EpiVax

Immune Camouflage

Originated with discovery about pathogens "copy/pasting" epitopes that looked like human Treg epitopes in their own genomes

Commensal pathogens e.g. CMV, EBV, HSV have the lowest number of T effector epitopes and the highest number of "self-like" putative Treg epitopes



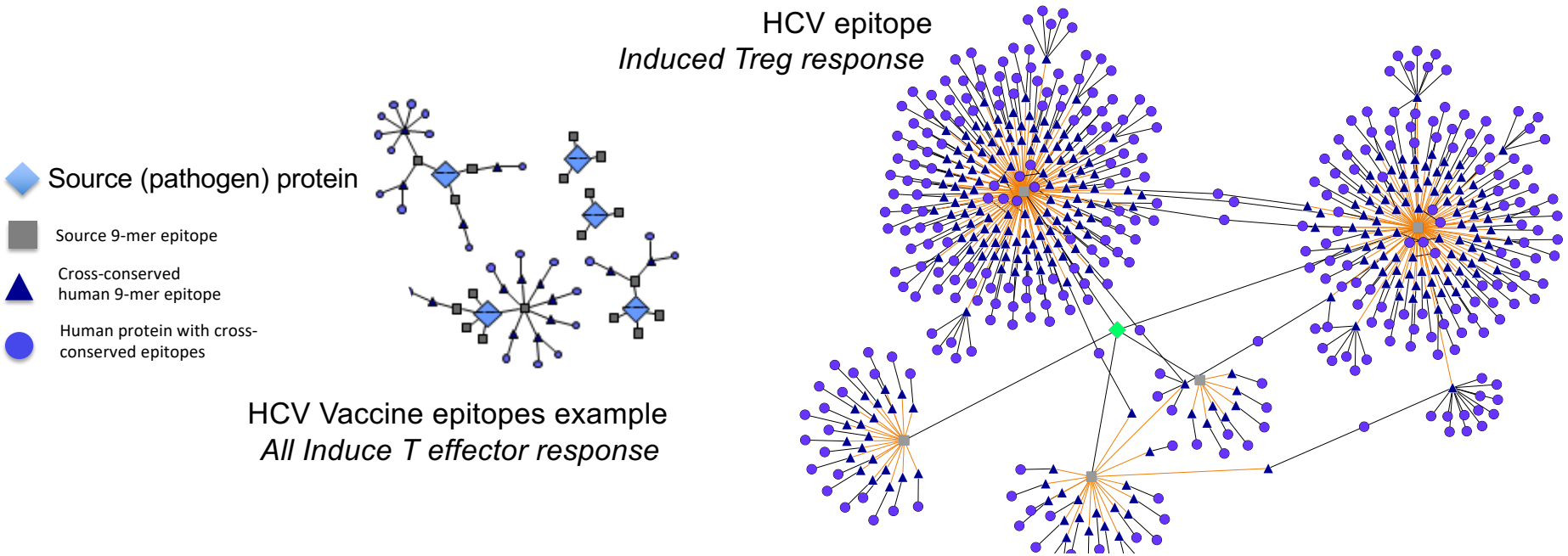
humanVACCINES & IMMUNOTHERAPEUTICS

Hum Vaccin Immunother. 2014 Dec; 10(12): 3570–3575. PMCID: PMC4514035
Published online 2014 Nov 1. doi: 10.4161/hv.36134 PMID: 25483703

Immune camouflage: Relevance to vaccines and human immunology

Anne S De Groot,[1,2,*] Lenny Moise,[1] Rui Liu,[2] Andres H Gutierrez,[2] Ryan Tassone,[2] Chris Bailey-Kellogg,[3] and William Martin[1]

