European Immunogenicity Platform
Lisbon
February 2014

Tregitopes: Their role in the prediction and mitigation of immunogenity

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EpiVax, Inc., Institute for Immunology and Informatics

Institute for
Immunology and
Informatics

Outline

- Who?
- What?
- Where?
- How?
- Why?

Who are We? (EpiVax)

- Biotech company based in Providence Rhode Island
- Owned and operated by Annie De Groot and Bill Martin

EpiVax designs and develops safer, more effective biologic products and vaccines

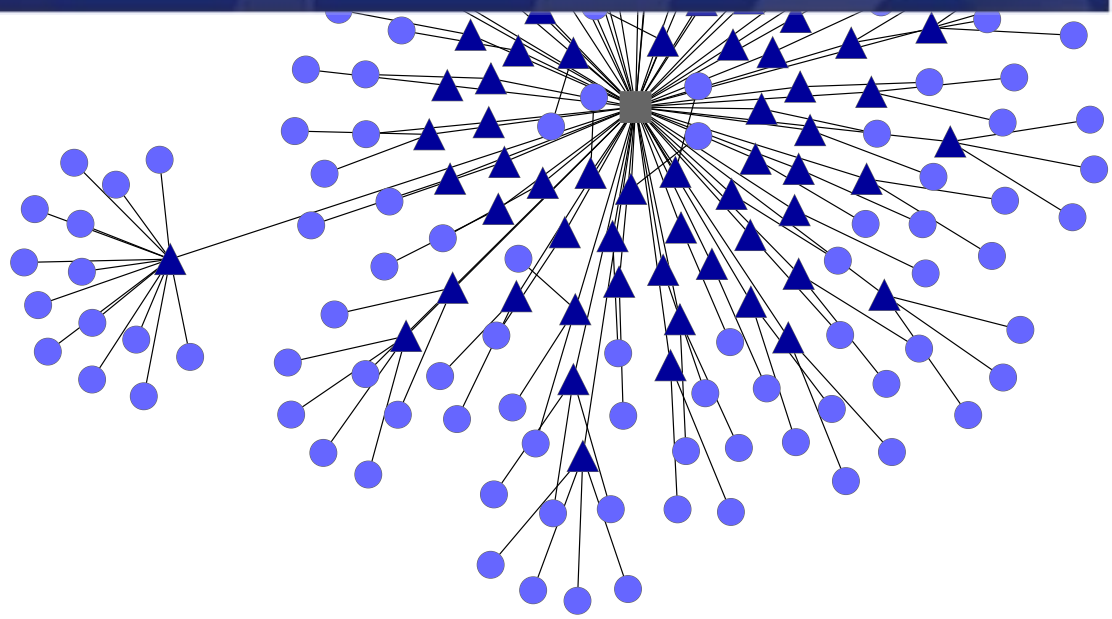
Cutting Edge	Trusted	Engaged
Continuously innovating	Pharma- and Peer- approved	"To improve human health everywhere"

EpiVax: Four Core Strengths

www.epivax.com

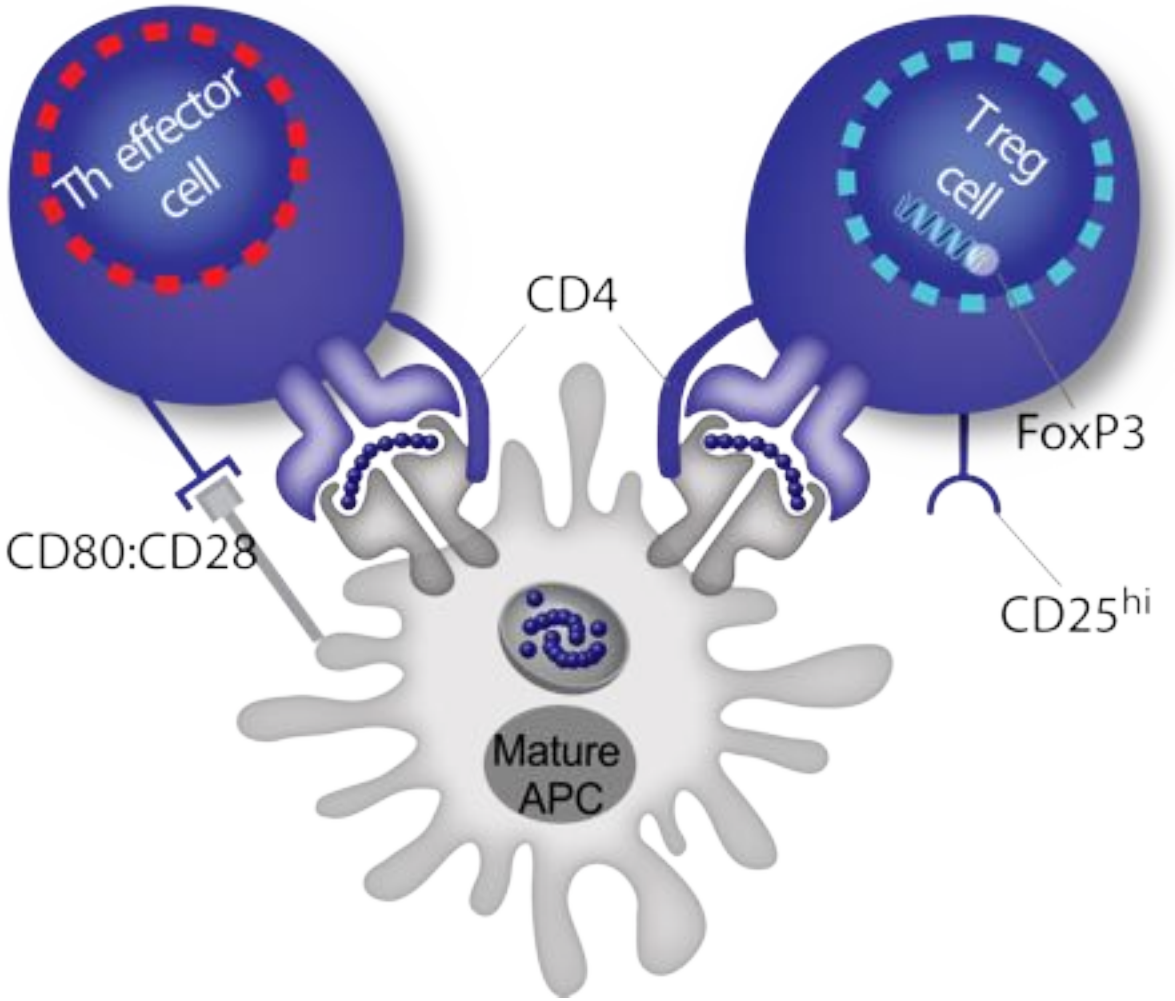
Immunogenicity Screening	➢ Epitope Mapping ➢ HLA Binding, Class I & II ➢ T cell assays (naïve, memory) ➢ In vivo assays (HLA Tg mice)	Fee for Service
Vaccines	➢ Grant funded R & D ➢ Excellent proof of principle	Grant Funded
Bio-Betters	➢ Select targets ➢ Funded research or joint developments ➢ Develop molecule and license	Sponsored research / Joint Development
Immuno-modulation	➢ Tregitopes ➢ In preclinical development - first in class - allergy, autoimmunity, transplant	Grant funded Options available for selected “Field of Use”

What are Tregitopes?



http://bit.ly/Tregi-Pubs

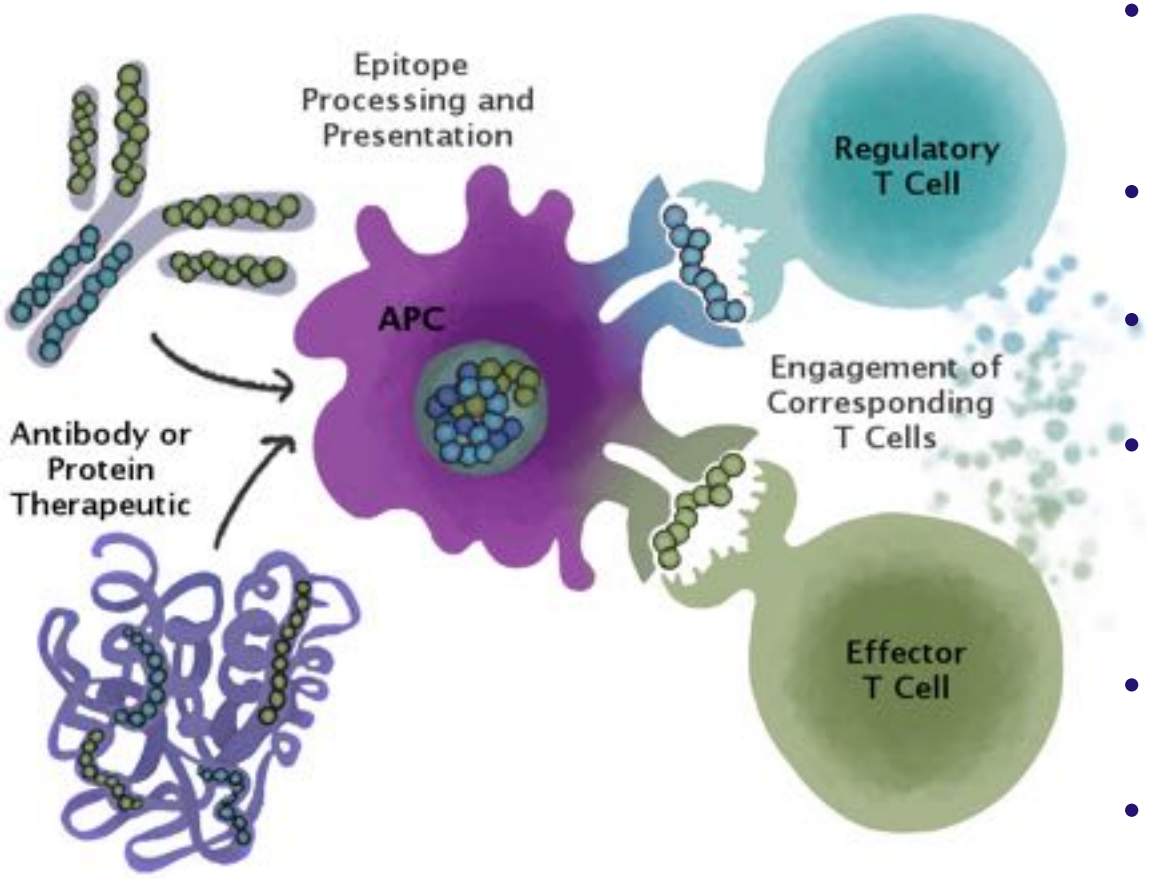
What are *Tregs?*



T cells that suppress immune response

(a.k.a. Suppressor T cells)

What are *Tregitopes*



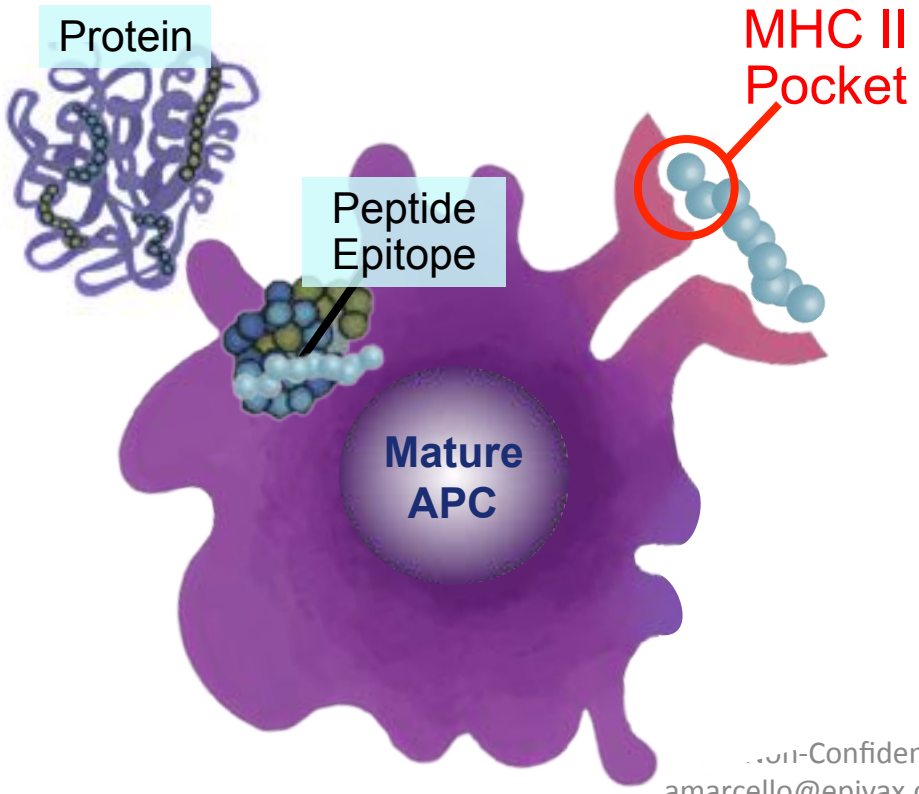
- Short, linear peptide sequences that activate *regulatory T cells*
- Highly conserved in IgG across species
- Tregitope role: to suppress inflammation
- Co-formulated or attached to proteins to provide antigen-specific tolerance
- . . . First in class therapeutic
- Wide range of applications

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

http://bit.ly/Treg1

Discovery of Tregitopes

- EpiMatrix maps putative epitopes
  - matrix based prediction algorithm using pocket profile method described by Sturniolo and Hammer
- Predict likelihood of sequence binding to HLA I and II complex



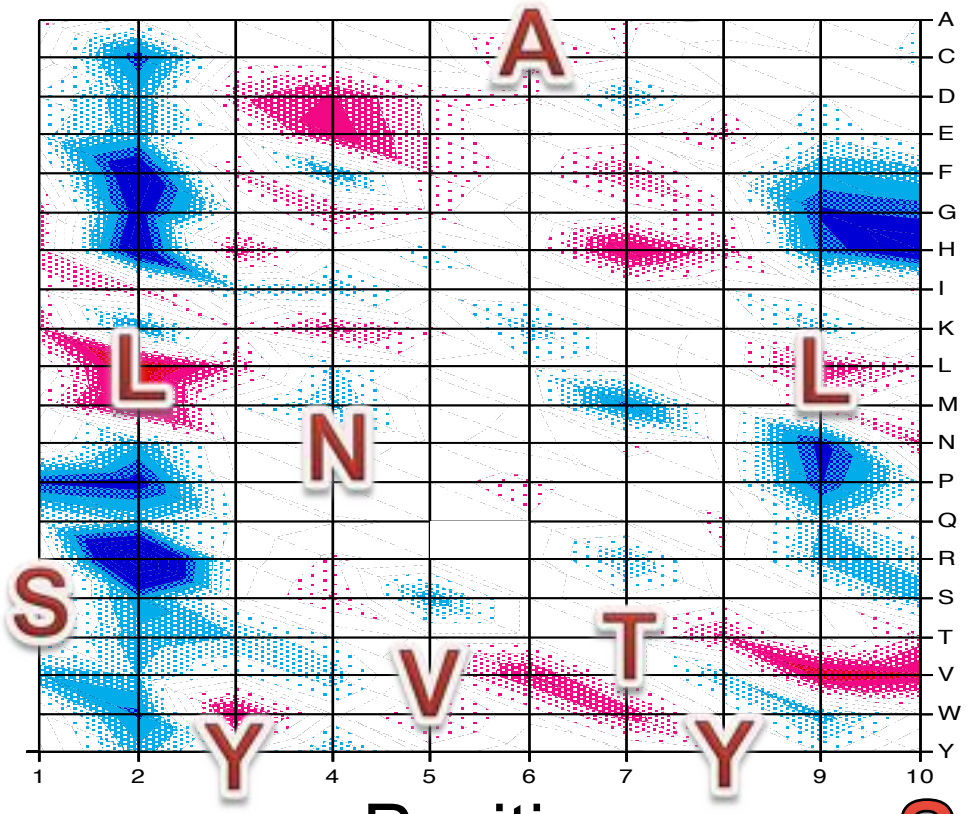
T cell epitopes are linear and directly derived from antigen sequence

Binding is determined by amino acid side chains (R groups) and 'encoded' in single letter code

Discovery of Tregitopes

- **Analyzes 9mer frames for binding likelihood based on matrix-based algorithm**

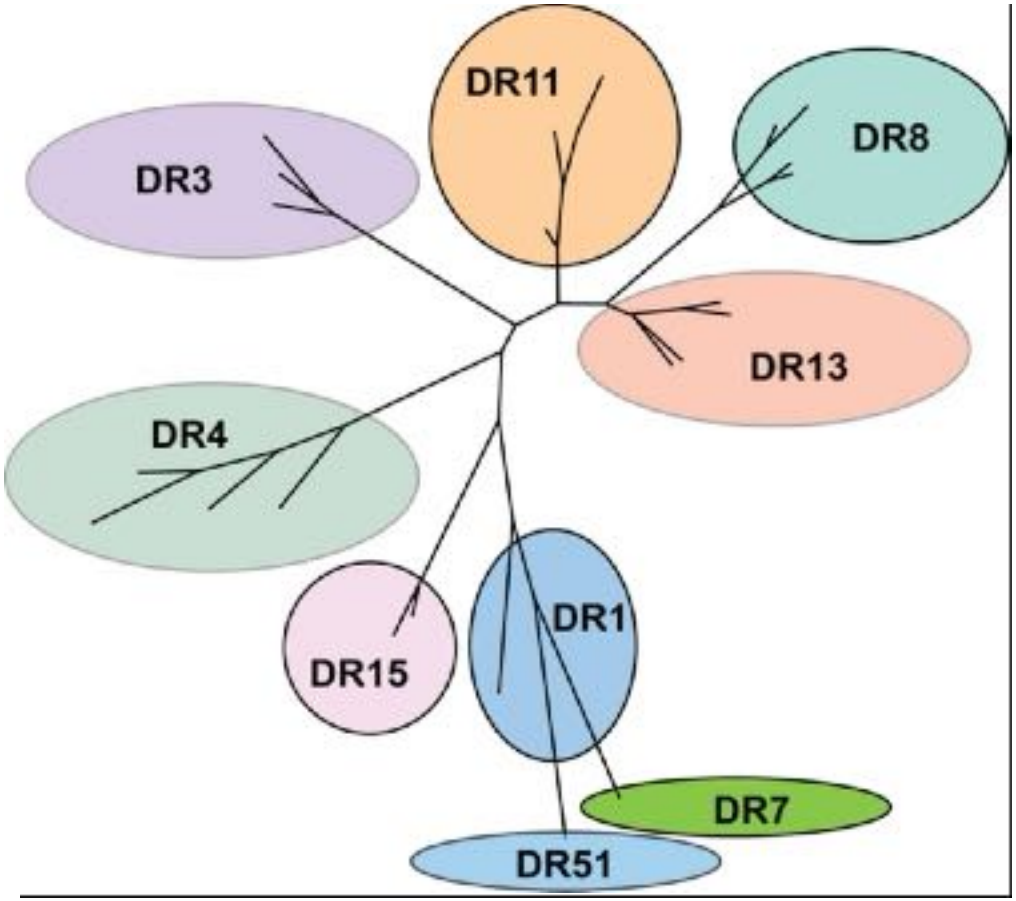
Graphical Representation of A*0201 Coefficient matrix



S+L+Y+N+V+A+T+Y+L

= indication of binding likelihood

Discovery of Tregitopes



- 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 >90% of human populations worldwide* without the necessity of testing each haplotype individually.

* Southwood et. al., Several Common HLA-DR Types Share Largely Overlapping Peptide Binding Repertoires. 1998. Journal of Immunology.

Discovery of Tregitopes

EpiMatrix Report

Accession: Influenza - Sequence: HA 306-318

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
306	PRYVKQNTL	314	1.34	1.40		2.06				1.28	1
307	RYVKQNTLK	315									
308	YVKQNTLKL	316	3.33	1.97	3.15	3.27	1.96	1.99	2.37	2.36	8
309	VKQNTLKLA	317						1.59	1.67		1
310	KQNTLKLAT	318									

Assessments performed: 40 **Deviation from Expectation: 17.62**

Z score indicates the potential of a 9-mer frame to bind to a given HLA allele; the strength of the score is indicated by the blue shading as shown below:

Cluster Regions Outlined	Z scores in top 1%	Z scores in top 5%	Z scores in top 10%*	remaining scores masked*

All scores in the Top 5% are considered "Hits". *non hits below 10% are masked for simplicity
Frames containing four or more alleles scoring above 1.64 are referred to as Epi-Bars and are highlighted in yellow.
These frames have an increased likelihood of binding to multiple HLA.

EpiBar : A common feature of highly immunogenic clusters

= "EpiBar"

Roberts CGP, Meister GE, Jesdale BM, Lieberman J, Berzofsky JA, A.S. De Groot, Prediction of HIV peptide epitopes by a novel algorithm, AIDS Research and Human Retroviruses, 1996, Vol. 12, No. 7, pp. 593-610.

ClustiMer - Locates highly immunogenic regions

In Silico Protein Re-engineering And Immunogenicity

- EpiMatrix – maps T cell epitopes
- ClustiMer - Promiscuous / Supertype
- BlastiMer - Avoiding "self" - autoimmu
- OptiMatrix - Deimmunize
- JanusMatrix – Analyze conservation v
- ISPRI HT- High Throughput analysis

Seamless
Biologics Design

Integrated toolkit is
unique to ISPRI

Bill Martin – EpiVax – Principle Architect of the ISPRI Toolkit

ISPRI Online Platform

Data Management

Accession Manager
Use this Link to Manage Uploaded Datasets

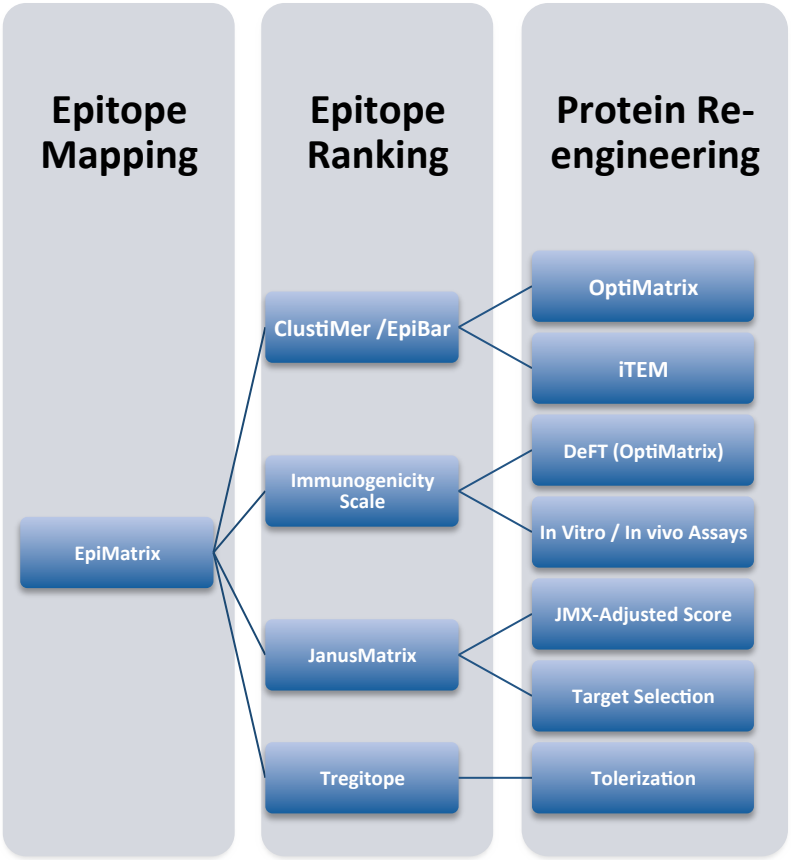
Upload Proteins
Use this Link to Upload Protein Data for Analysis

Upload Clusters
Use this Link to Upload T cell Epitope Clusters for Analysis

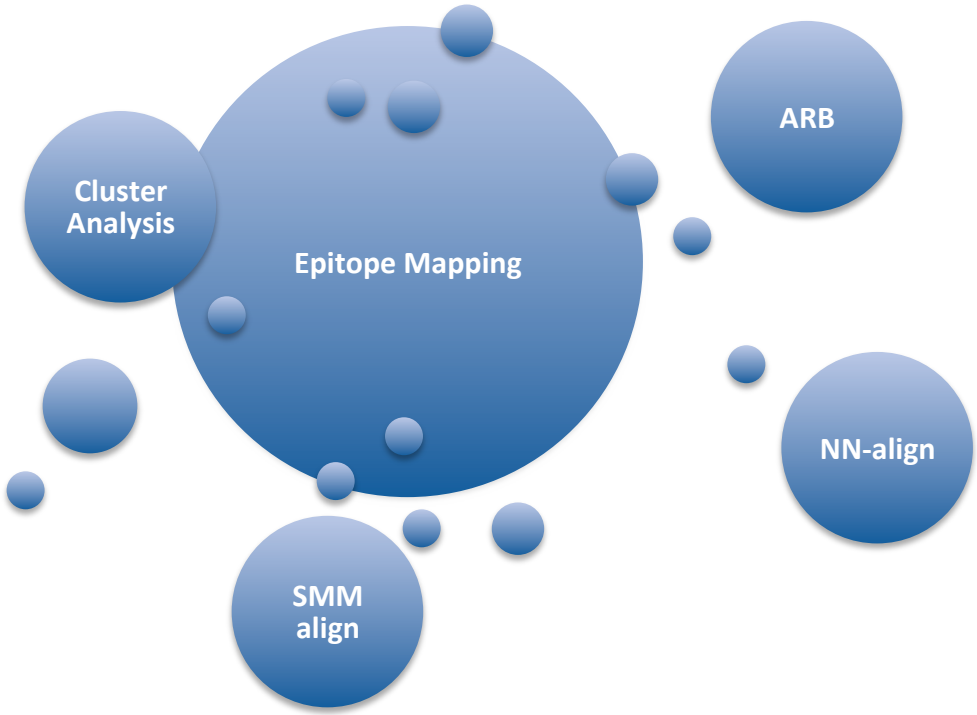
Upload Archive
Use this Link to Upload an Archived Analysis

ISPRI vs. IEDB in silico services

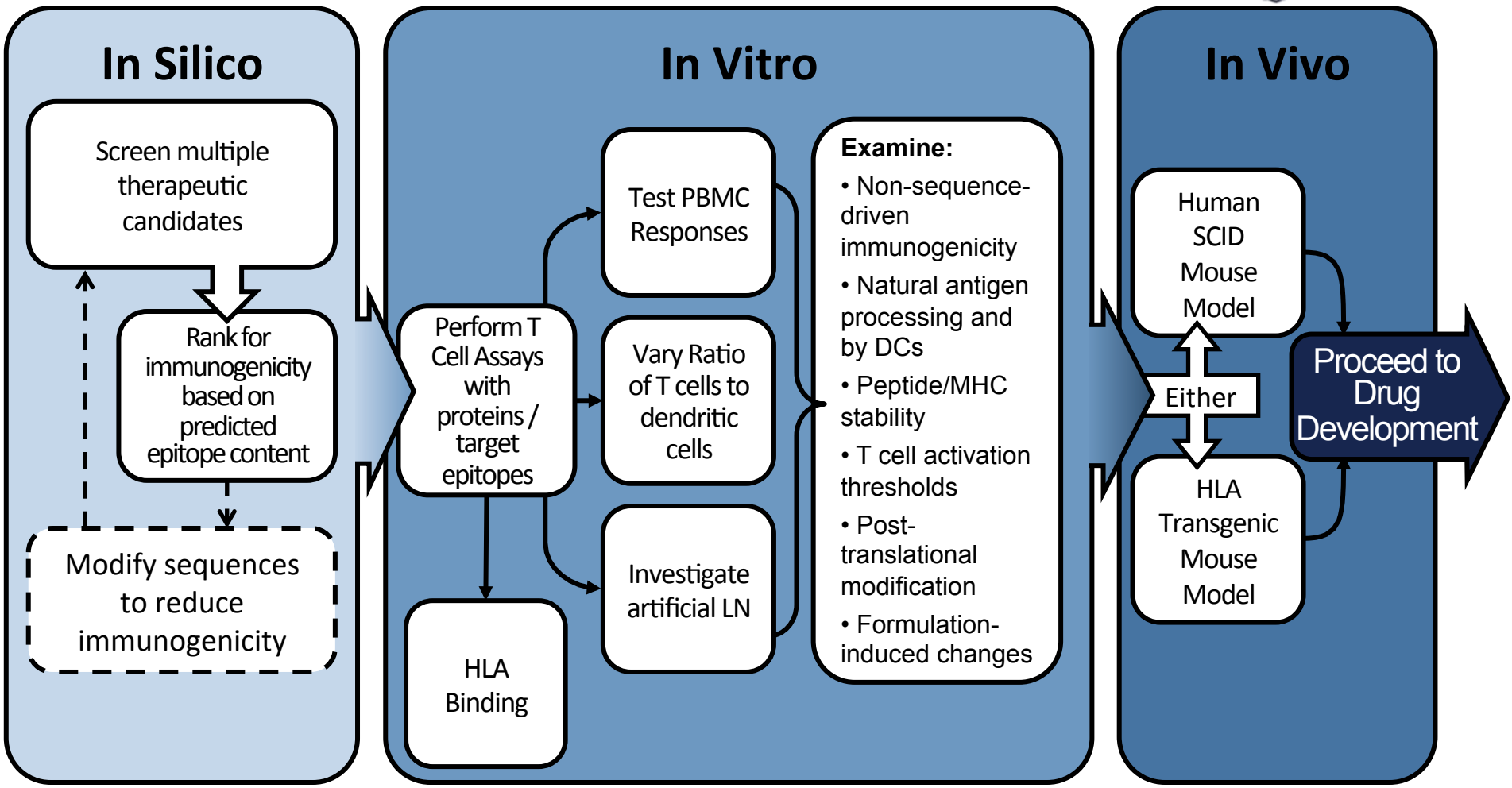
ISPRI is an ***integrated, interactive set of tools*** specifically designed for immunogenicity analysis. ISPRI provides the **depth of analysis that is** necessary to accurately predict clinical immunogenicity



IEDB is a collection of tools that are not integrated in any coherent fashion. Epitope prediction is possible, but seamless immunogenicity screening and protein re-engineering is not.

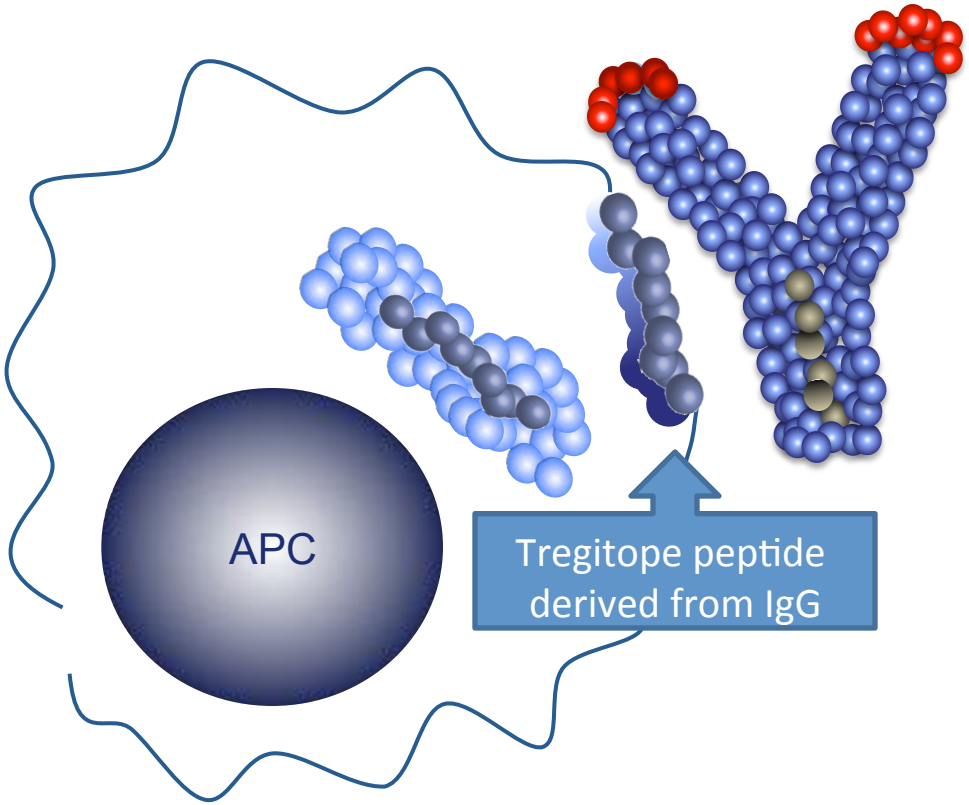


Our Recommended Approach to Immunogenicity Screening



From: http://bit.ly/The_TCWP - T cell "White Paper"

Discovery of Tregitopes



Screen Mabs For immunogenicity

Found Epitopes that are Highly conserved

Tolerated or ignored?

(David W. Scott connection)

Tregs?

. . . Test hypothesis

Original Publication

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]

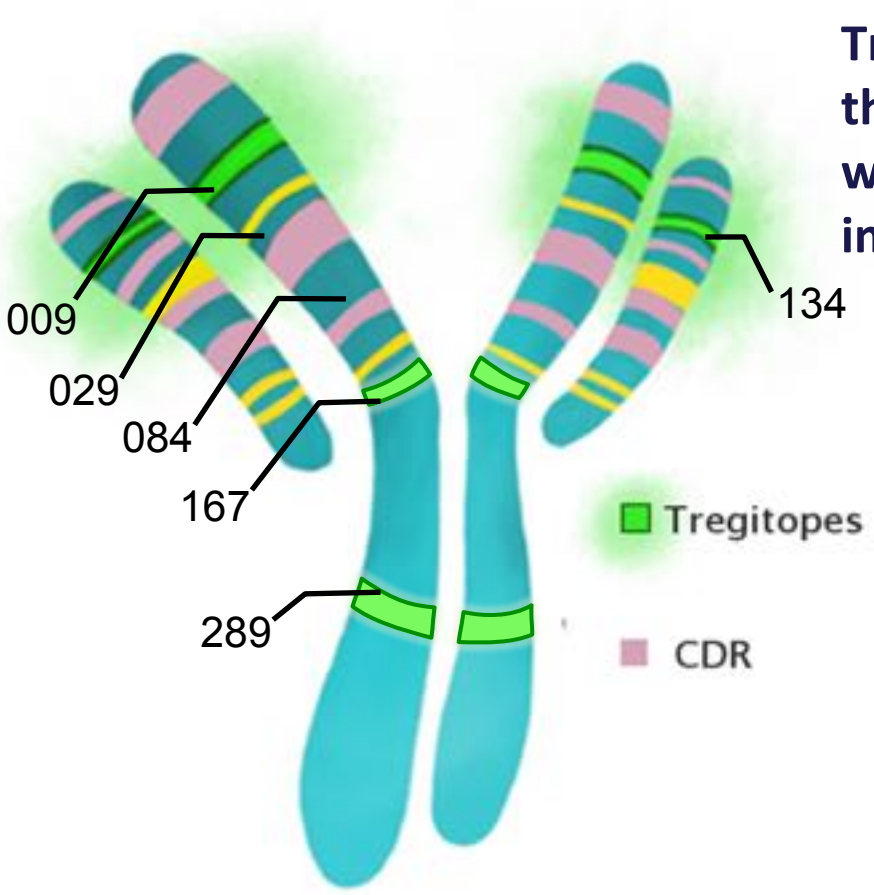
[1]EpiVax, Providence, RI; [2]University of Rhode Island, Providence, RI; [3]Stallergenes, Anthony, France; [4]University of Maryland, College Park, MD

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)

Outline

- Who are we?
- What is a Tregitope and . . . What do they do?
- Where are they located?
- How they can be applied to protein therapeutics
- Why they are important to immunogenicity

Where are Tregitopes



Tregitopes are highly conserved regions of the IgG sequence that represent what appear to be highly relevant, immunosuppressive or regulatory epitopes.

Tregitope definition:

- *15-20 mer peptides in conserved regions*
- *Strong signals for T cells ("EpiBars")*
- *Highly conserved among IgG molecules*
- *Not conserved across Immunoglobulin subtypes*
- *Conserved across species (mouse, cat, camel)*
- *Induce natural Tregs to modify immune response*
- *Potentially one of the mechanism of action of IVIG*