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

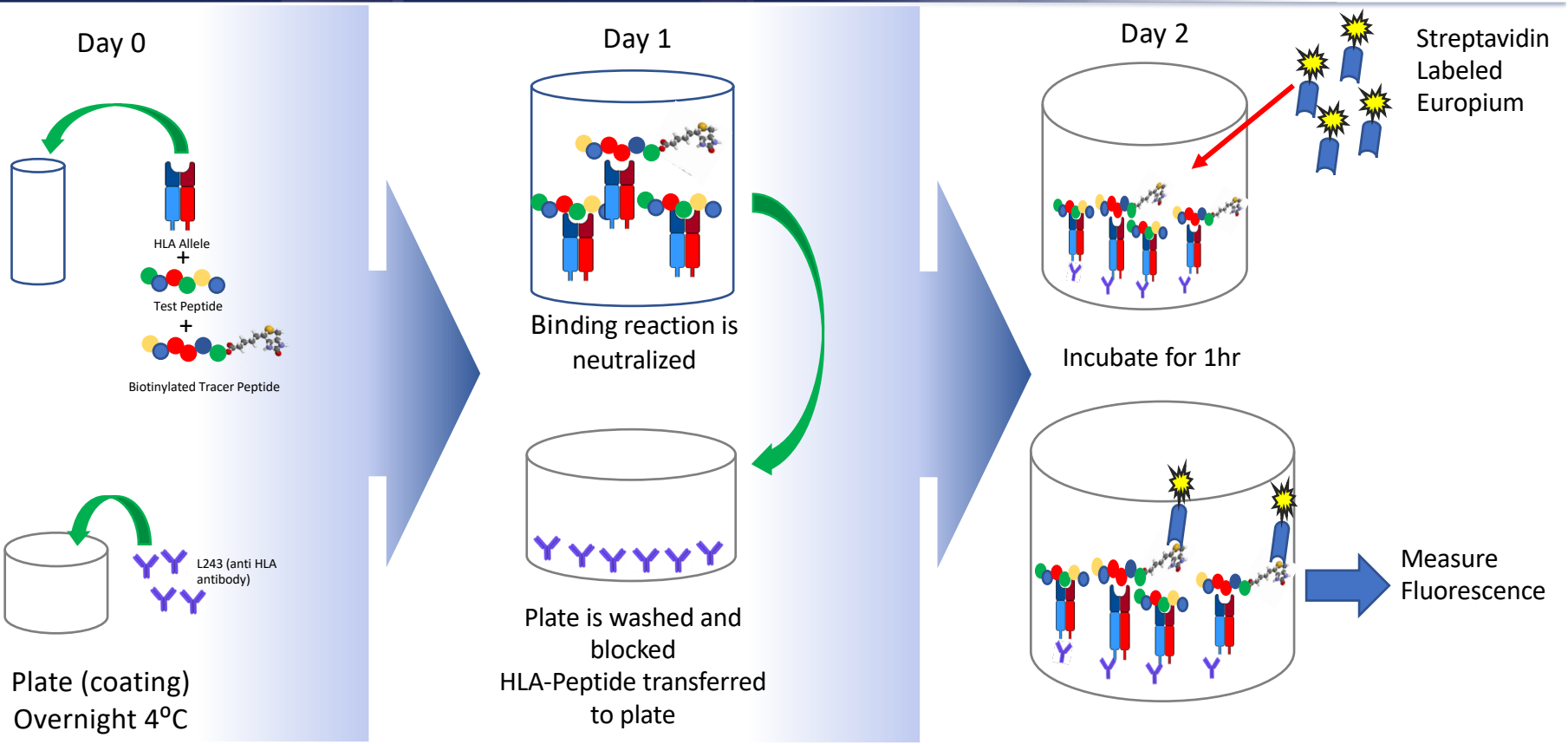


Losikoff PT, Mishra S, Terry F, Gutierrez A, Ardito MT, Fast L, Nevola M, Martin WD, Bailey-Kellogg C, De Groot AS, Gregory SH. **HCV Epitope, Homologous to Multiple Human Protein Sequences, Induces a Regulatory T Cell Response in Infected Patients.** J Hepatol. 2014 Aug 22. pii: S0168-8278(14)00613-8.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

Outline

- Defining T cell Epitopes In Silico – Yes, We Can.
- Comprehensive Immunogenicity Risk Assessment (Includes In Vitro)
  - Binding and T cell Assays (including Treg assays)
- Cutting Edge Tools: The two-faced T cell epitope and Tregitopes
- Immune Engineering Immunogenicity and Tolerance
- T cell epitopes and Generic Peptide PANDA-monium

HLA-Binding Assay Overview



Not all HLA binding assays are the same

More sensitive assays may uncover binding that was missed

EpiVax

Characterization of CD4 T Cell Epitopes of Infliximab and Rituximab Identified from Healthy Donors

*Moustafa Hamze[1], Sylvain Meunier[1], Anette Karle[2], Abdelaziz Gdoura[1], Amélie Goudet[1], Natacha Szely[3], Marc Pallardy[3], Franck Carbonnel[4], Sebastian Spindeldreher[2], Xavier Mariette[5], Corinne Miceli-Richard[5] and Bernard Maillère[1]**

[1] CEA-Saclay, Institut de Biologie et Technologies, Université Paris-Saclay, Gif sur Yvette, France, [2] Novartis Pharma AG, Basel, Switzerland, [3] INSERM UMR 996, Faculté de Pharmacie, Université Paris-Sud, Chatenay Malabry, France, [4] Service de gastro-entérologie, Hôpitaux Universitaires Paris-Sud, Le Kremlin-Bicêtre, France, [5] INSERM UMR 1184, Assistance Publique-Hôpitaux de Paris, Service de Rhumatologie, Hôpitaux Universitaires Paris-Sud, Université Paris-Sud, Le Kremlin-Bicêtre, France*

Peptide As Published – Repeat Assay

20_IL1-15 Cluster: 1

Frame Start	AA Sequence	Frame Stop	Hydro-phobicity	DRB1*0101 Z-Score	DRB1*0401 Z-Score	DRB1*0701 Z-Score	DRB1*1101 Z-Score	DRB1*1501 Z-Score
1	DILLTQSPA	9	0.42	0.75	0.23	0.4	0.59	-0.02
2	ILLTQSPAI	10	1.31	2.27	1.65	2.45	1.24	2.64
3	LLTQSPAIL	11	1.23	2.07	1.51	1.32	1.76	1.35
4	LTQSPAILS	12	0.72	2	2.06	1.73	1.89	1.91
5	TQSPAILSV	13	0.77	-0.61	0.17	0.81	-0.27	0.15
6	QSPAILSVS	14	0.76	-0.66	-0.23	-0.83	-0.05	0.1
7	SPAILSVSP	15	0.97	-0.16	0.28	-0.11	-0.7	-0.22

Two strong EpiBars

Summarized Results	DRB1*0101	DRB1*0401	DRB1*0701	DRB1*1101	DRB1*1501
Maximum Single Z score	2.27	2.06	2.45	1.89	2.64
Publication Results (R.B.A)	B	NB	B	NB	B
EpiVax Assessment	B	B	B	B	TBD

Observed HLA binding in EpiVax HLA binding assay (8 point curve) where publication (yes/no binding) did not observe binding.

Centering HLA DR binding motif improves HLA binding assay performance

ORIGINAL Peptide (Overlapping 15-mer from Hamze, et al.

EpiMatrix Cluster Detail Report

RH36-50 Cluster: 36

Frame Start	AA Sequence	Frame Stop	Hydro-phobicity	DRB1*0101 Z-Score	DRB1*0401 Z-Score	DRB1*0701 Z-Score	DRB1*1101 Z-Score	DRB1*1501 Z-Score
36	WVKQTPGRG	44	-1.3	2.26	1.93	1.24	2.31	1.22
37	VKQTPGRGL	45	-0.78	1.89	0.82	1.9	0.56	1.33
38	KQTPGRGLE	46	-1.63	-1.45	-1.83	-1	-0.9	-0.61
39	QTPGRGLEW	47	-1.3	-0.3	-0.29	0.22	-1.07	-0.45
40	TPGRGLEWI	48	-0.41	-1.98	-2.91	-1.66	-1.94	-1.72
41	PGRGLEWIG	49	-0.38	-1.19	-1.31	-1.56	-0.44	-0.59
42	GRGLEWIGA	50	0	-0.14	0.11	0.3	-0.04	0.34
Summarized Results				DRB1*0101	DRB1*0401	DRB1*0701	DRB1*1101	DRB1*1501
Maximum Single Z score				2.26	1.93	1.9	2.31	1.33
Publication Results				B	NB	NB	NB	NB
EpiVax Binding Data IC50 (nM)				1237	32143	TBD	1424	TBD
EpiVax Assessment				B	B	--	B	--

OPTIMIZED Peptide (Centered Motif)

EpiMatrix Cluster Detail Report

RH36-50MOD Cluster: 33

Frame Start	AA Sequence	Frame Stop	Hydro-phobicity	DRB1*0101 Z-Score	DRB1*0401 Z-Score	DRB1*0701 Z-Score	DRB1*1101 Z-Score	DRB1*1501 Z-Score
33	NMHWVKQTP	41	-0.27	-1.53	-0.52	-1.08	-0.07	-0.55
34	MHWVKQTPG	42	-0.19	1.07	0.48	0.26	0.36	0.71
35	HWVKQTPGR	43	-0.35	-0.64	-0.52	-1.05	-0.11	-1.02
36	WVKQTPGRG	44	-1.3	2.26	1.93	1.24	2.31	1.22
37	VKQTPGRGL	45	-0.78	1.89	0.82	1.9	0.56	1.33
38	KQTPGRGLE	46	-0.35	-1.45	-1.83	-1	-0.9	-0.61
39	QTPGRGLEW	47	-0.28	-0.3	-0.29	0.22	-1.07	-0.45
40	TPGRGLEWI	48	-0.09	-1.98	-2.91	-1.66	-1.94	-1.72
Summarized Results				DRB1*0101	DRB1*0401	DRB1*0701	DRB1*1101	DRB1*1501
Maximum Single Z score				2.26	1.93	1.9	2.31	1.33
EpiVax Binding Data IC50 (nM)				192	4444	422	206	TBD
EpiVax Assessment				B	B	B	B	--

In original publication, the HLA DR binding motifs are located at flanks of the peptide. Binding results do not correlate with predictions.

(1) More sensitive HLA binding assay (7 point binding assessment) confirms **two** more HLA-binding correlations ("B") in original peptide as tested, than original one point binding assay as performed by Hamze et al.

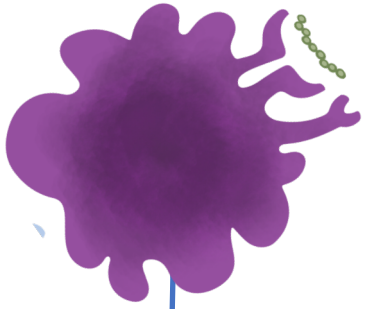
(2) Peptide is optimized (motif is centered). Predicted HLA DR binding motifs are centered in optimized version of Rituximab cluster.

(3) Repeat sensitive HLA binding assay. Using optimized version of Rituximab peptide, HLA binding assay performance improves and predicted binding affinities are **validated (B).**

T cell assays used by Industry

In vitro immunogenicity Protocol or "IVIP"

MAPPS Assay

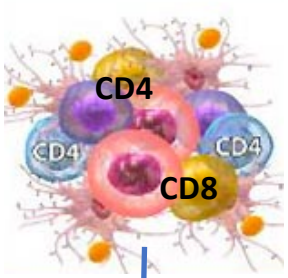


MHC immunoprecipitation

MS sequencing of peptides

Value added: peptide processing/competition

PBMC Assay - IVIP



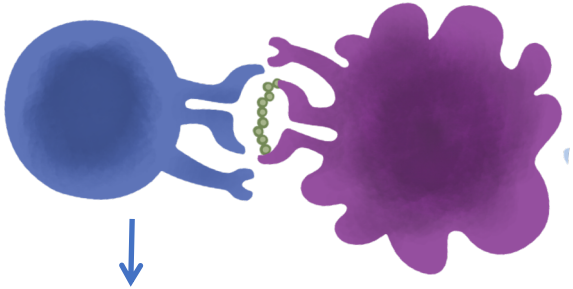
TNFa, IL2, IFNg

Luminex/Elispot/ICS /Proliferation

Validation of immunogenicity/ high sample numbers;; low sensitivity for primary responses

DC/T cell Assay

Generate moDC



TNFa, IL2, IFNg

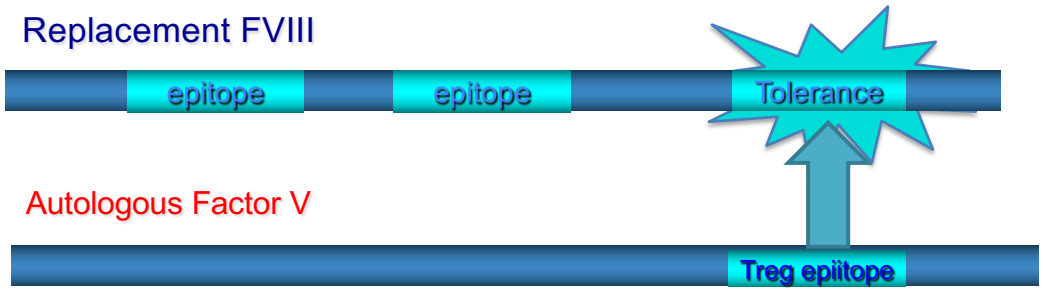
Luminex/Elispot/ICS /Proliferation

High Sensitivity, Technically Complex

Factor V has a Tregitope (Amy Rosenberg/Bill Martin) Unpublished assays by Eduardo Guillen/Sandra Lelias

EpiVax

Other Autologous Proteins with Similar (Homologous) Epitopes may be Tolerogenic



We ask:

- *Do Autologous T reg epitopes (in FV) regulate immune response to FVIII?*
- *Could these autologous Treg epitopes be used to induce FVIII-specific tolerance?*
- ***We think YES***

Outline

EpiVax

- Defining T cell Epitopes In Silico – Yes, We Can.
- Comprehensive Immunogenicity Risk Assessment (Includes In Vitro)
- Cutting Edge Tools: The two-faced T cell epitope and Tregitopes
- Immune Engineering Immunogenicity and Tolerance

Immune Engineering – What is it

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.