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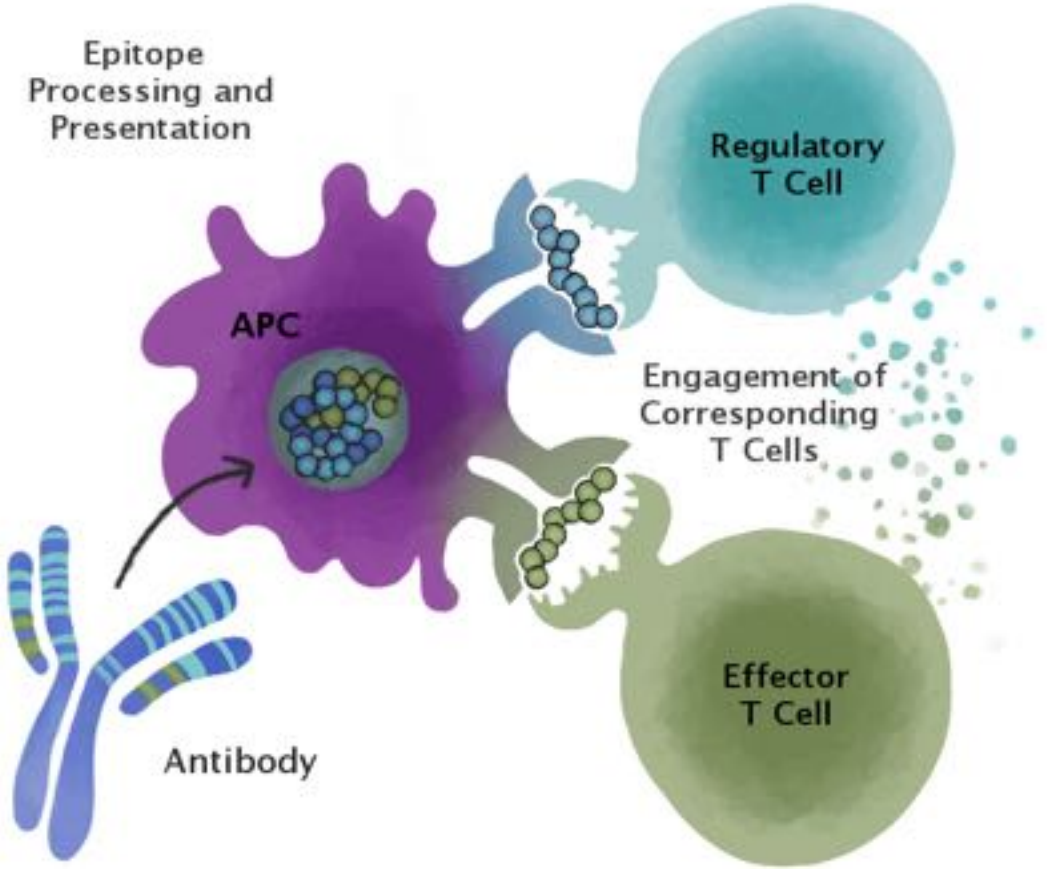
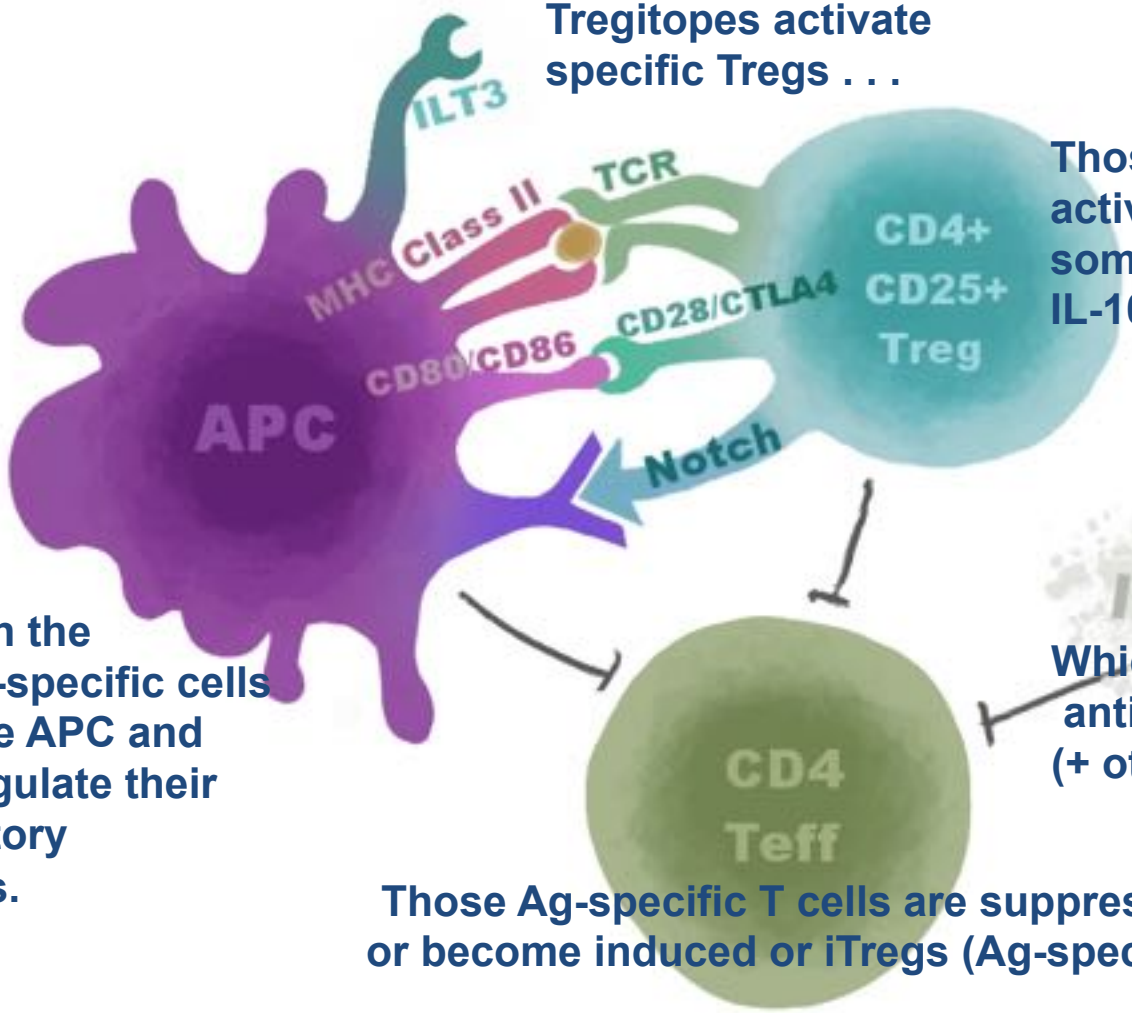
Hypothesis: Treg epitopes are there to balance Teff epitopes



Tregitopes are Treg epitopes. . .

Tregitopes activate specific Tregs . . .



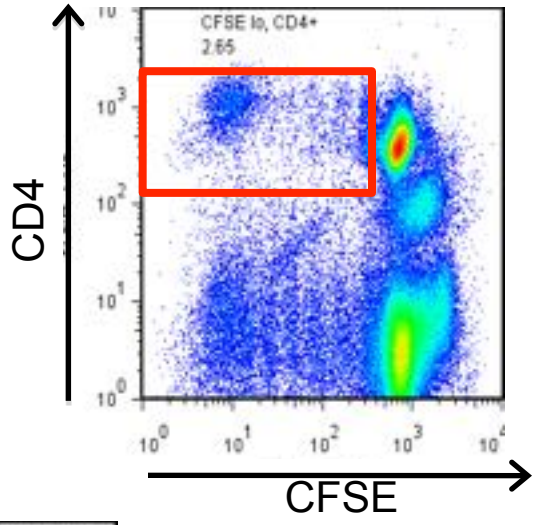
Those Tregs get activated and expand; some of them make IL-10 . . .

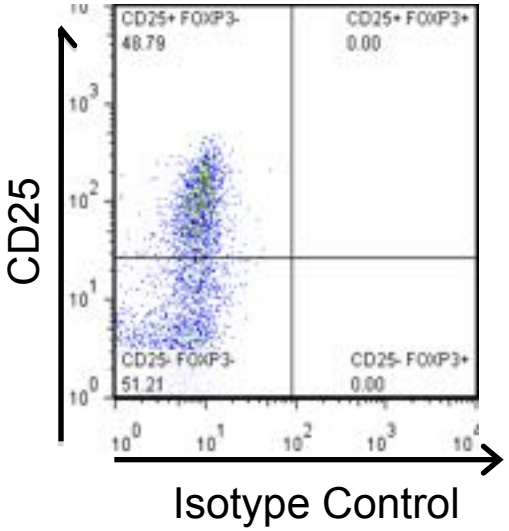
Which modifies nearby antigen-specific T cells (+ other mechanisms) . . .

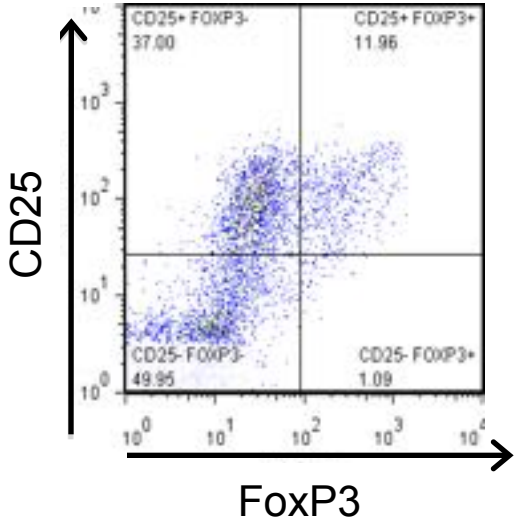
In addition the Tregitope-specific cells modify the APC and down –regulate their inflammatory properties.

Those Ag-specific T cells are suppressed or become induced or iTregs (Ag-specific)!

In culture, Tregitopes expand Tregs
FACS: Staining for CD25 and FoxP3+

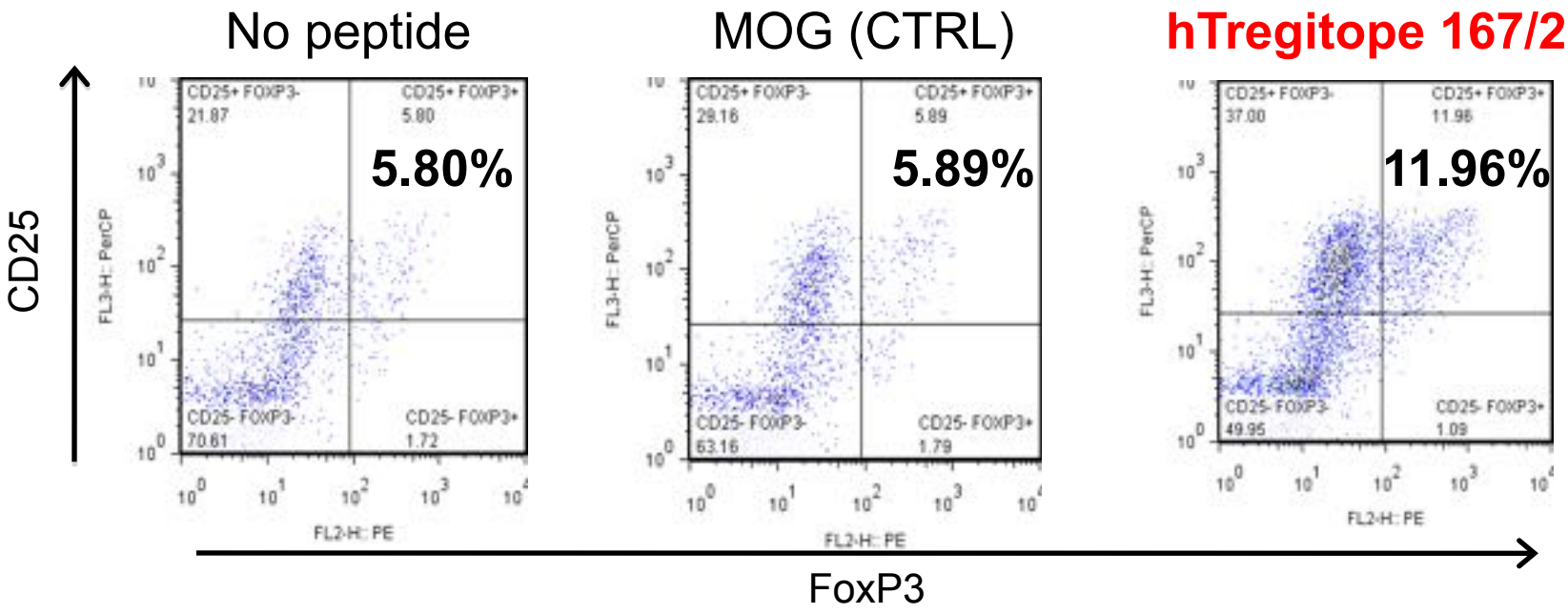






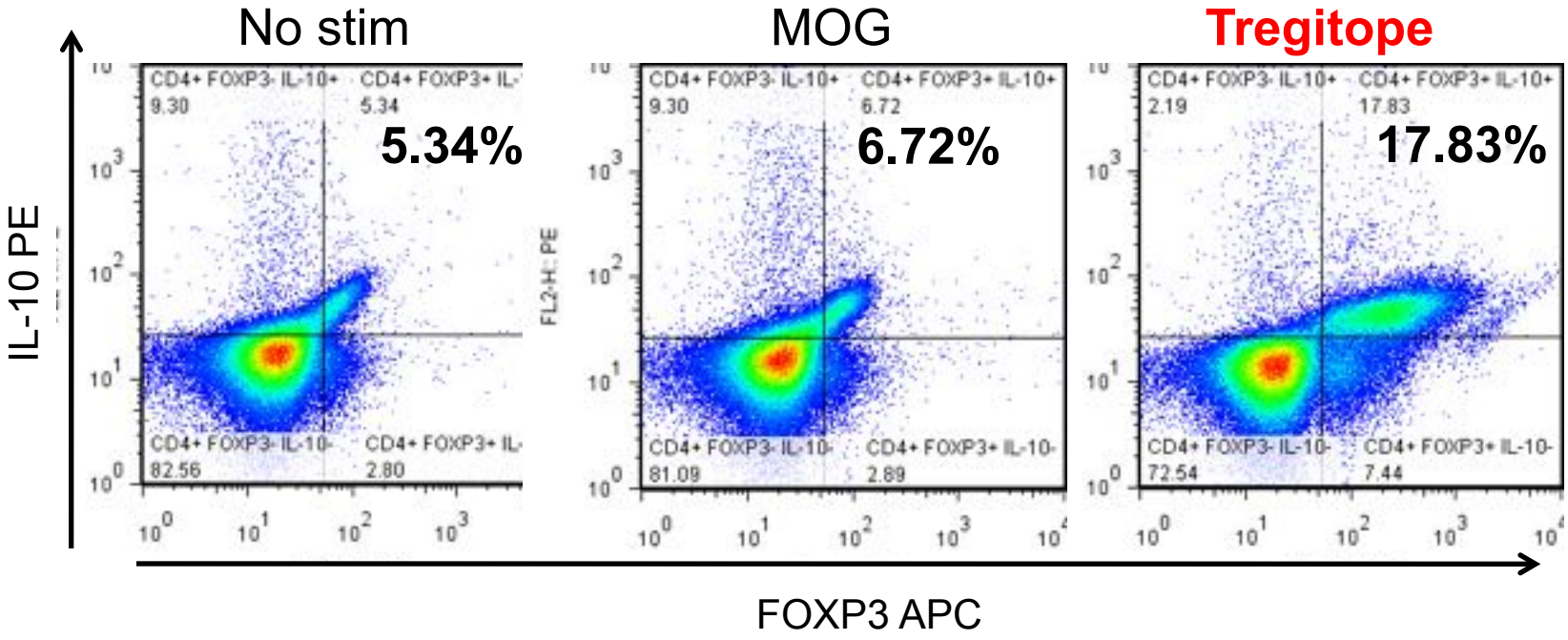
Unpublished

hTregitopes 167/289 induce Treg proliferation



Unpublished

CD4+ T cells producing IL-10 are CD25^{high}, FoxP3+



Unpublished

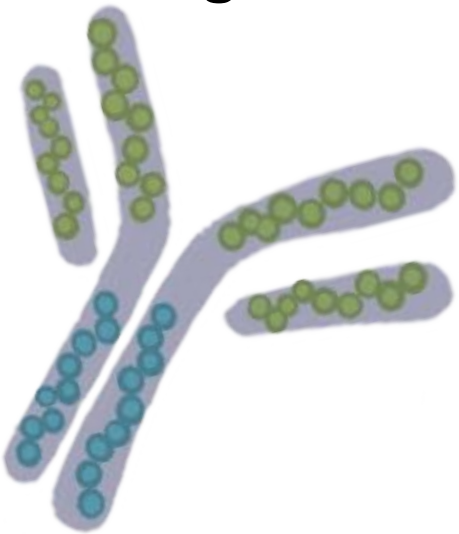
Tregitope MOA Parallels IVIG

- **Inhibition of maturation and function of dendritic cells by intravenous immunoglobulin. Blood. 2003 Jan 15;101(2):758-65**. Epub 2002 Aug 29. Bayry J, Lacroix-Desmazes S, Carbonneil C, Misra N, Donkova V, Pashov A, Chevailler A, Mouthon L, Weill B, Bruneval P, Kazatchkine MD, Kaveri SV.

- **Intravenous immunoglobulin abrogates dendritic cell differentiation induced by interferon-alpha present in serum from patients with systemic lupus erythematosus.** Arthritis Rheum. 2003 Dec;48(12):3497-502. Bayry J, Lacroix-Desmazes S, Delignat S, Mouthon L, Weill B, Kazatchkine MD, Kaveri, SV.

Tregitope MOA Parallels IVIG

Patients treated with high-dose intravenous immunoglobulin show selective activation of regulatory T cells

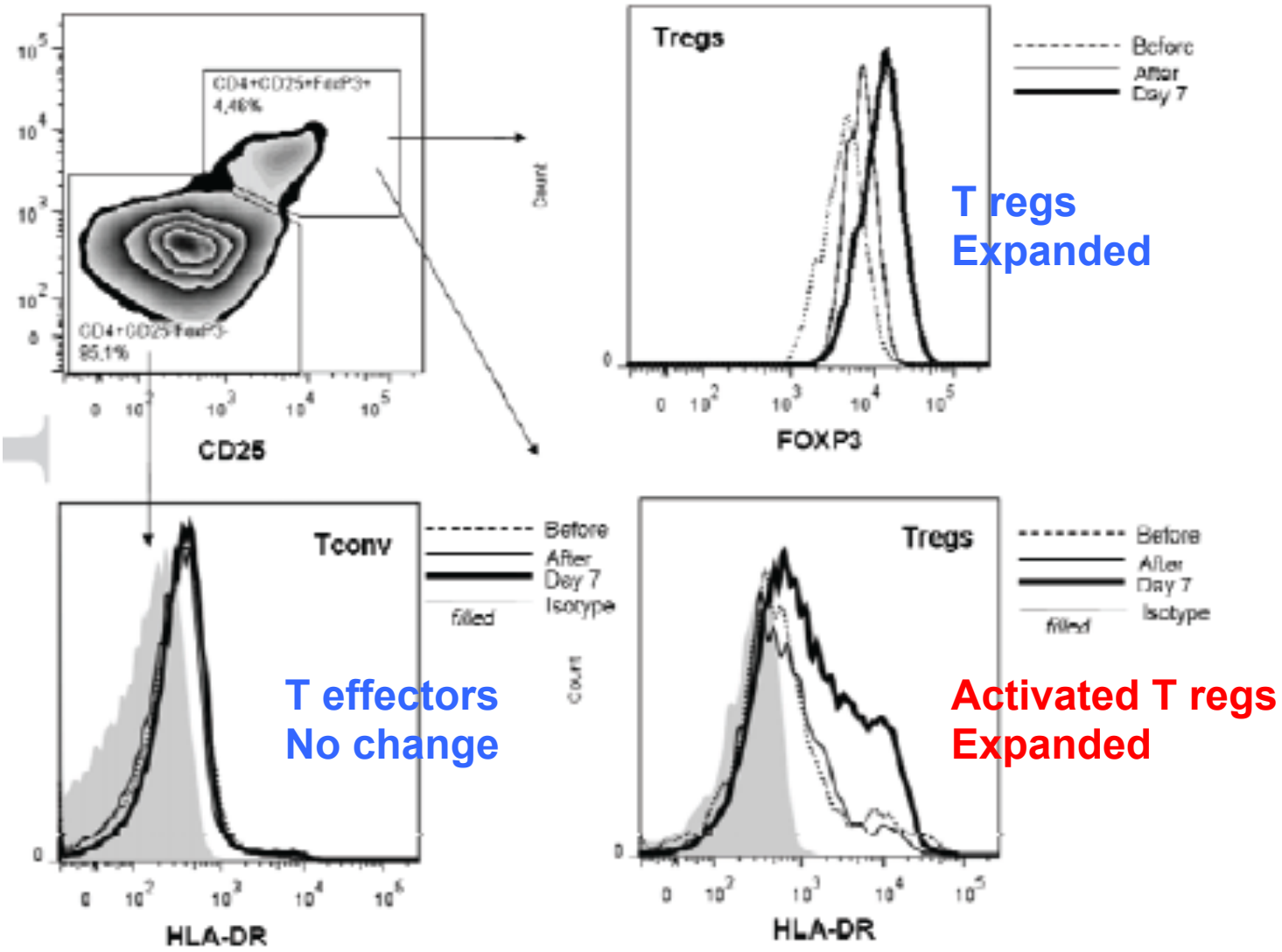


Clinical and Experimental Immunology
2013 Aug;173(2):259-67. doi: 10.1111/cei.12102
http://bit.ly/Kwekkeboom.