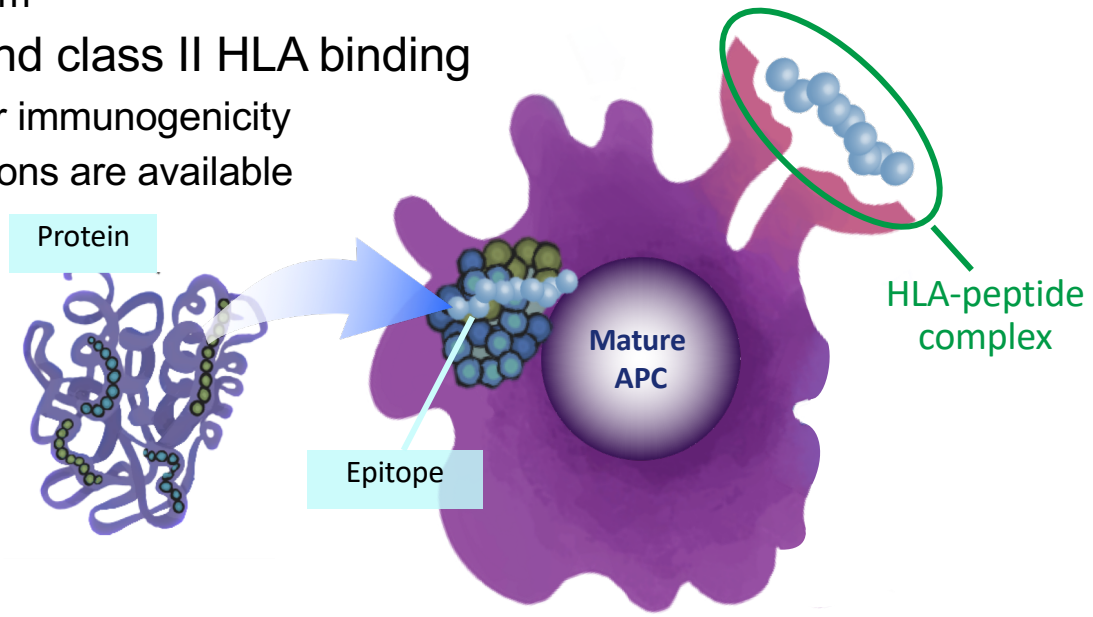
Engineer out regulatory T cell epitopes

EpiMatrix – T cell Epitope Prediction

Identifying Putative T cell Epitopes in peptides and proteins

EpiVax

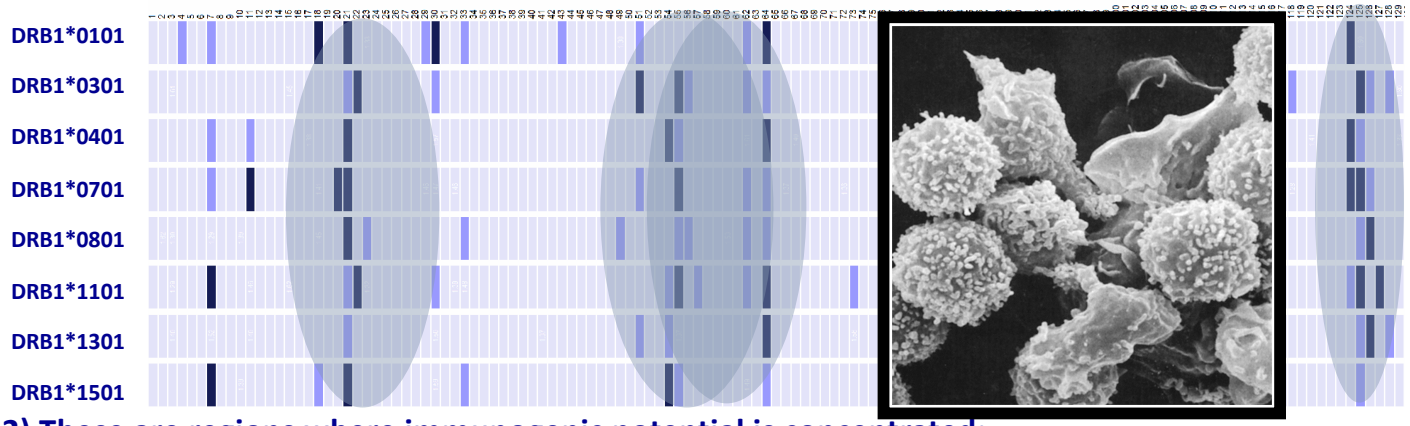
- EpiVax uses EpiMatrix to predict T cell epitopes
  - Matrix-based prediction algorithm
- EpiVax predicts both class I and class II HLA binding
  - HLA binding is a prerequisite for immunogenicity
  - Full suite of HLA-based predictions are available



Regions of Epitope Clustering: ClustiMer

Maximum impact with few AA changes

1) Identify regions where "positive scores" cluster across alleles



2) These are regions where immunogenic potential is concentrated:

Strong bands suggest binding across all "pockets": Promiscuous epitopes

OptiMatrix – In Silico Immune Engineering

Use OptiMatrix to redesign potentially immunogenic clusters

EpiVax

DEIMMUNIZE

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

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

2014 FDA Guideline: 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

OptiMatrix – Tolerization Function

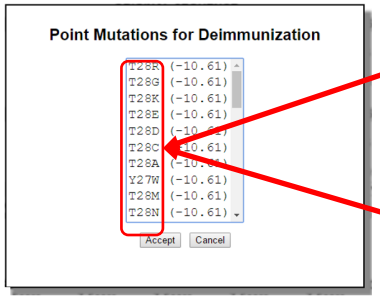
Maintaining or Introduce Existing Tregitopes*

EpiVax

DEIMMUNIZE

According to regulatory guidelines, when de-immunzing monoclonal antibodies, the removal of regulatory T cell epitopes, **Tregitopes**, should be avoided.

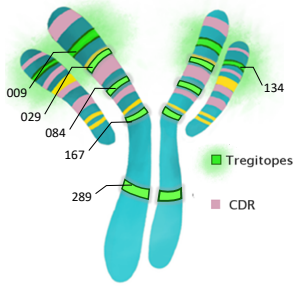
If the original sequence contains a Tregitope, then OptiMatrix attempts to deimmunize the sequence **without interfering** with the Tregitopes



Example: List of deimmunization options will try to remove the epitopes in frames 11 and 20 by changes made to L11, Y27, or T28 below (residues that don't overlap with the Tregitopes).

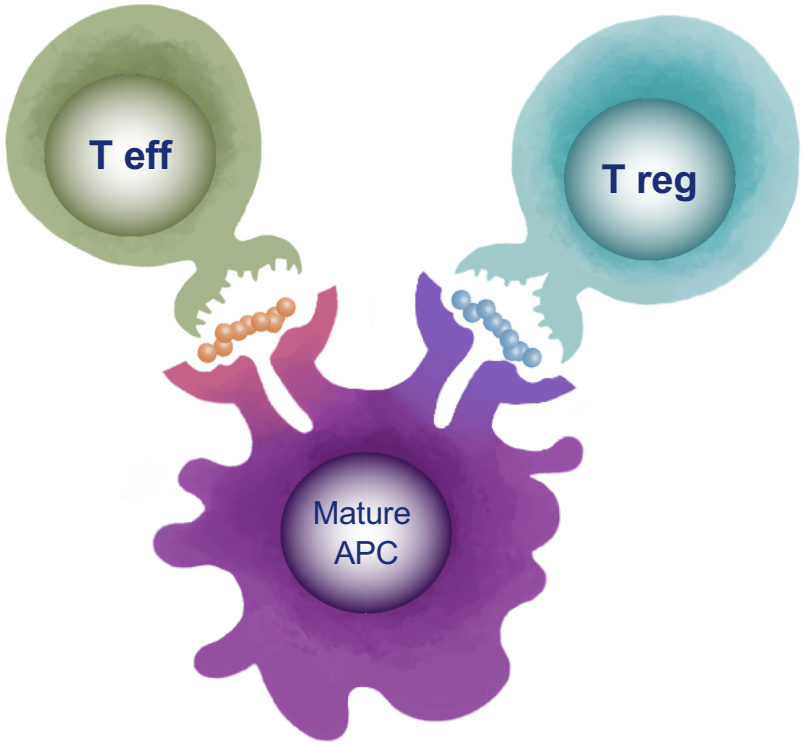
EpiMatrix Cluster Detail Report

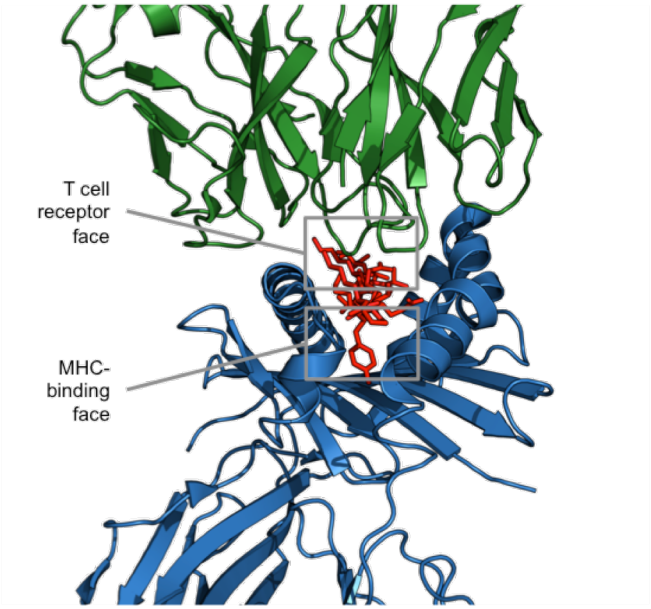
Frame Start	AA Sequence	Frame Stop	Hydro-phobicity	DRB1*0101 Z-Score	DRB1*0301 Z-Score	DRB1*0401 Z-Score	DRB1*0701 Z-Score	DRB1*0801 Z-Score	DRB1*1101 Z-Score	DRB1*1301 Z-Score	DRB1*1501 Z-Score	Hits
9	GGLVQPGGS	17	0.01	0.20	0.18	-0.03	0.14	-0.11	0.41	-0.70	0.36	0
10	GLVQPGGSL	18	0.11	0.76	-0.07	-0.22	0.44	-0.36	-0.32	-0.43	0.48	0
11	LVQPGGSLR	19	0.01	0.82	1.22	0.78	0.35	0.51	-0.15	1.75	1.04	1
12	VQPGGSLRL	20	0.07	1.49	1.56	1.45	1.88	0.45	0.82	1.23	2.24	2
13	QPGGSLRLS	21	-0.49	-1.18	-0.51	-0.92	-1.74	-0.87	0.08	-1.41	-0.77	0
14	PGGSLRLSC	22	0.18	-0.10	-0.09	-1.01	0.10	-0.37	0.09	-0.27	0.14	0
15	GGSLRLSCA	23	0.56	-0.30	-0.07	-0.21	-0.49	0.03	0.49	0.06	0.32	0
16	GSLRLSCAA	24	0.80	0.39	-1.42	-0.77	0.57	-0.72	0.19	-0.45	0.05	0
17	SLRLSCAAS	25	0.76	0.58	0.34	1.64	-0.45	0.80	1.35	0.78	0.09	0
18	LRLSCAASG	26	0.80	2.44	2.45	2.40	1.32	2.15	2.51	2.29	1.84	7
19	RLSCAASGY	27	0.23	-0.28	0.67	0.09	-0.75	0.03	-0.90	0.71	-0.04	0
20	LSCAASGYT	28	0.66	1.07	0.43	0.83	1.65	0.59	0.38	-0.67	1.71	2
21	SCAASGYTF	29	0.12	-0.08	0.13	0.31	0.31	-0.27	-0.42	-0.02	0.20	0
22	CAASGYTFT	30	0.12	0.11	-1.42	-0.90	0.14	-1.62	-1.61	-1.72	-0.46	0
23	AASGYTFTN	31	-0.02	-0.30	-0.96	-0.75	-0.79	-0.99	-0.68	-1.45	0.11	0



JanusMatrix to find Treg/Tolerated epitopes

EpiVax





DeFT re-engineering of Alpha Interferon
Remove Epitopes But Preserve Funcation

Clinical Immunology 176 (2017) 31–41

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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]

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[b] EpiVax, Inc., Providence, RI, USA
[c] Institute for Immunology and Informatics, University of Rhode Island, RI, USA