Authors:

Angela S.W. Tjon, MD, Thanyalak Tha-In, MD, Herold J.Metselaar, PhD, Rogier van Gent, PhD, Luc J.W. van der Laan, PhD, Zwier M.A. Groothuismink, BSc, Peter A.W. te Boekhorst, MD, PhD, P. Martin van Hagen,MD, PhD, Jaap Kwekkeboom, PhD

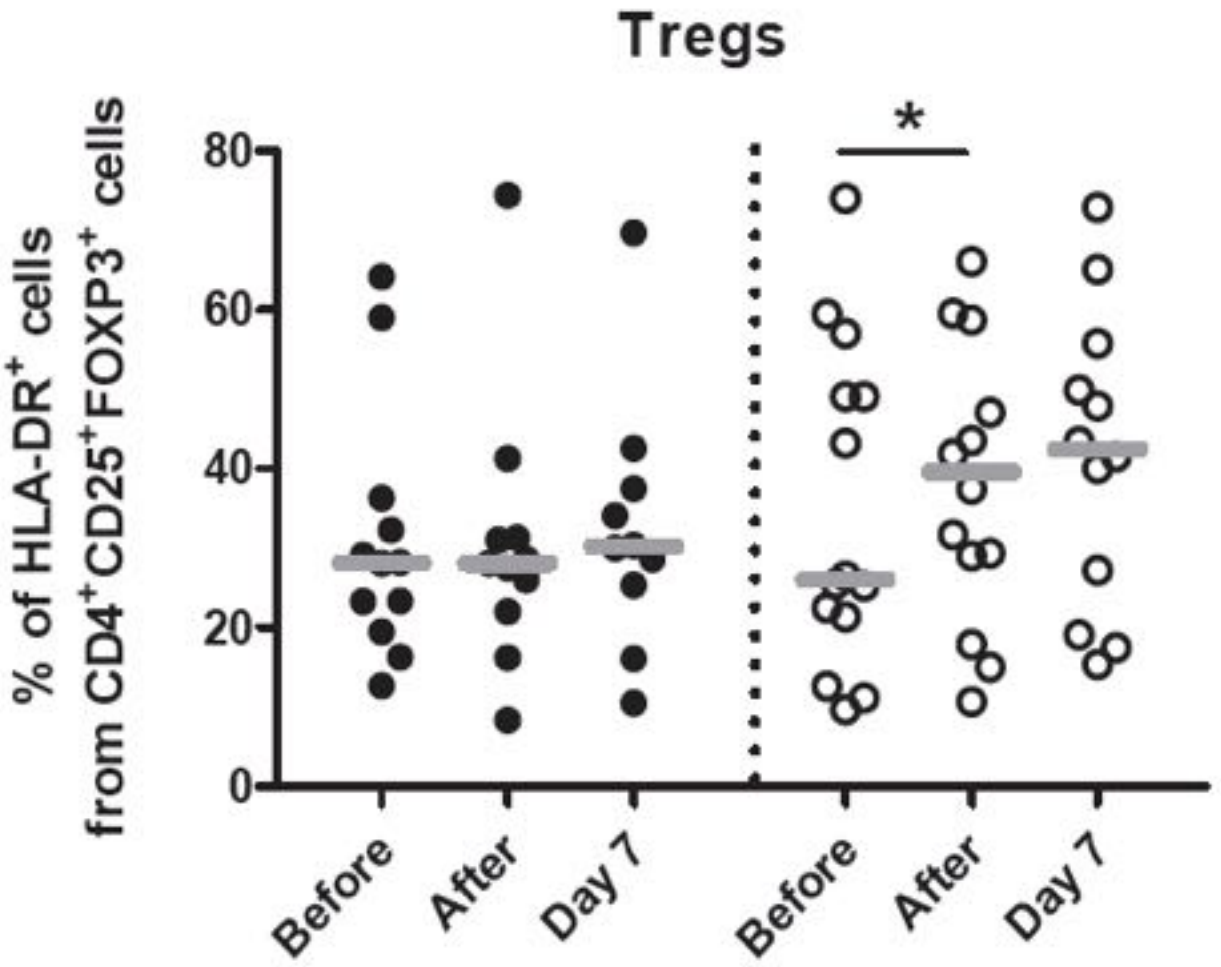
IVIG expands Tregs in Human Diseases Tjon et al.



Tjon et al. 2013

IVIG expands Tregs in Human Diseases: Dose dependent Tjon et al.

Dose Related



Low dose (n=12)
High dose (n=15)

Higher Dose:

Activated Tregs More Expanded

Tjon et al. 2013

Tregitope Applications

Autoimmunity

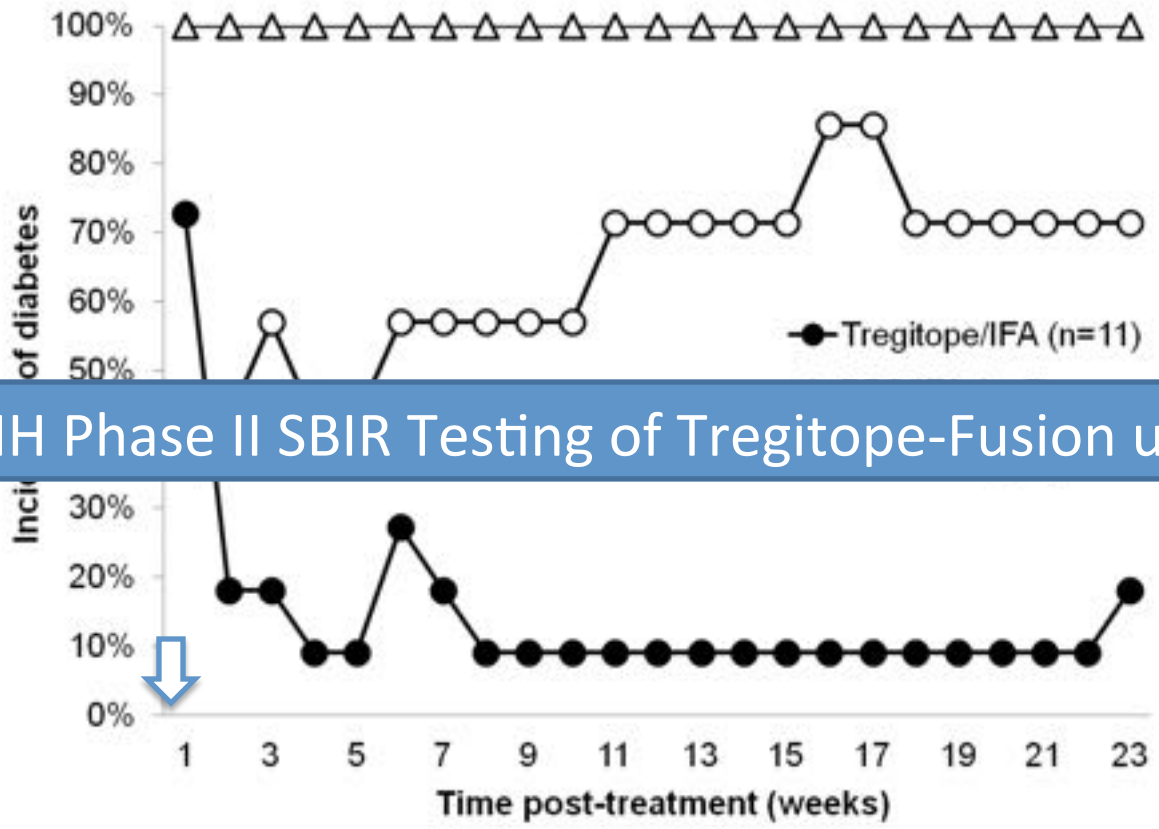
Allergy/Asthma

Transplant Rejection

Immunogenicity of Protein Therapeutics

Tregitopes in T1D

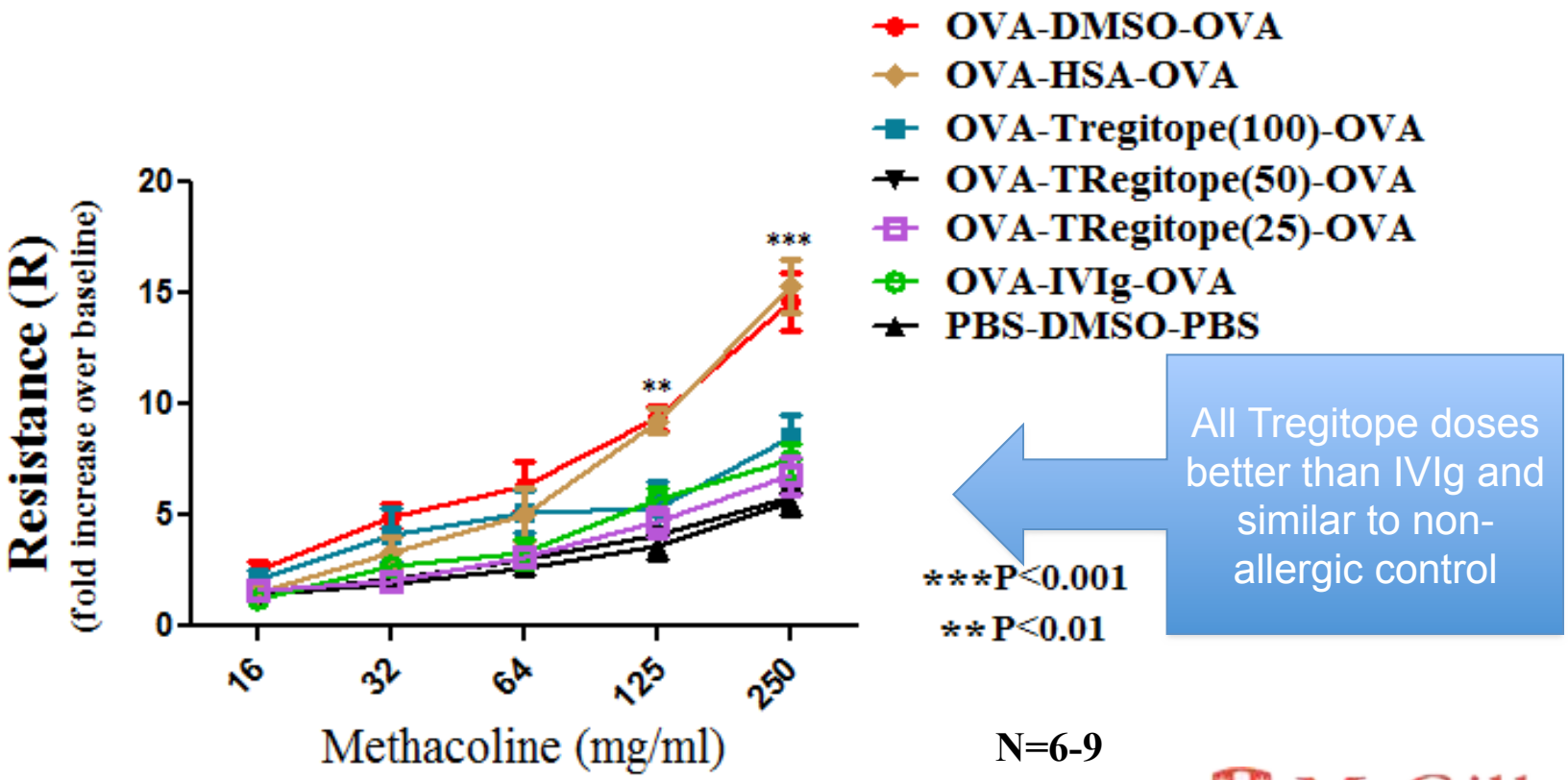
Effect of Tregitopes at onset of Diabetes, NOD mice, Single Treatment IP



$2.5M NIH Phase II SBIR Testing of Tregitope-Fusion underway

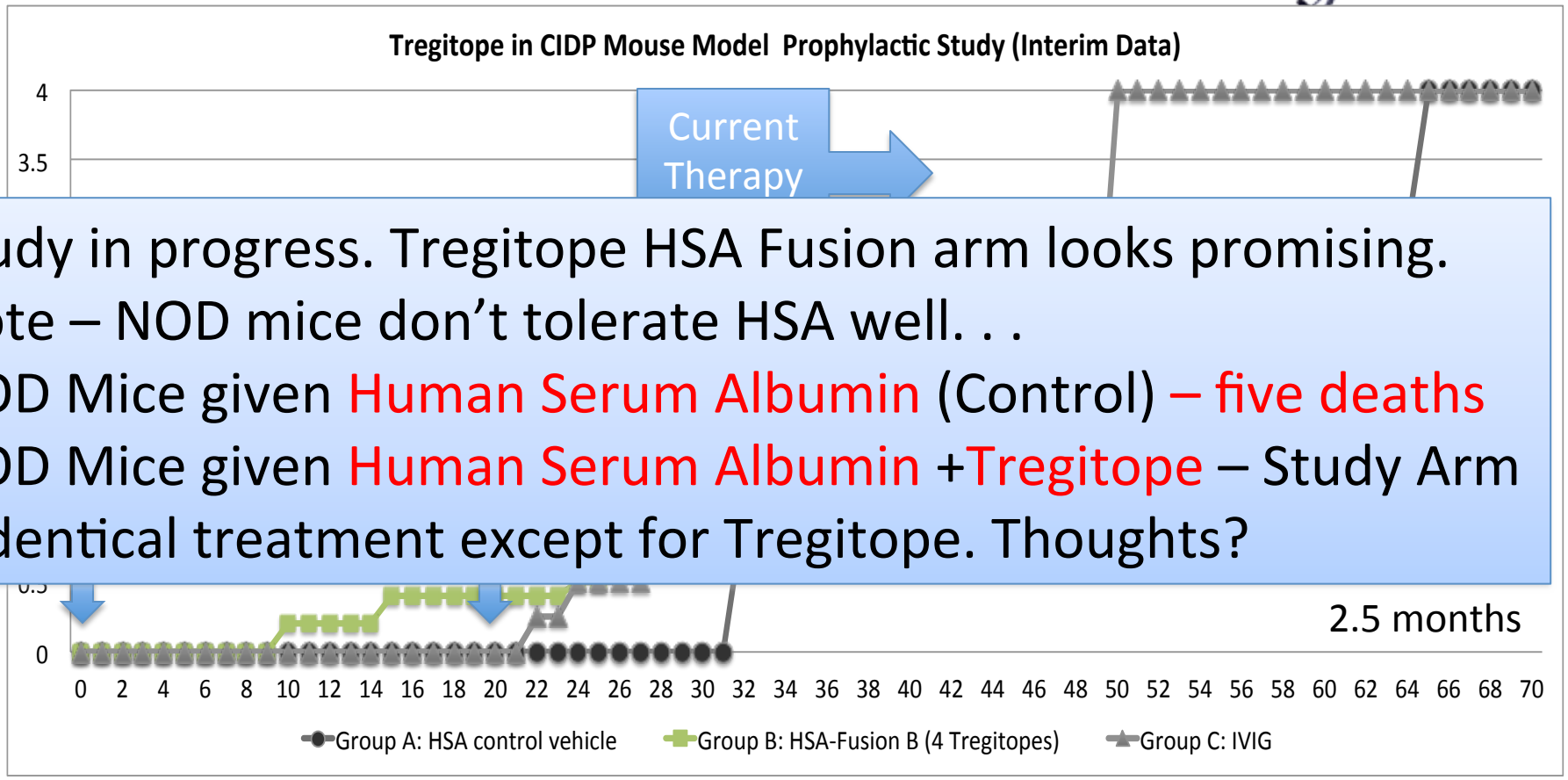
J Diabetes Res. 2013 http://bit.ly/Tregitope_T1D

In vivo studies: Tregitopes suppress Airway Hypersensitivity Reaction



- AHR to Mch (pulmonary resistance) was measured by flexiVent

Tregitope in CIDP Mouse Model
Prophylactic Study (Interim Data)



Study in progress. Tregitope HSA Fusion arm looks promising.
Note – NOD mice don't tolerate HSA well. . .
NOD Mice given Human Serum Albumin (Control) – five deaths
NOD Mice given Human Serum Albumin +Tregitope – Study Arm
- Identical treatment except for Tregitope. Thoughts?

Prophylactic: Mice enter study at 16wks of age = Day0
Injected on Days 0 and 21

Tregitope Validation Checklist

- ✓ Tregitopes induce adaptive tolerance in C57Bl/6, D011.10, OTII
- ✓ Tregitopes suppress/treat diabetes in NOD model (Scott/EpiVax)
- ✓ Tregitopes suppress transplant rejection in CD28 KO mice (Najafian)
- ✓ Tregitopes suppression = IVIG in OVA/Allergy Model (Mazer)
- ✓ Tregitopes suppress immune responses to AAV capsid (Mingozzi)
- ✓ Tregitopes suppress immune responses to GAA (Myozyme) (Koeberl)
- ✓ *Tregitopes cause expansion of Tregs – iTreg or nTreg?*

Continued publications – see here: http://bit.ly/TregPub

http://bit.ly/Treg1

Non-Confidential contact amarcello@epivax.com (US/ other) or pdegroot@epivax.com (EUR) for more info

Tregitope Validation (Primary Articles)

- Hui, DJ, Basner-Tschakarjan E, Chen Y, Davidson RJ, Buchlis G, Yazicioglu M, Pien, GC Finn JD Haurigot V, Tai A, Scott DW, Cousens LP, Zhou S, De Groot AS, Mingozzi F. **Modulation of CD8+ T cell responses to AAV vectors with IgG-derived MHC class II epitopes**. Mol Ther. 2013 Jul 16. doi:10.1038/mt.2013.166.
- Yan Su, Robert Rossi, Anne S. De Groot, and David W. Scott**. Regulatory T cell epitopes** (**Tregitopes) in IgG induce tolerance in vivo and lack immunogenicity per** se. J. Leukocyte Biology. J Leukoc Biol. 2013 May 31. http://bit.ly/Tregitopes_Per_Se
- Leslie P. Cousens, Yan Su, Elizabeth McClaine, Xin Li, Frances Terry, Robert Smith, Jinhee Lee, William Martin, David W. Scott, **Anne S. De Groot**. **Application of IgG-derived natural Treg epitopes (IgG Tregitopes) to antigen-specific tolerance induction in a murine model of Type 1 Diabetes.** Experimental Diabetes Research. J Diabetes Res. 2013:621693. doi: 10.1155/2013/621693. Epub 2013 Apr 23. http://bit.ly/Tregitope_T1D
- Cousens LP, Najafian N, Mingozzi F, Elyaman W, Mazer B, Moise L, Messitt TJ, Su Y, Sayegh M, High K, Khoury SJ, Scott DW, **De Groot AS**. **In Vitro and In Vivo Studies of IgG-Derived Treg Epitopes (Tregitopes): A Promising New Tool for Tolerance Induction and Treatment of Autoimmunity.** J Clin Immunol. 2013**. January; 33(1): 43–49.** PMID:22941509**.** http://bit.ly/TregInVivo12
- van der Marel S, Majowicz A, Kwikkers K, van Logtenstein R, te Velde AA, De Groot AS, Meijer SL, van Deventer SJ, Petry H, Hommes DW, Ferreira V. **Adeno-associated virus mediated delivery of Tregitope 167 ameliorates experimental colitis**. World J Gastroenterol. 2012 Aug 28;18(32):4288-99. http://bit.ly/TregAAV12
- De Groot AS, Moise L, McMurry JA, Wambre E, Van Overvelt L, Moingeon P, Scott DW, Martin W. **Activation of Natural Regulatory T cells by IgG Fc-derived Peptide "Tregitopes"**. Blood. 2008 Oct 15;112(8): 3303-11. http://bit.ly/Blood08

Tregitope Validation (Reviews with Data)

- Cousens, Mingozzi, van der Marel, Su, Garman, Ferreira, Martin, Scott, and De Groot. **Teaching tolerance: New approaches to enzyme replacement therapy for Pompe disease**. Hum Vacc & Immunother. (OCT 2012) 8(10): http://bit.ly/TregPompes
- Cousens, Tassone, Mazer, Ramachandiran, Scott, De Groot**. Tregitope update: Mechanism of action parallels IVIg.** Autoimmun Rev. 2012 Aug 28. [Epub ahead of print]. PMID: 22944299. http://bit.ly/TregIVIG2012
- De Groot AS, Frances Terry, Leslie Cousens and William Martin. **Beyond Humanization and De-immunization: Tolerization as a Method for Reducing the Immunogenicity of Biologics. Expert Review of Clinical Pharmacology**.13-Aug-2013 (accepted)
- **Chapter.** Vibha Jawa; Leslie Cousens and Anne S. De Groot**. Immunogenicity of Therapeutic Fusion proteins: Contributory Factors and Clinical Experience**. Chapter in: Fusion Protein Technologies for Biopharmaceuticals: Applications and Challenges. Stefan R. Schmidt (Editor). John Wiley and Sons, Inc. March 2013. http://bit.ly/Amgen_Prospective_Tregitope_Data
- Elyaman W, Khoury SJ, Scott DW, De Groot AS**. Potential Application of Tregitopes as Immunomodulating Agents in Multiple Sclerosis.** Neurol Res Int. APR 2011;2011:256460 http://bit.ly/TregMS11
- De Groot, AS**. Do Tregitopes have the potential to impact the current treatment landscape of autoimmune diseases?** Clinical Immunology Reviews, Invited editorial. Submitted. September 2013.