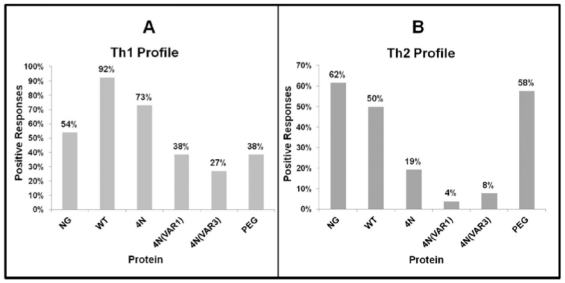
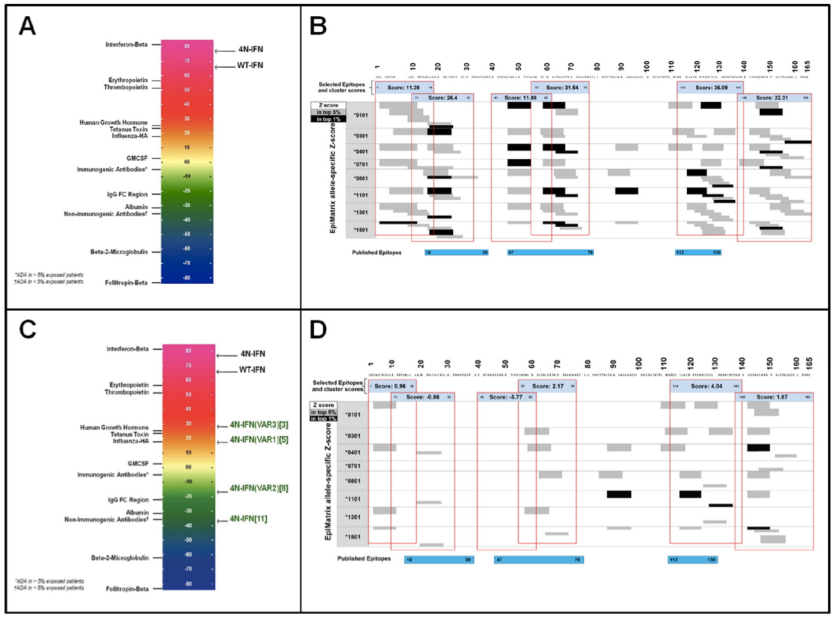
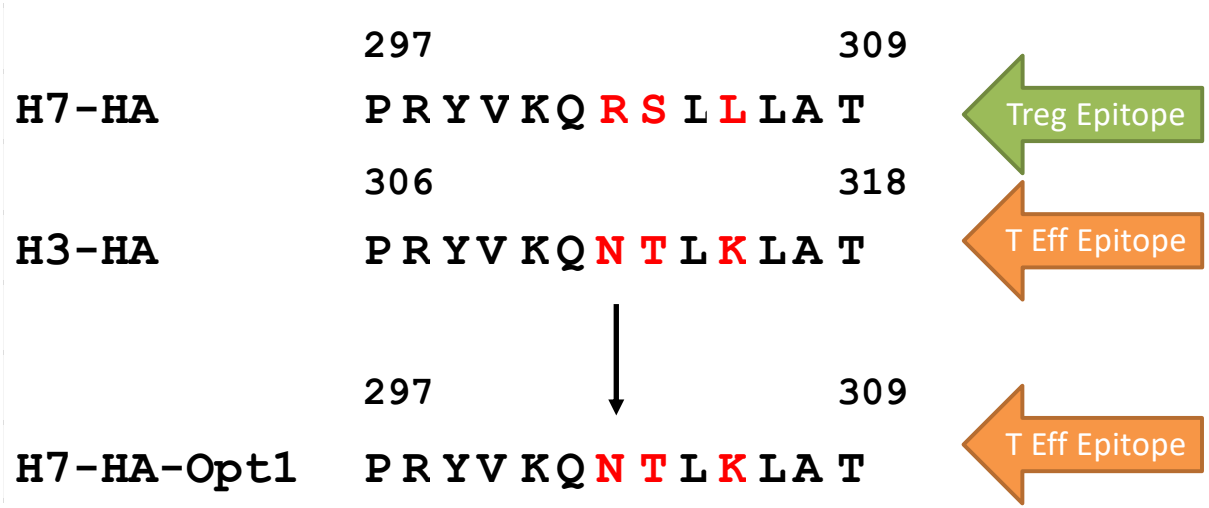


Fig. 6. 4N-IFN de-immunized variants showed a marked reduced immunogenicity in comparison with other IFN versions. *Ex vivo* cytokine secretion by T-cells after incubation with IFN-pulsed dendritic cells. Data were obtained from 26 donors. A Stimulation Index (SI) was defined as a ratio of the cytokine 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.

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



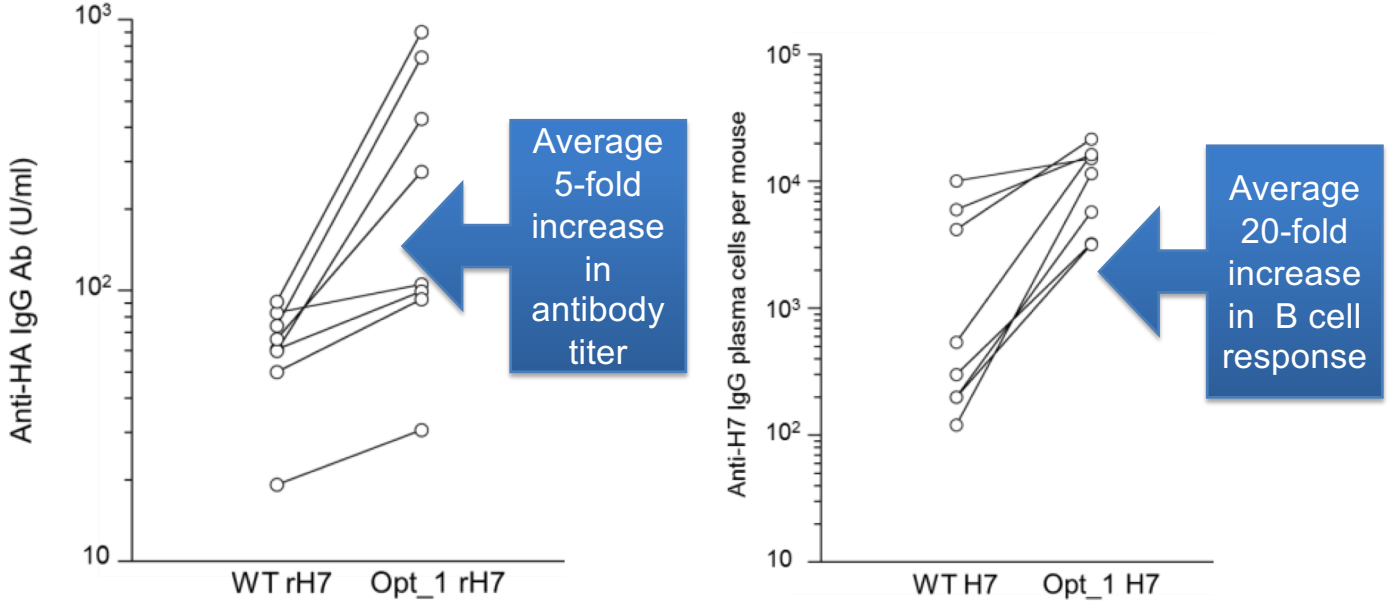
simultaneous Treg epitope knock-out and Teff epitope knock-in

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

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**

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

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[4], Masato Tashiro[4], Ganjana Lertmemongkolchai[3], Haruko Takeyama[2], Anne S. De Groot[5,6], Manabu Ato[1] & Yoshimasa Takahashi[1]