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- Why are these epitopes Treg epitopes ?
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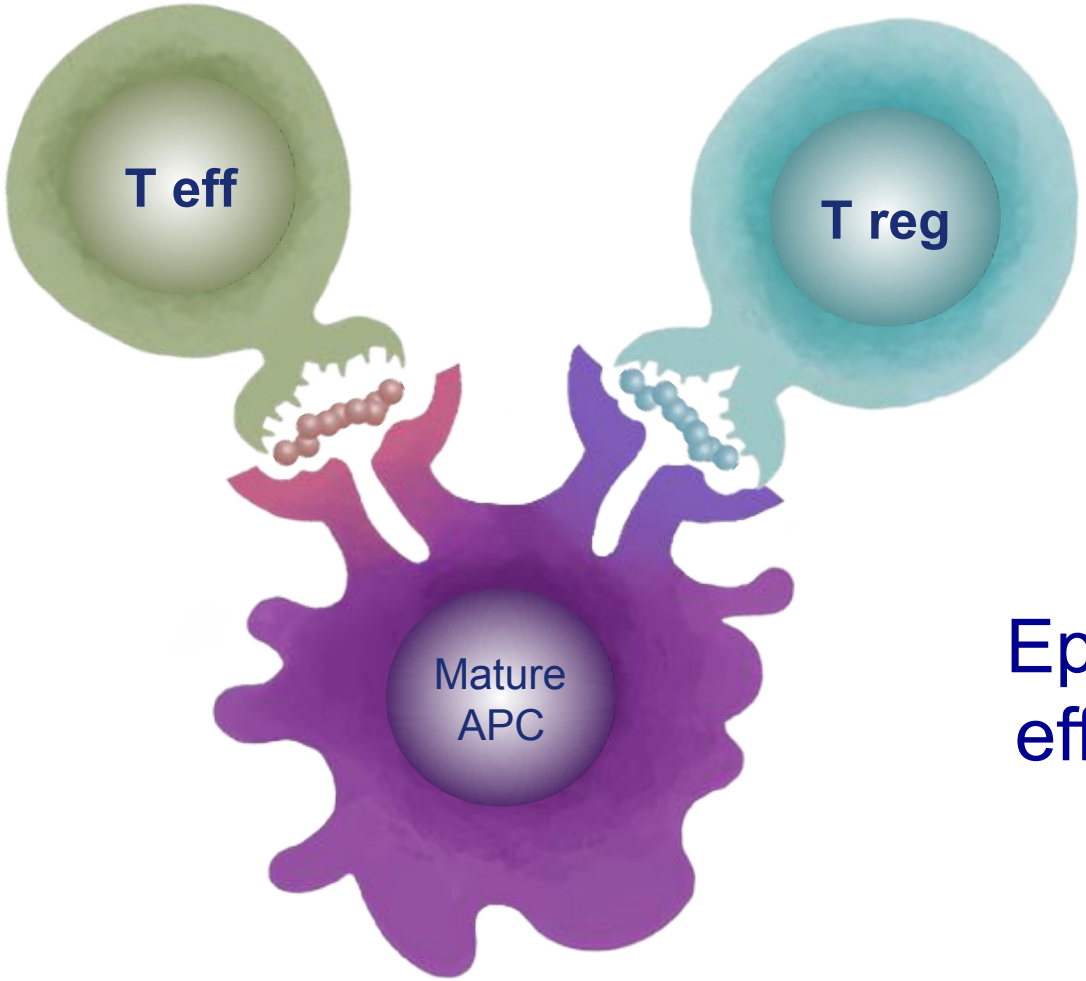
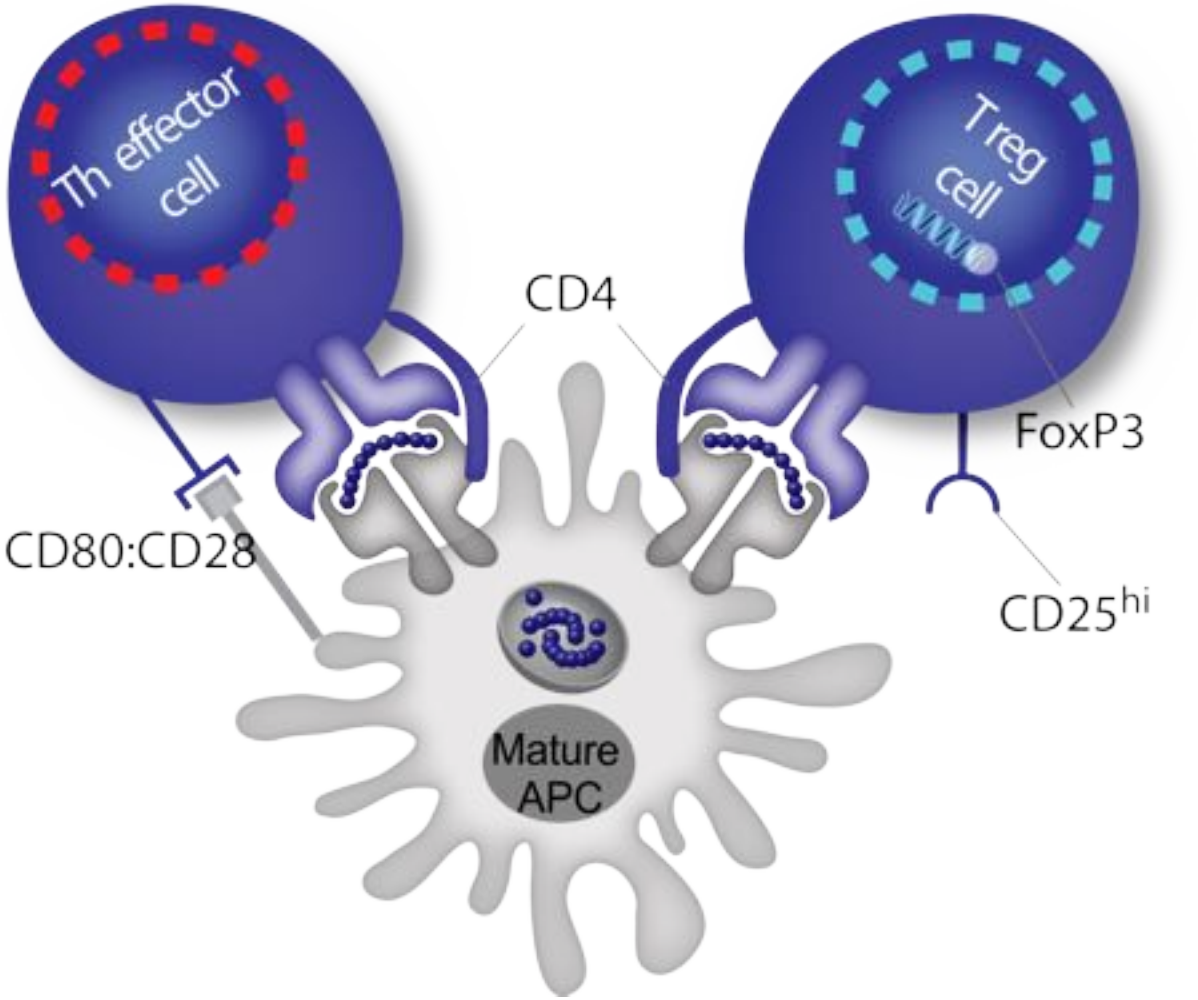
Presence of epitope indicates immune potential



Epitope can be *either* effector or regulatory

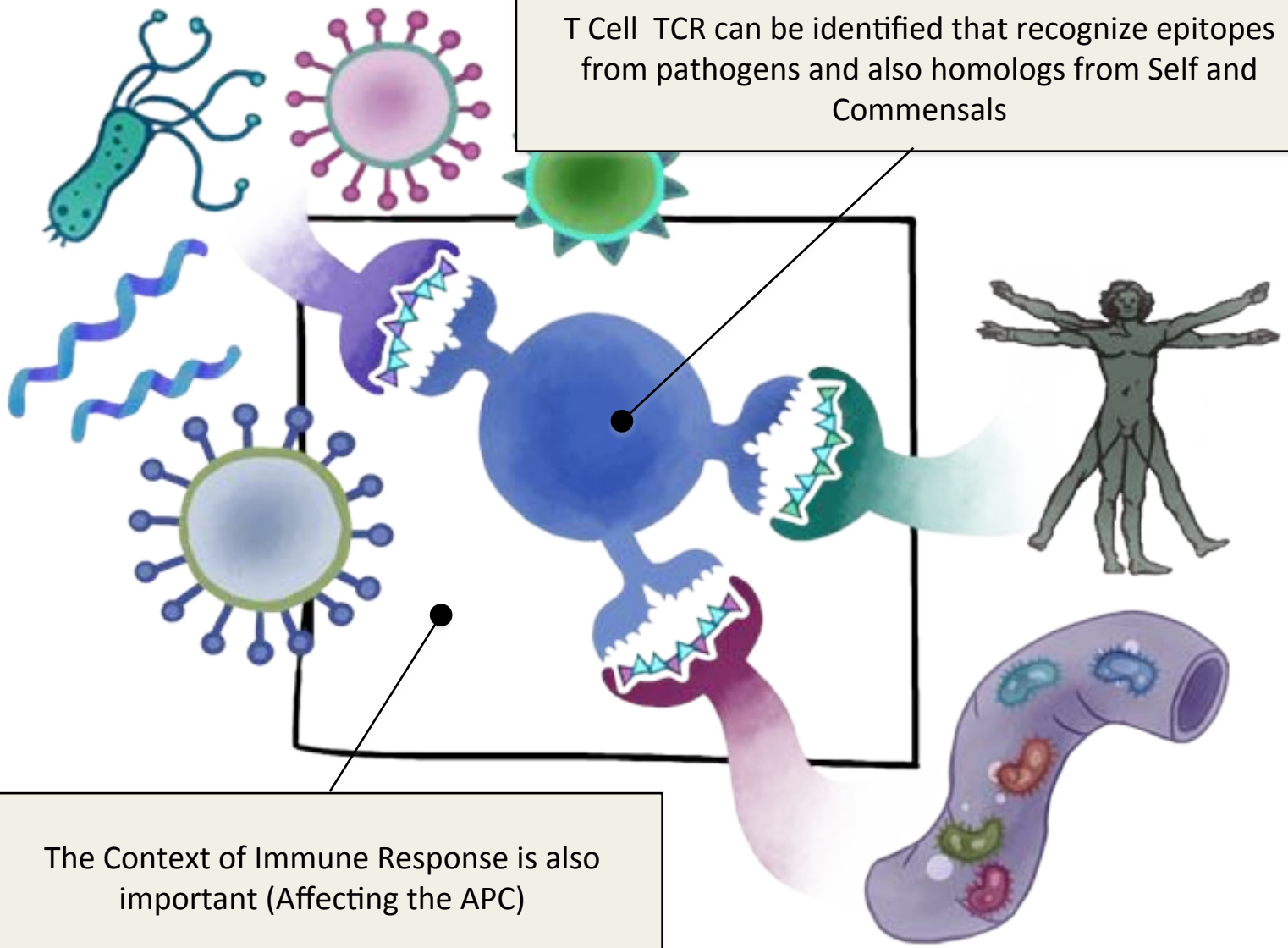
NO difference in Treg/Teff epitopes ‘in silico’



Treg and Teff epitopes *in silico* signatures are identical …

(How do Tregitopes become Treg epitopes? . . .)

New Research on the "two-faced" T cell epitope



JanusMatrix (Based on EpiMatrix)

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human VACCINES & IMMUNOTHERAPEUTICS

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Research Paper

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

Volume 9, Issue 7 July 2013

Keywords: T cell epitope, T cell receptor, TCR, agretope, computational immunology, cross-reactivity, epitope, immunodominance, immunoinformatics, regulatory T cell, vaccine

Authors: Lenny Moise, Andres H. Gutierrez, Chris Bailey-Kellogg, Frances Terry, Qibin Leng, Karim M. Abdel Hady, Nathan VerBerkmoes, Marcelo B. Sztein, Phyllis Losikoff, William D. Martin, Alan Rothman and Anne S. De Groot

View affiliations

Abstract:

Advances in the field of T cell immunology have contributed to the understanding that cross-reactivity is an intrinsic characteristic of the T cell receptor (TCR), and that each TCR can potentially interact with many different T cell epitopes. To better define the potential for TCR cross-reactivity between epitopes derived from the human genome, the human microbiome, and human pathogens, we developed a new immunoinformatics tool, JanusMatrix, that represents an extension of the validated T cell epitope mapping tool, EpiMatrix. Initial explorations, summarized in this synopsis, have uncovered what appear to be important differences in the TCR cross-reactivity of selected regulatory and effector T cell epitopes with other epitopes in the human genome, human microbiome, and selected human pathogens. In addition to exploring the T cell epitope relationships between human self, commensal and pathogen, JanusMatrix may also be useful to explore some aspects of heterologous immunity and to examine T cell epitope relatedness between pathogens to which humans are exposed (Dengue serotypes, or HCV and Influenza, for example). In Hand-Foot-Mouth disease (HFMD) for example, extensive enterovirus and human microbiome cross-reactivity (and limited cross-reactivity with the human genome) seemingly predicts immunodominance. In contrast, more extensive cross-reactivity with proteins contained in the human genome as compared to the human microbiome was observed for selected Treg epitopes. While it may be impossible to predict all immune response influences, the availability of sequence data from the human genomes, the human microbiome, and an array of human pathogens and vaccines has made computationally-driven exploration of the effects of T cell epitope cross-reactivity now possible. This is the first description of JanusMatrix, an algorithm that assesses TCR cross-reactivity that may contribute to a means of predicting the phenotype of T cells responding to selected T cell epitopes. Whether used for explorations of T cell phenotype or for evaluating cross-conservation between related viral strains at the TCR face of viral epitopes, JanusMatrix further studies may contribute to developing safer, more effective vaccines.

Received: February 28, 2013; **Accepted:** April 9, 2013; **Published Online:** April 12, 2013

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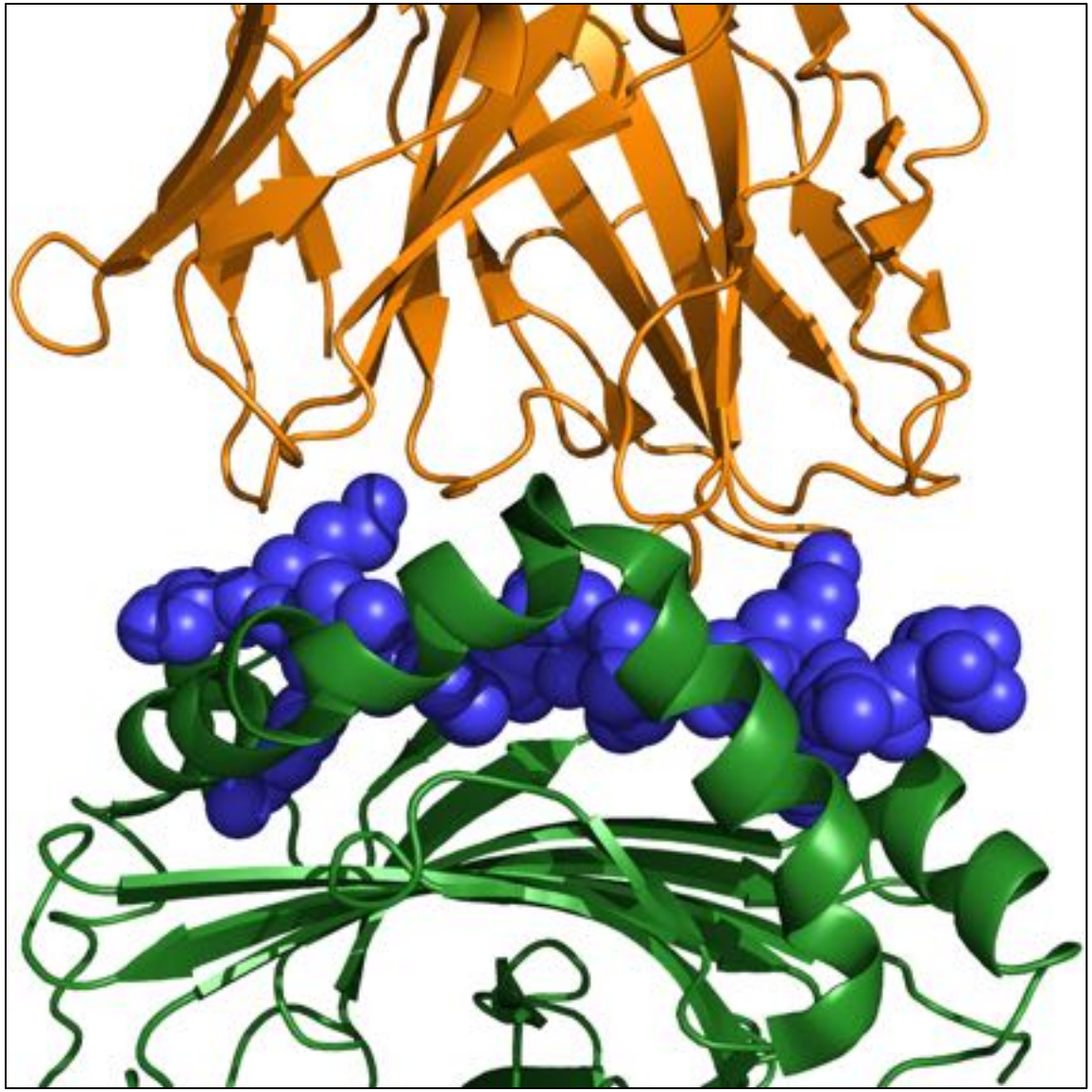