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

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

secukinumab (COSENTYX) anti-IL17A: in silico

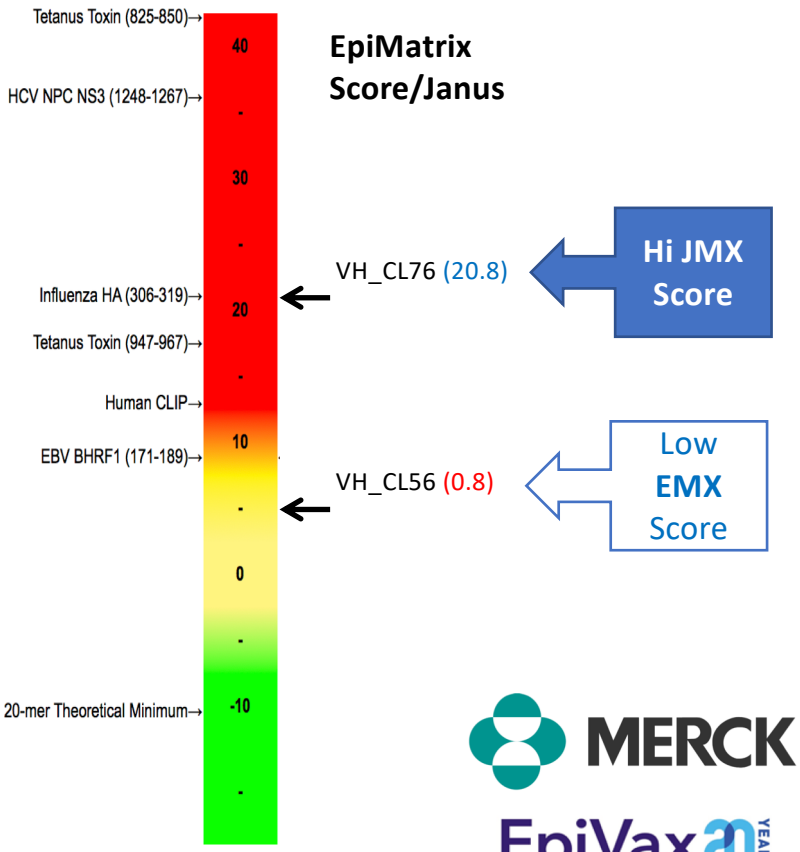
>secukinumab_H COSENTYX_aIL17a
EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMNWVRQAPGKGLEWVAAINQDGSEKYYVGSVKGRF
SRDNAKNSLYLQMNSLRVEDTAVYYCVRDYYDILTDYYIHYWYFDLWGRGTLVTVSSASTKGPSVFPLA
KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH
NTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
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%)

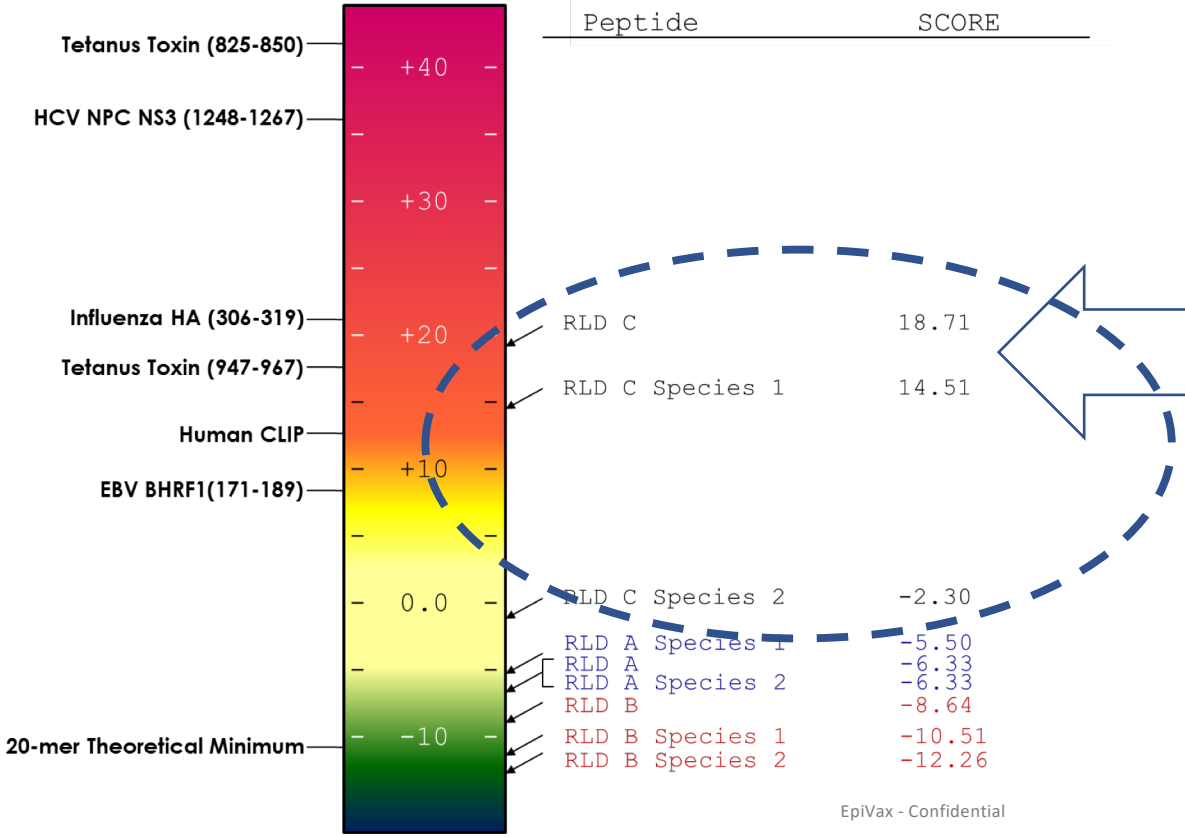


Presentation by D. Montgomery at PEGS Analysis: Jad Maamary

MERCK

EpiVax 20 YEARS Fearless Science

PANDA: In Silico Screening
Example Peptide RLDs – Peptide Impurities



RLD C (Peptide Generic) predicted to be immunogenic, impurities slightly less

But: **3% ADA in the clinic**

In Vitro Immunogenicity: IFNγ Fluorospot for RLD C / Peptide Generic

Much Less Immunogenic Than Predicted

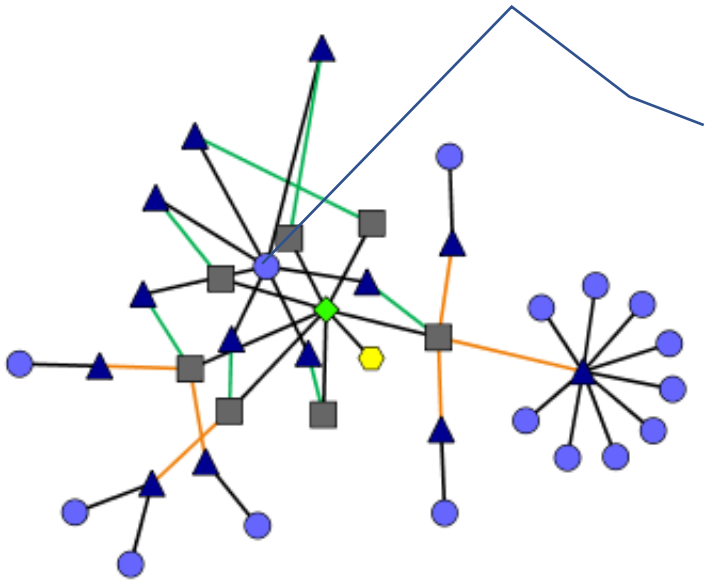
. . .Do JanusMatrix Analysis . . .