T cell receptor

peptide epitope

MHC II

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THE UNIVERSITY OF RHODE ISLAND
Center for Vector-Borne Disease
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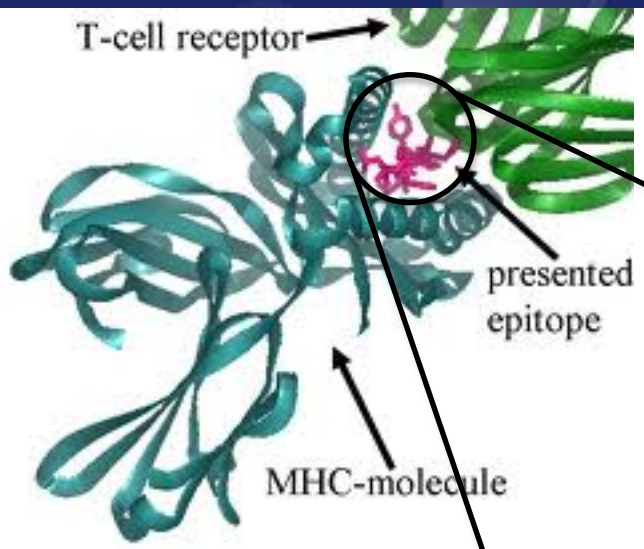
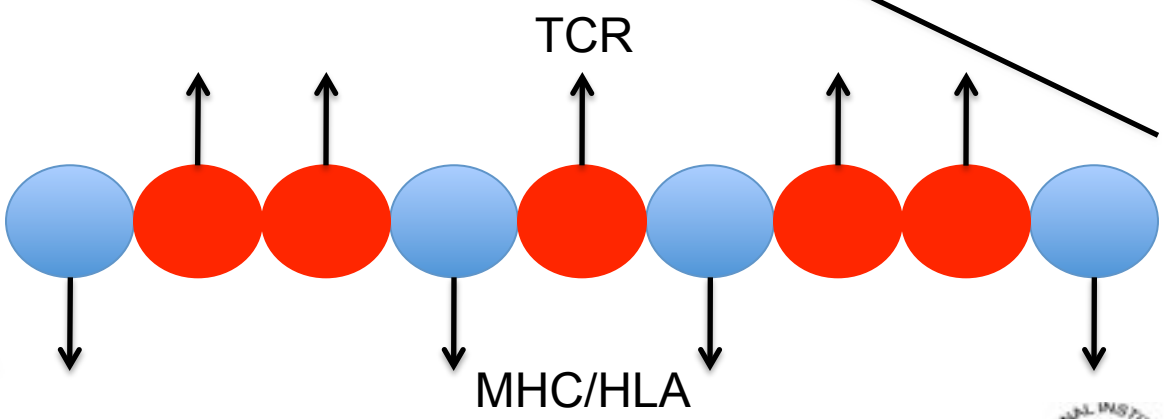
Approach: TCR face vs. MHC binding face



For the purpose of comparison:

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



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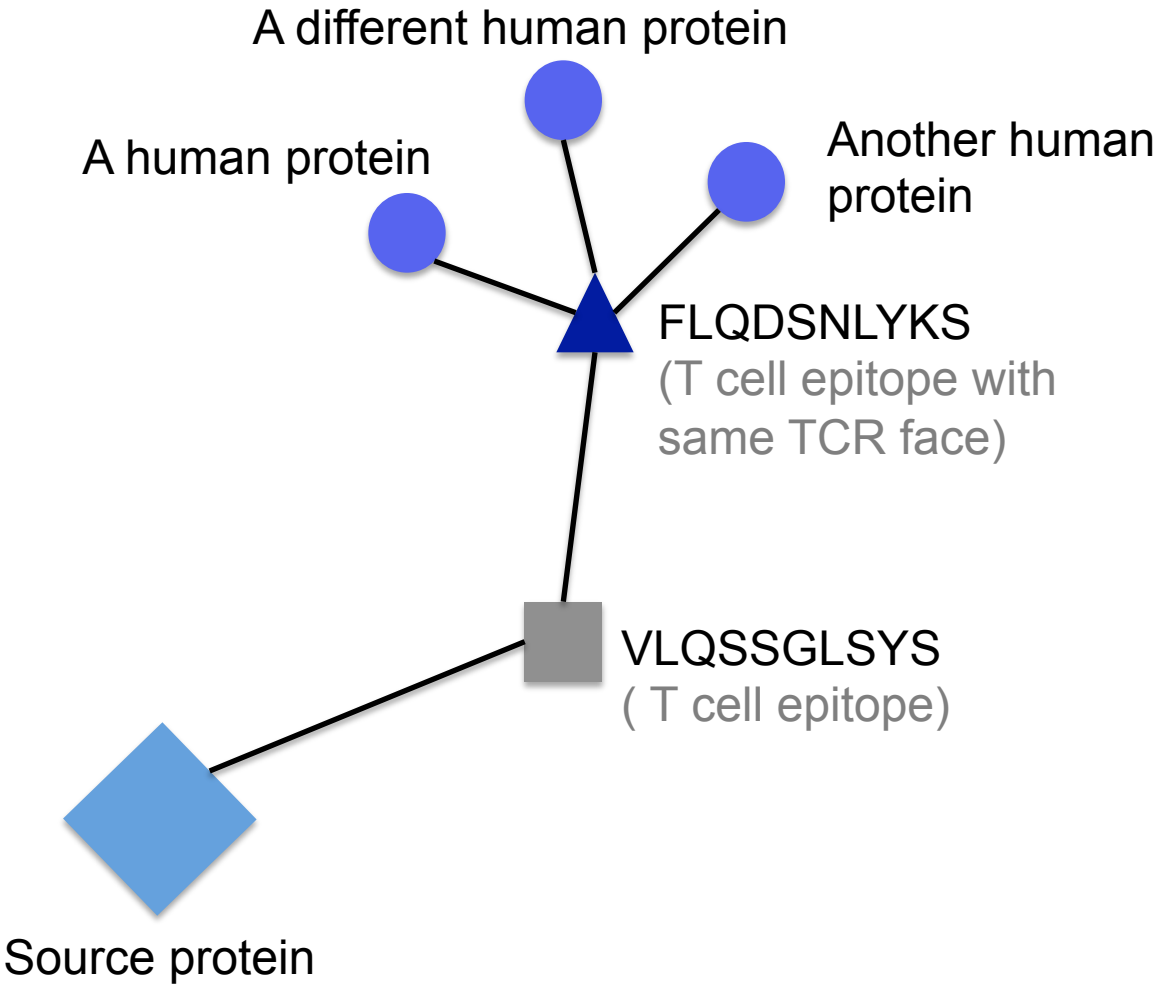
Epitope Networks: Cytoscape



Source Protein or large peptide

Predicted 9-mer epitope from a source protein

9-mer from human prevalent proteome, 100% TCR face identical to source epitope

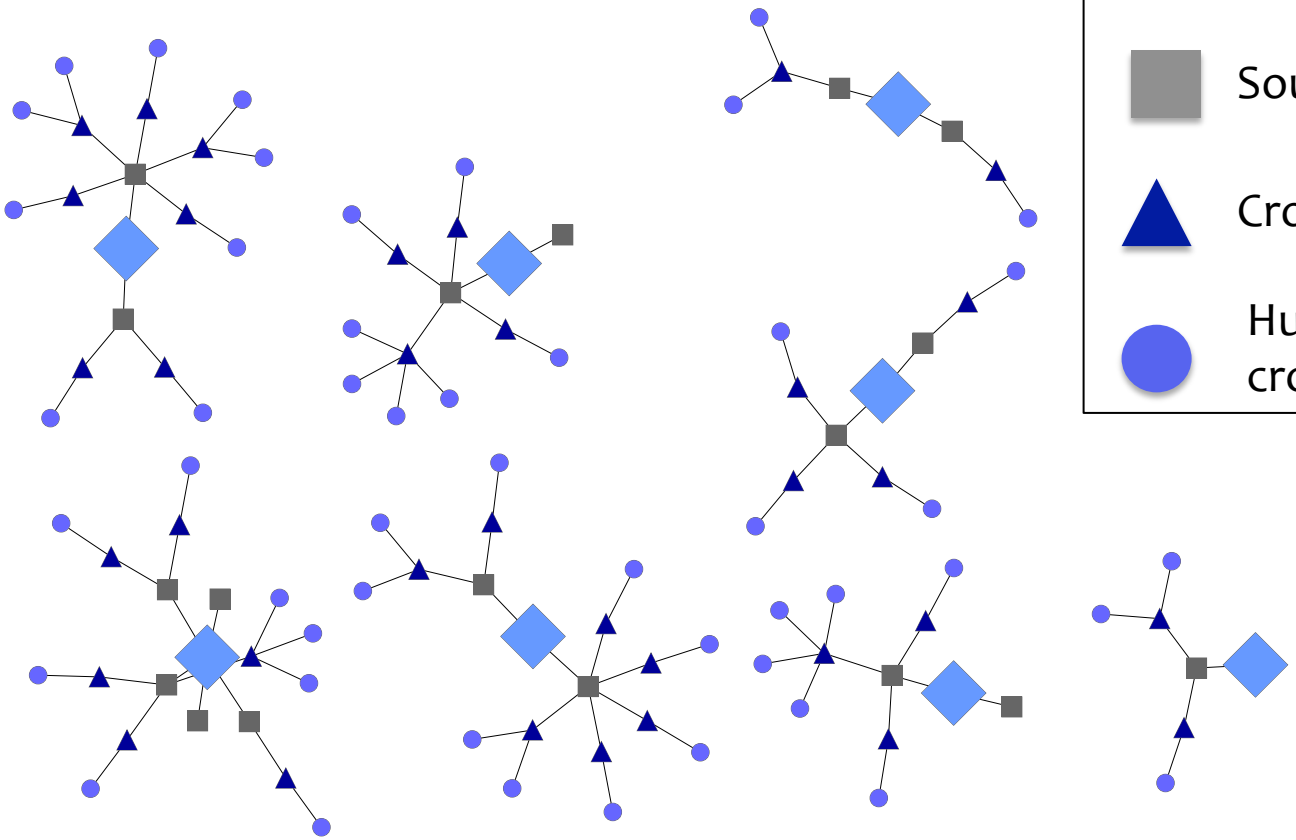
Human protein where cross-reactive epitopes are present

http://www.cytoscape.org/

U19AI082642

Visualizing Cross-reactivity Patterns - Cytoscape

CEFT Peptides (immunogenic)



Source (pathogen) protein

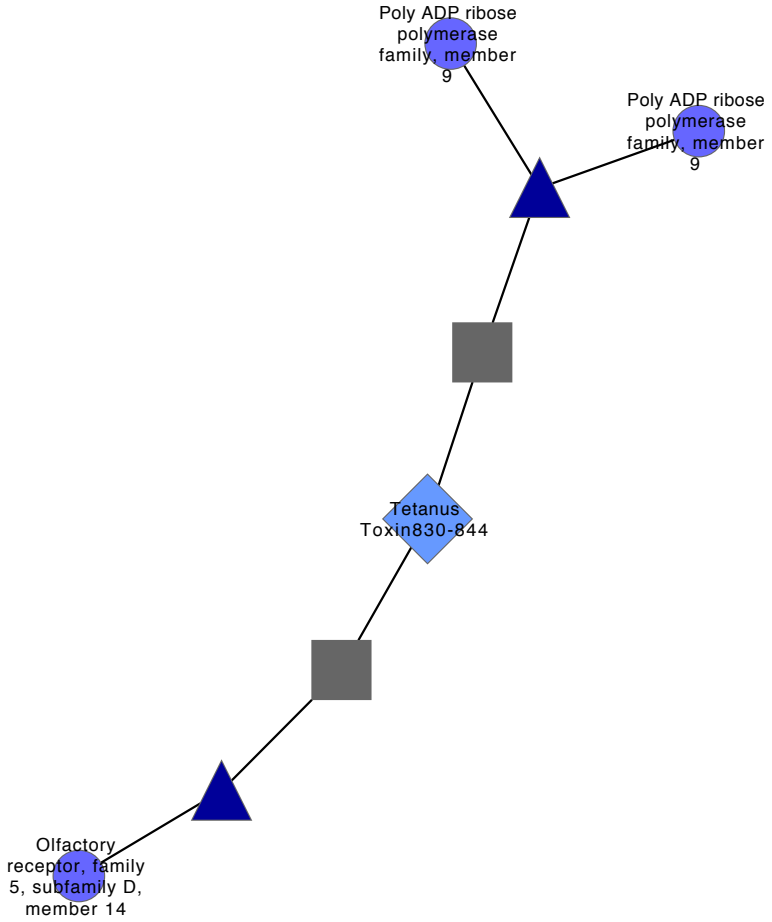
Source epitope

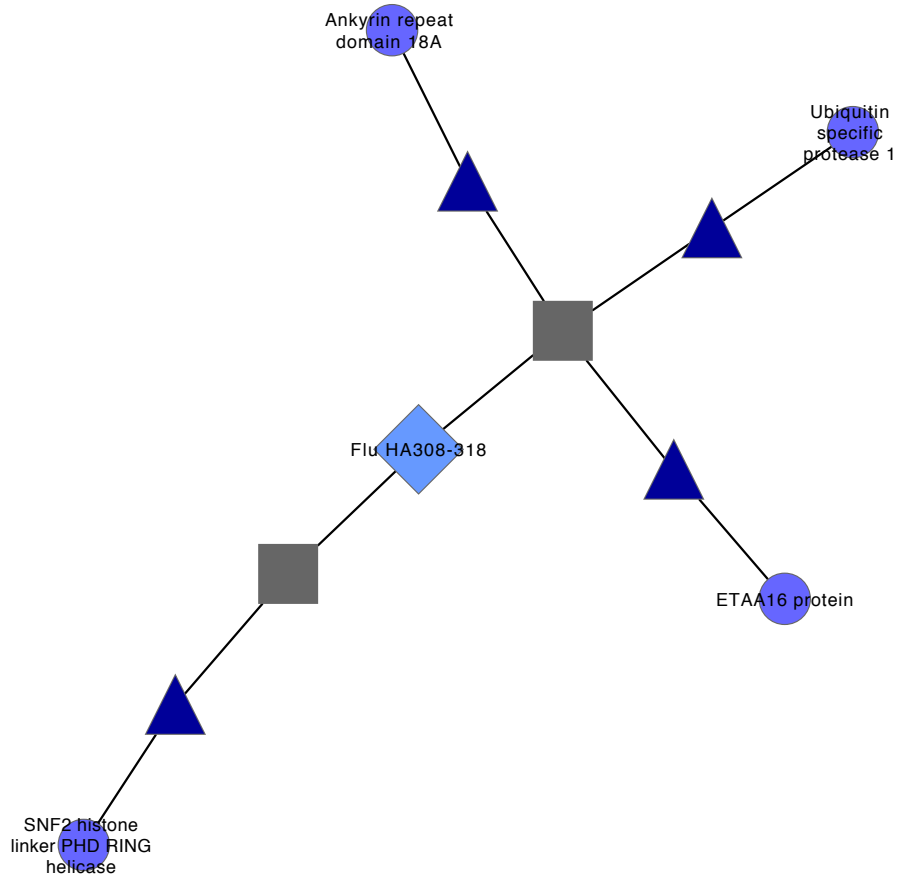
Cross-reactive human epitope

Human protein with cross-reactive epitopes

U19AI082642

Flu and Tet tox epitopes





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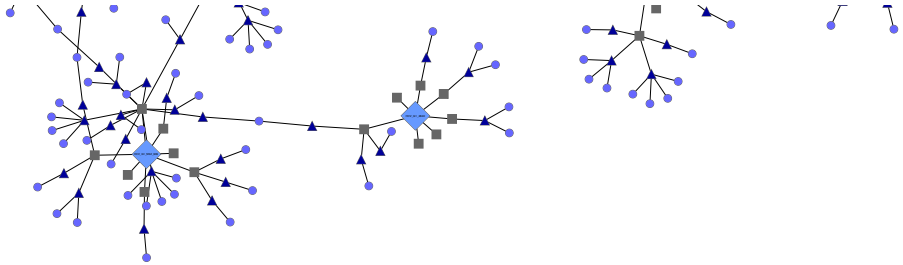
HCV Vaccine project

Conserved T cell epitopes predicted with EpiMatrix



All induced Teff response

Gregory et al.
Submitted for publication

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Lifespan
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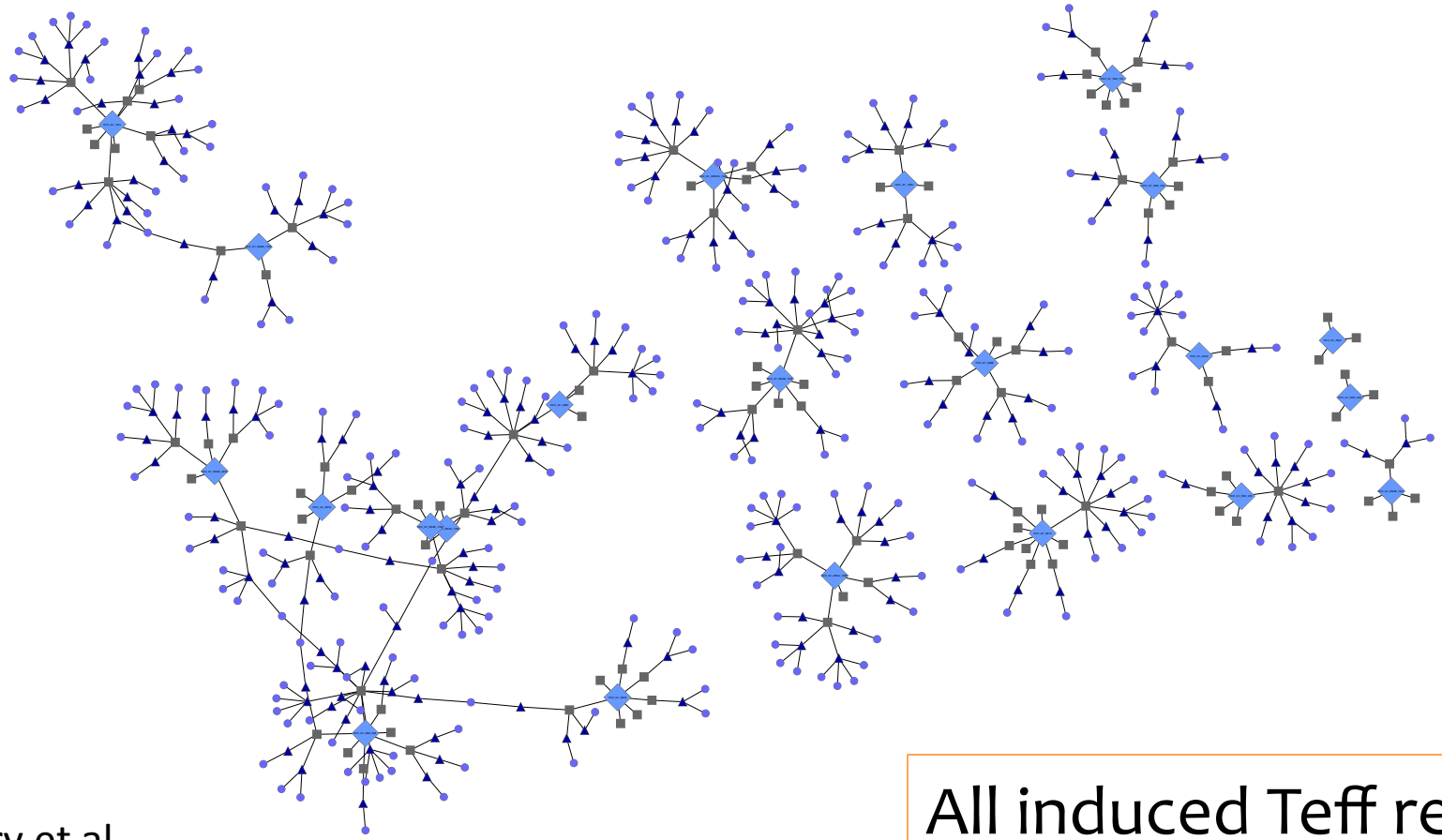

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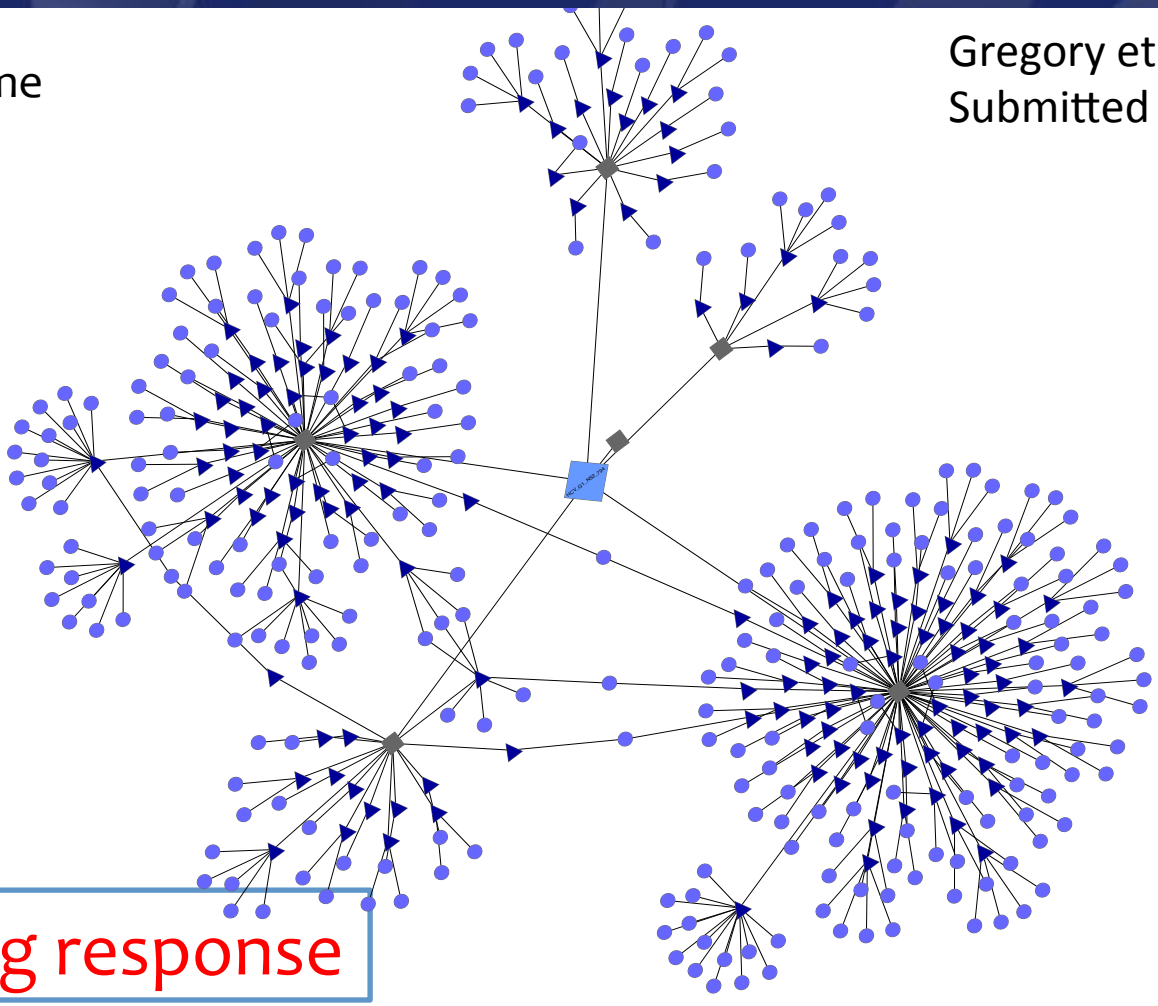
U19AI082642

HCV "Tregitope"

vs. human proteome

Gregory et al.
Submitted for publication



Induced Treg response

U19AI082642

Lu He & Chris Bailey Kellog in collaboration with EpiVax

Integrated assessment of predicted MHC binding and cross-conservation with self reveals patterns of viral camouflage

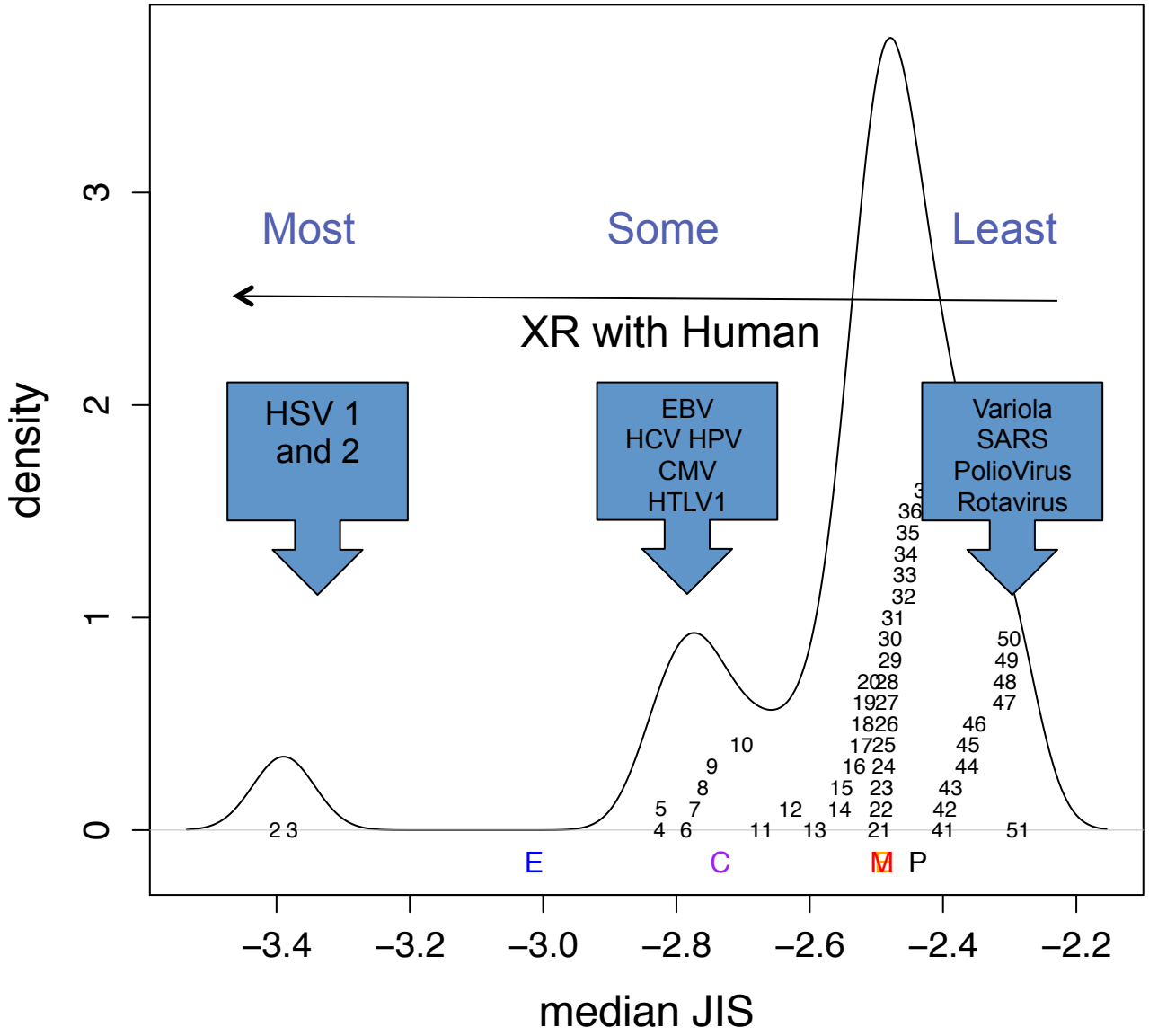
Lu He[1]
Anne S. De Groot[2,3]
Andres H. Gutierrez[2]
William D. Martin[3]
Lenny Moise[2,3]
Chris Bailey-Kellogg[1*]

[1]Department of Computer Science, Dartmouth College, Hanover, NH, USA
[2]Institute for Immunology and Informatics, University of Rhode Island, Providence, RI, USA
[3]EpiVax Inc., Providence, RI, USA

How do Viruses Escape

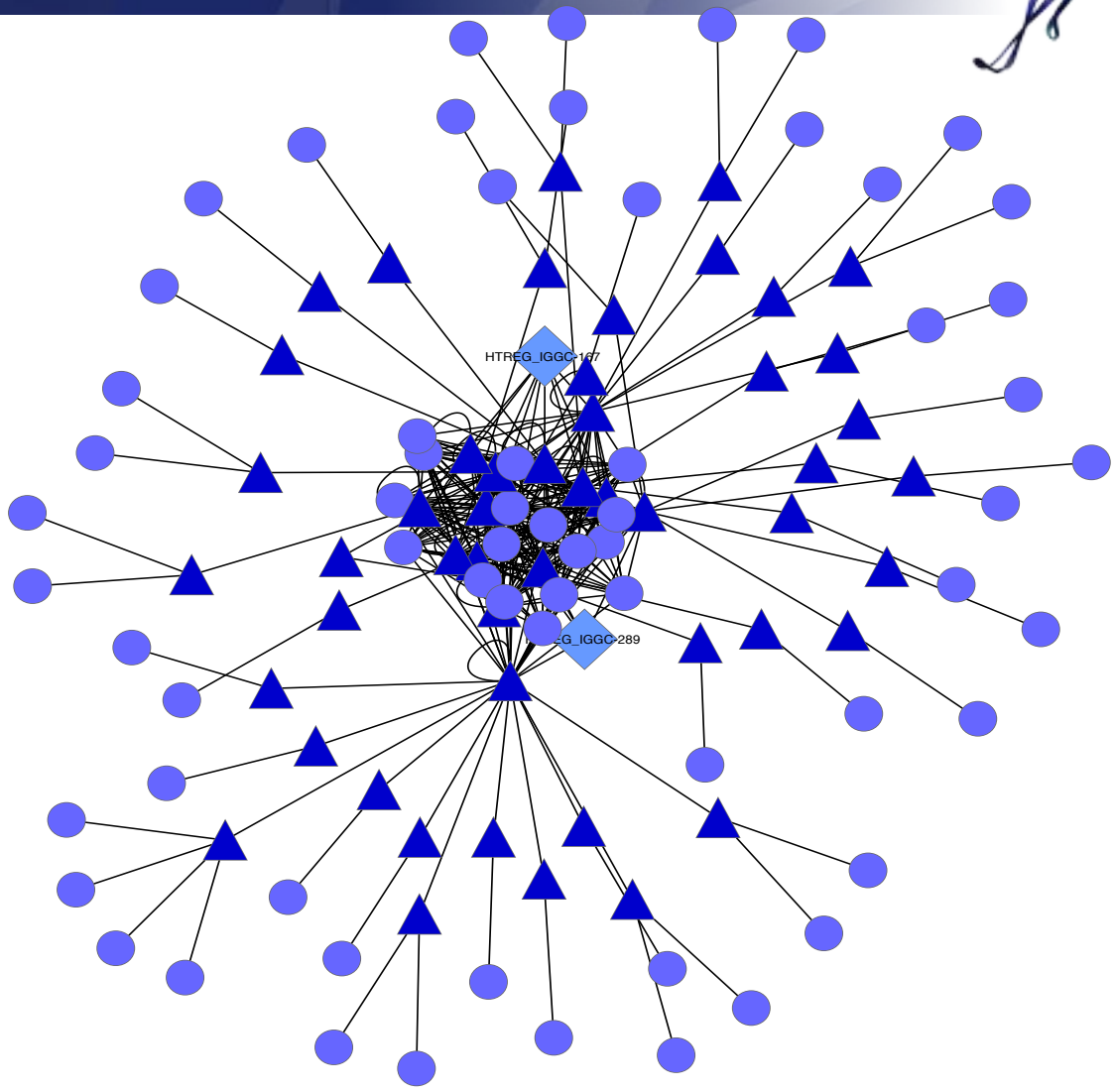
- *Remove* T cell epitopes that human may recognize
  - HIV, HCV
  - 'Hit and Stay viruses' vs, 'Hit and Run'
- *Increase cross-conservation* with common human T cell epitopes

Evaluation of viruses for XR with Human Genome
Human Genome/Human Microbiome/Human Pathogens

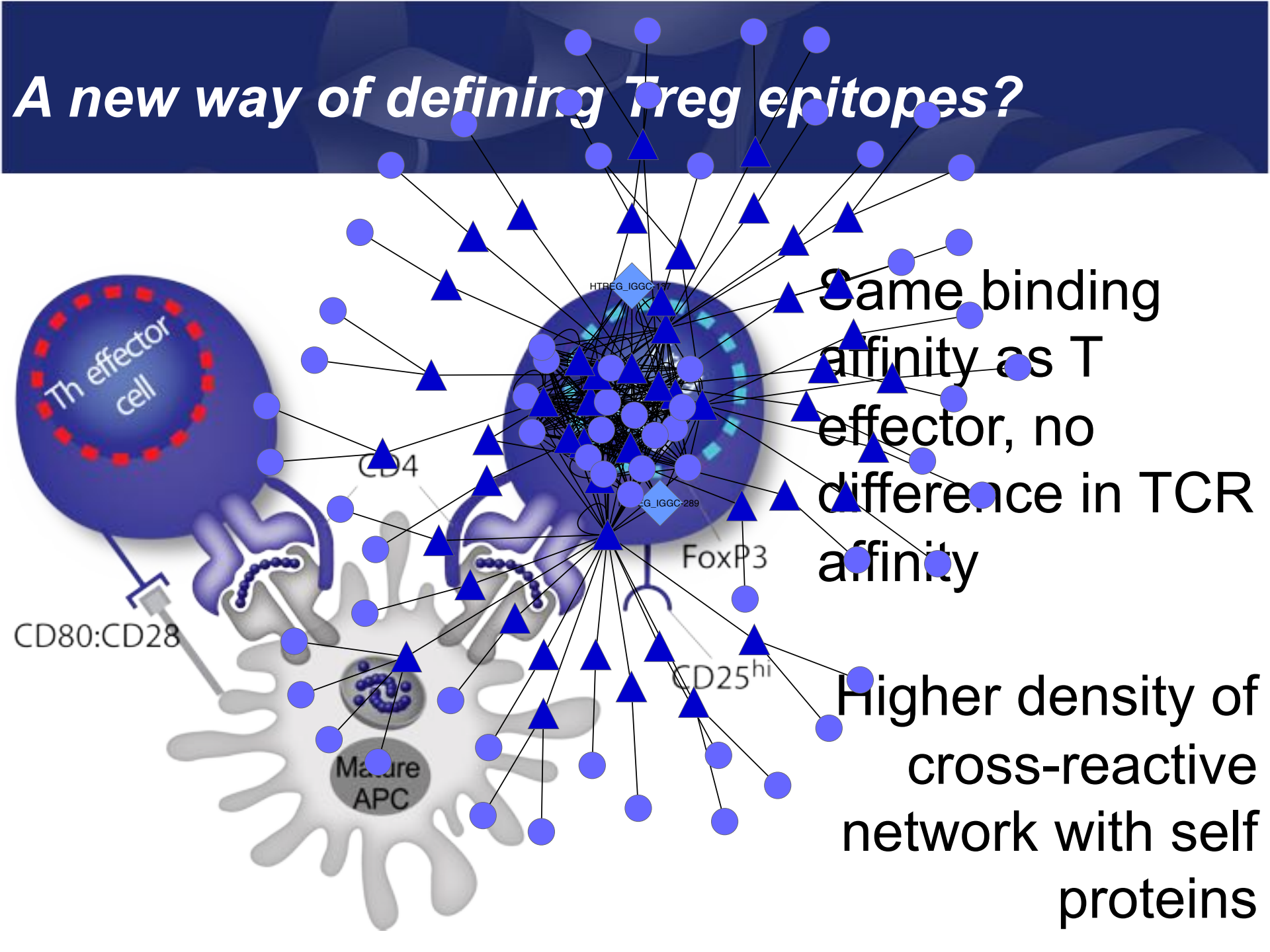


1 Rubella [not shown]
2 Human herpesvirus 2
3 Human herpesvirus 1
4 Human astrovirus
5 Human adenovirus C
6 Human herpesvirus 4 type 2 (EBV)
7 Hepatitis C virus
8 Human papillomavirus type 16
9 Human herpesvirus 5 strain AD169 (CMV)
10 Hepatitis B virus
11 Human T-lymphotropic virus 1
12 Dengue1
13 Human herpesvirus 8
14 Human adenovirus type 12
15 Human adenovirus type 17
16 Human immunodeficiency virus 2
17 Yellow fever virus
18 Dengue2
19 Japanese encephalitis virus
20 West Nile virus
21 Influenza B virus
22 Influenza A H5N1 virus
23 Human herpesvirus 3
24 Zaire ebolavirus
25 Lymphocytic choriomeningitis virus
26 Dengue3
27 Human parainfluenza virus 3
28 Human papillomavirus type 6b
29 Human parvovirus B19
30 JC polyomavirus
31 Rabies virus
32 Human coronavirus OC43
33 Dengue4
34 Human enterovirus D
35 Human metapneumovirus
36 Human rhinovirus A
37 Influenza A H3N2 virus
38 Mumps virus
39 Human enterovirus C
40 Hantavirus Z10
41 Measles
42 Poliovirus
43 Human herpesvirus 6A
44 Human enterovirus A
45 Human respiratory syncytial virus
46 SARS coronavirus
47 Hepatitis A virus
48 Monkeypox virus
49 Human parechovirus
50 Variola virus
51 Human rotavirus G3

hTregitope-167
hTregitope-289



A new way of defining Treg epitopes?



Same binding affinity as T effector, no difference in TCR affinity

Higher density of cross-reactive network with self proteins

Outline

- Who are we?
- What is a Tregitope and . . . What do they do?
- Where are they located?
- Why are these epitopes Tregitopes?
- How they can be applied to protein therapeutics
- Why they are important to immunogenicity

Our approach to analyzing mAbs . . .