RLD C Summary of IFNγ Fluorospot responses across donors

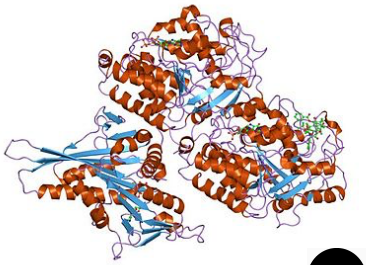
	DONOR	1	2	3	4	5	6	7	8	9	10	TOTAL # of Positive Responses*
	DRB1 Allele	01:03	01:02	04:01	07:01	01:01	07:01	01:01	11:01	04:04	01:01	
		07:01	04:02	13:02	11:01	03:01	13:01	04:04	11:04	11:03	04:04	
	EpiMatrix Hits: Allele	2	1	1	3	2	3	2	4	1	2	
		3	2	2	4	1	2	1	4	4	1	
RLD	1	-	-	+	-	-	+	-	+	-	-	3/10
	2	-	-	-	-	-	-	-	-	-	-	0/10
	3	-	-	-	-	-	-	-	-	-	-	0/10
	4	-	-	-	-	-	-	-	-	-	+	1/10
	5	-	-	-	+	-	-	-	-	-	-	1/10

JanusMatrix Analysis of Generic Peptide

Immunogenic by T cell epitope analysis.
But Both in vitro assay and clinical data (3% ADA) . . . much Less Immunogenic than predicted . . .Do JanusMatrix Analysis:
Conserved with very common intracellular protein, Tubulin.

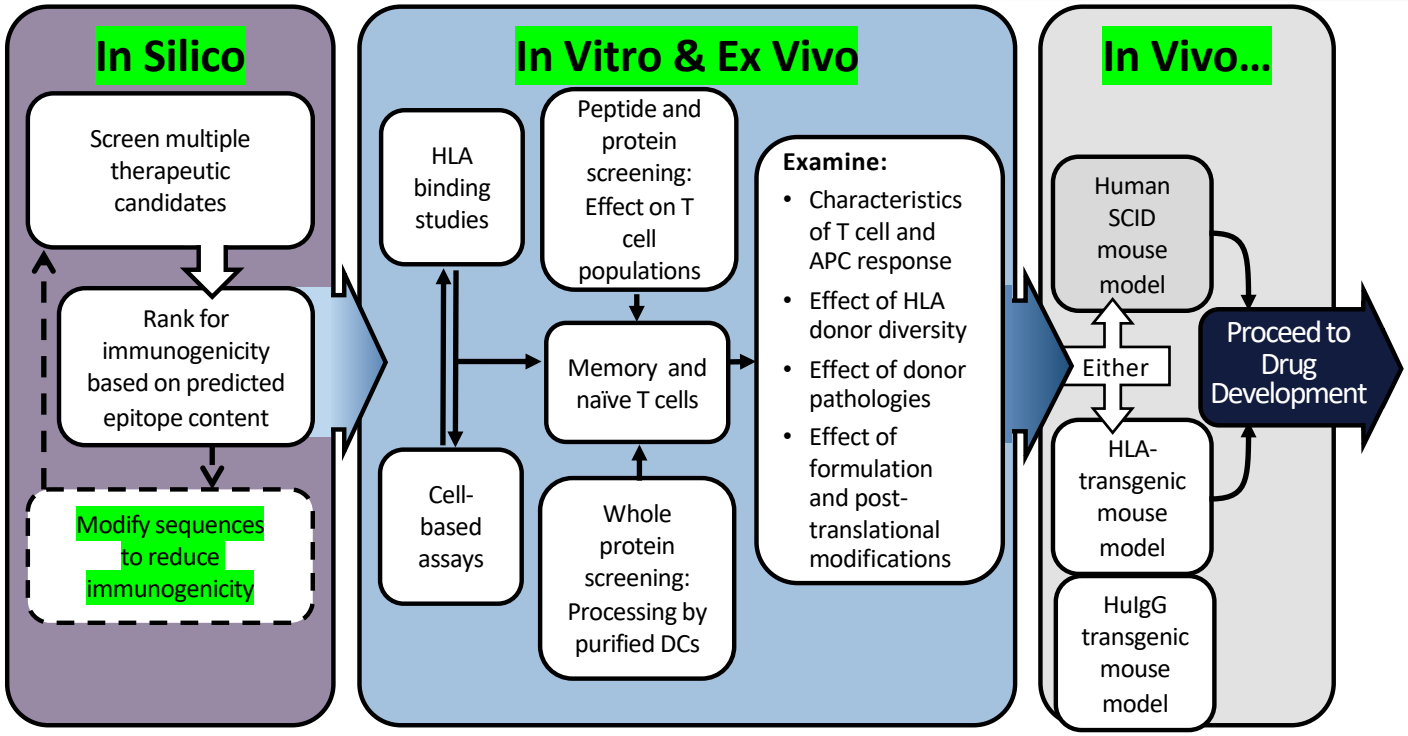


Tubulin
tubulin protein superfamily of globular proteins, or one of the member proteins of that superfamily. α- and β-tubulins polymerize into microtubules, a major component of the eukaryotic cytoskeleton.



Immune Engineering Utilizes Many Platforms

EpiVax



T-cell dependent immunogenicity of protein therapeutics: Preclinical assessment and mitigation.
Jawa V, Cousens LP, Awwad M, Wakshull E, Kropshofer H, **De Groot** AS. Clin Immunol. 2013 Dec;149(3):534-55. doi:10.1016/j.clim.2013.09.006. Epub 2013 Sep 25. Review. PMID: 24263283

Conclusions

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!*

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

Questions?