Immune Response = Sum of Epitopes
Sum includes + (T effectors) and – (Tregs) scores

Protein Therapeutic

1 + 1 - Treg = Response

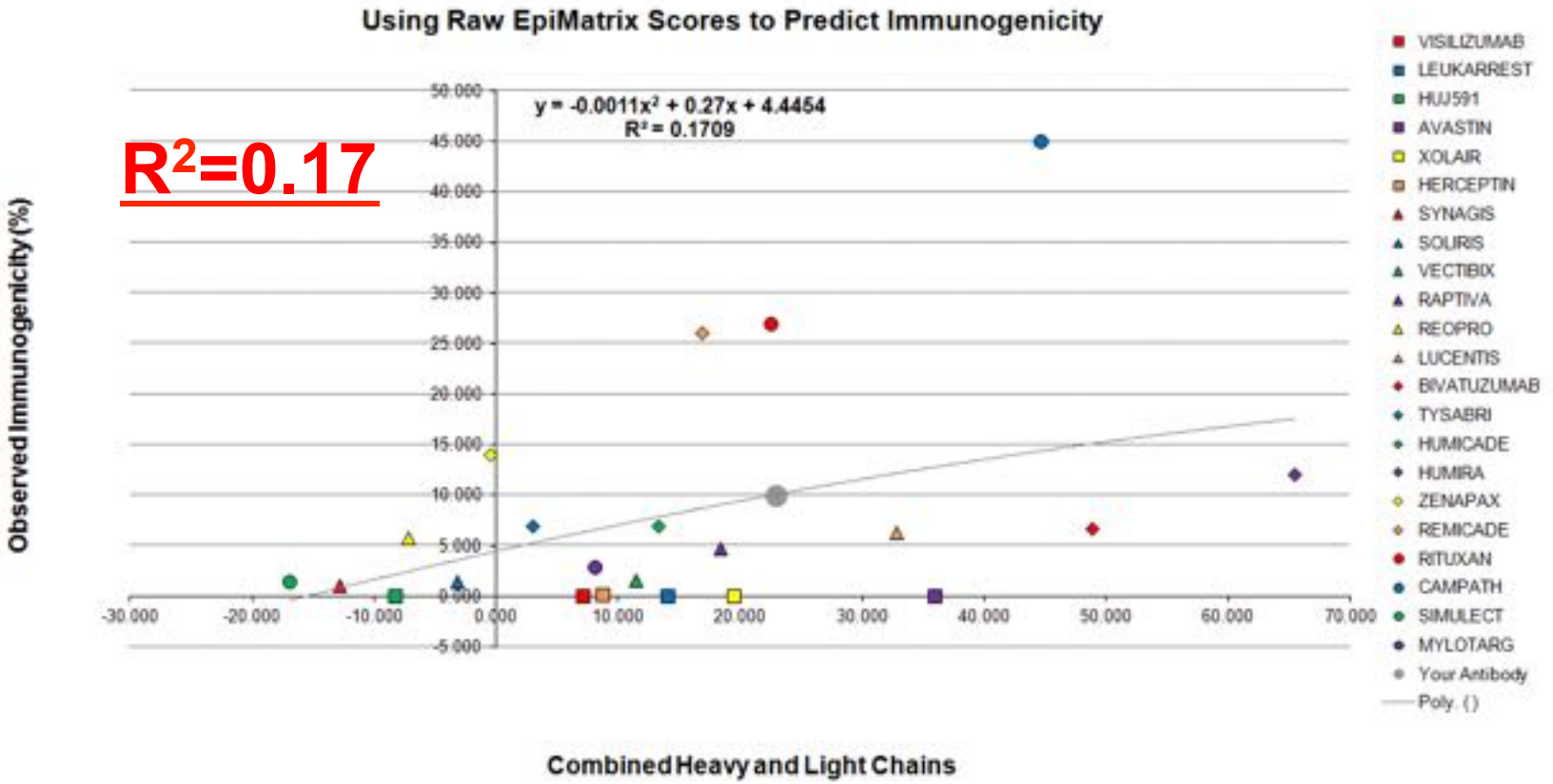
T cell response depends on:

T cell epitope content x HLA – Treg Epitope content x HLA

Protein Immunogenicity can be Ranked

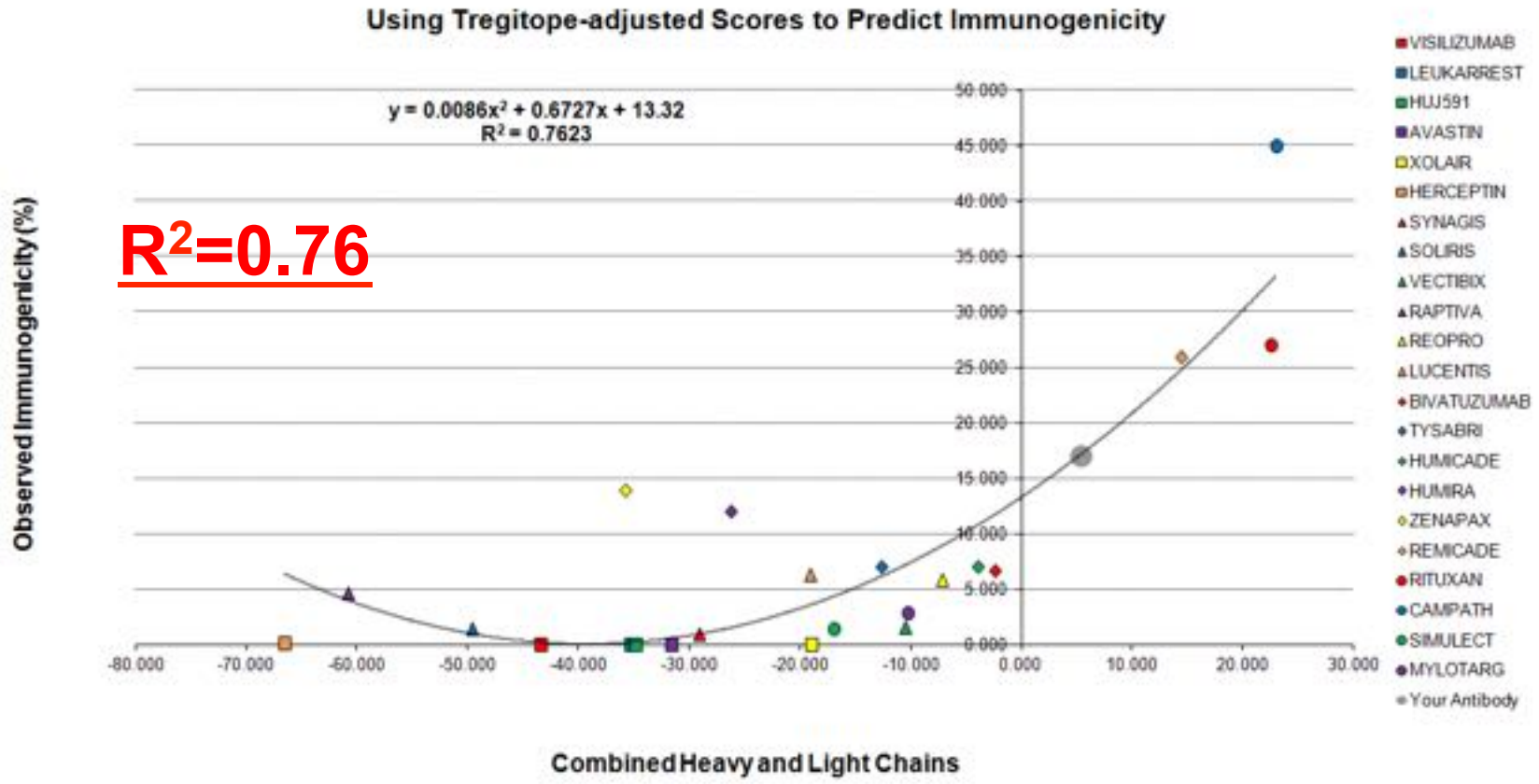
Correlation of antibody immunogenicity without Tregitope adjustment

Correlation to observed Immunogenicity before accounting for Tregitopes



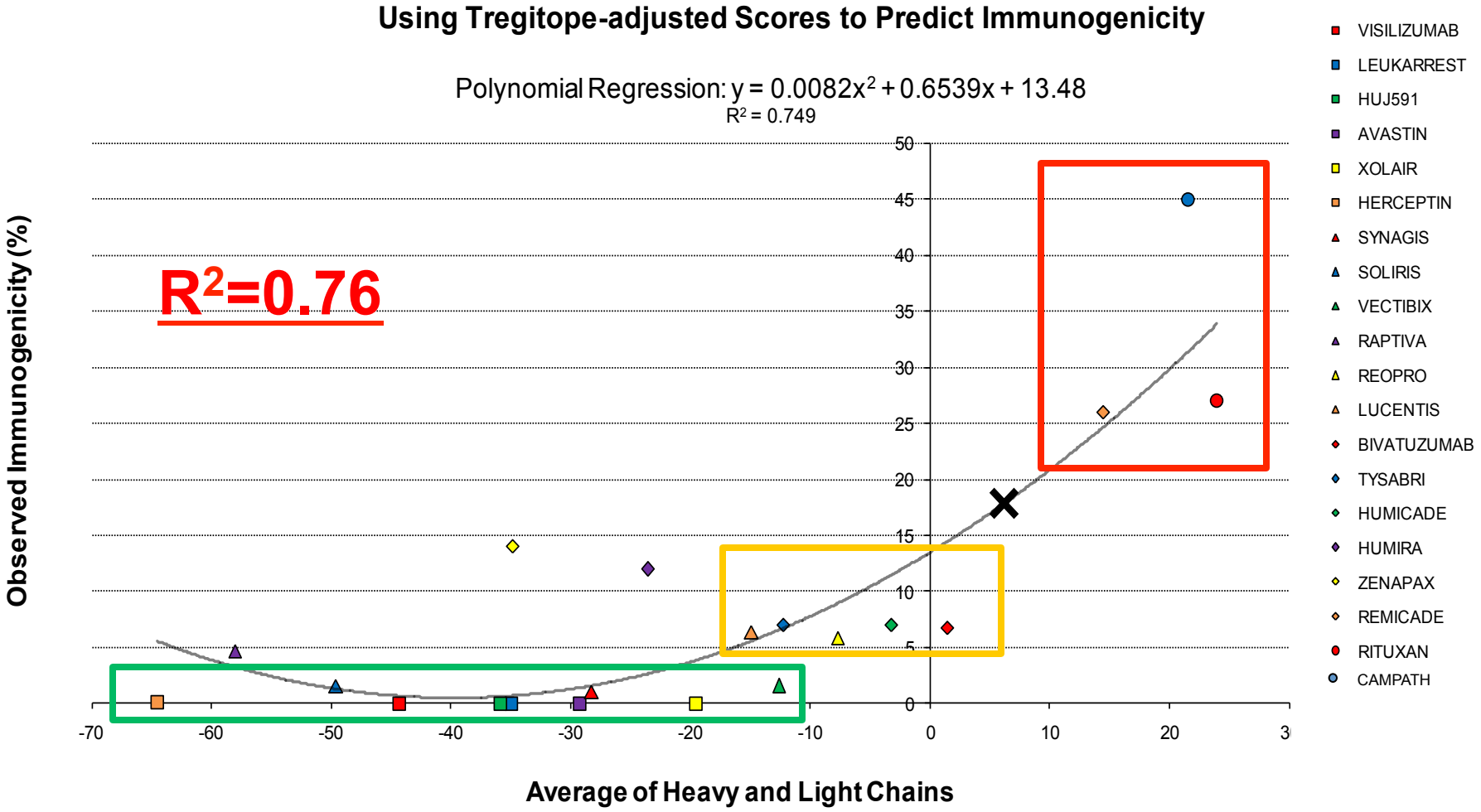
Correlation of antibody immunogenicity with Tregitope adjusted EPX Scores

Correlation to observed immunogenicity after accounting



Accounting for Tregitopes results in more accurate predictions.

Correlation of antibody immunogenicity with Tregitope adjusted EPX Scores

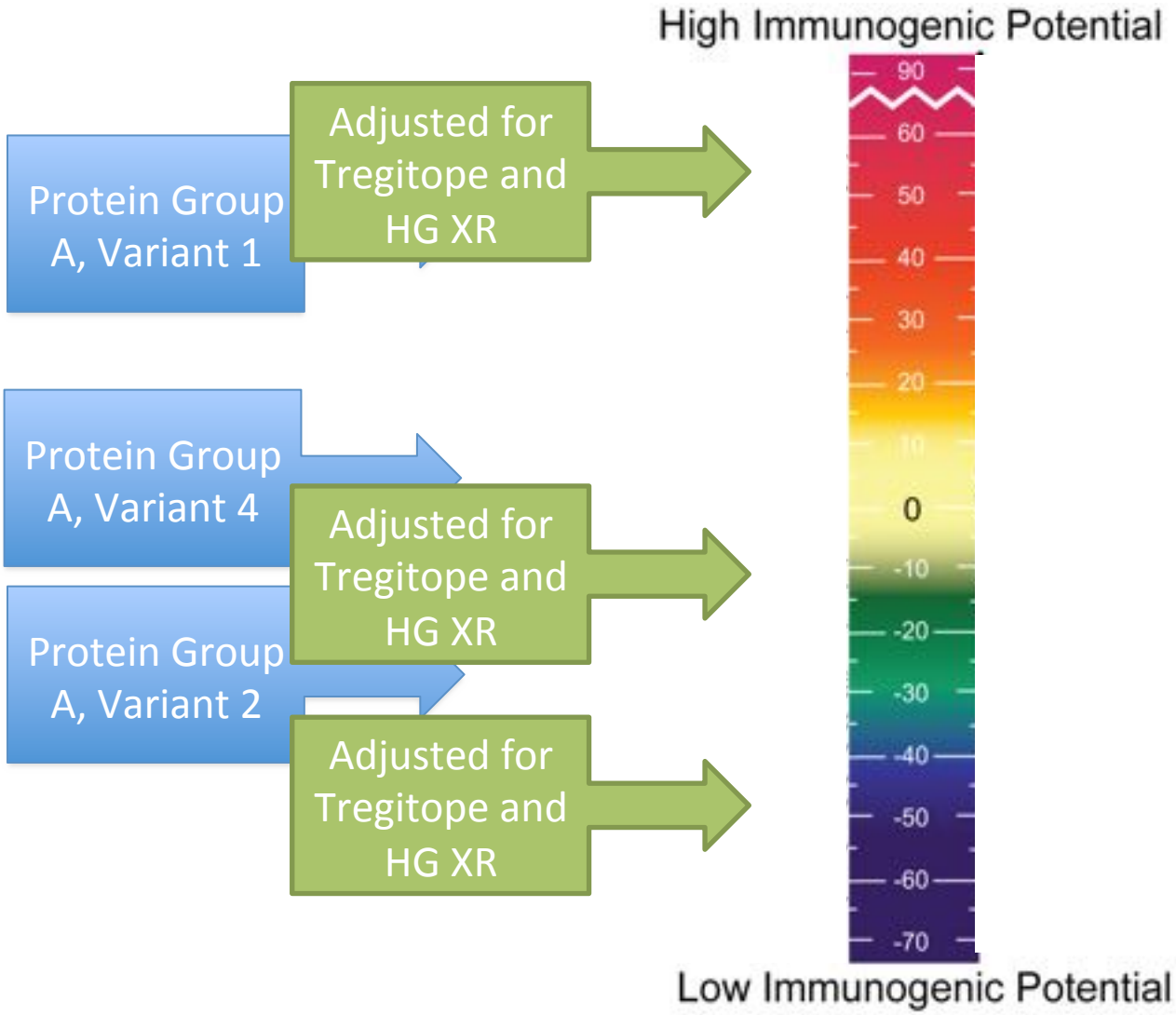


Tregitope (negative) EpiMatrix Scores and Immunogenicity in Human Clinical Studies

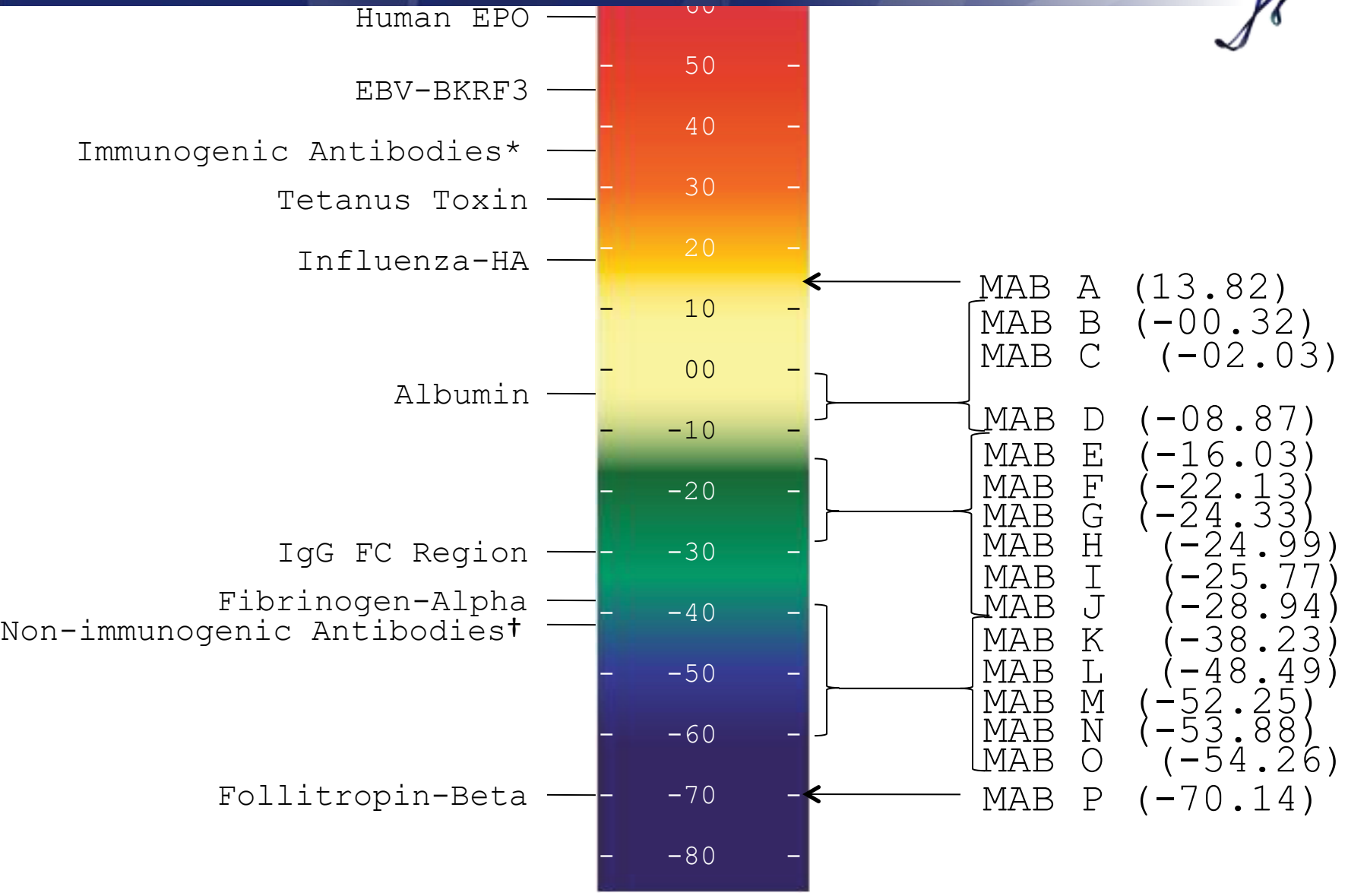
Protein	FPX 1	FPX 2	FPX 3	FPX 4	FPX 5
EpiMatrix score	21.97	34.37	1.62	-1.76	-111.25
Binding Antibodies	37%	53%	7.8%	5.6%	9.3%
Neutralizing Antibodies	40%	12%	0.5%	NA	0%

Client Adjusted Approach – began Prospective immunogenicity screening

Immunogenicity Scale/Biologics

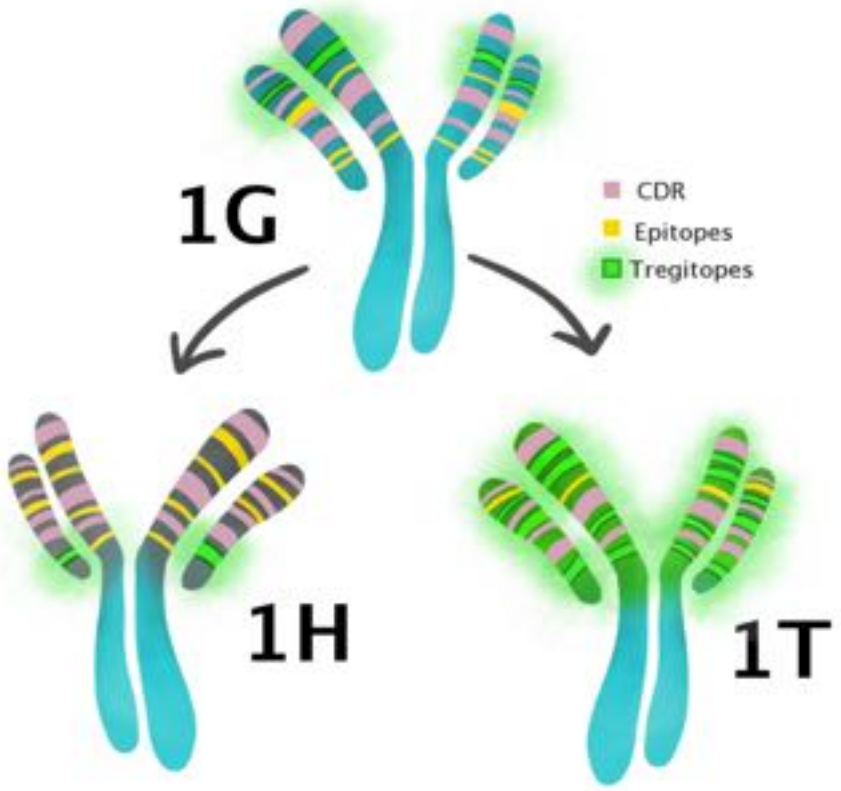


Immunogenicity Scale/Germlines



Humanization vs Tolerization

Campath Project

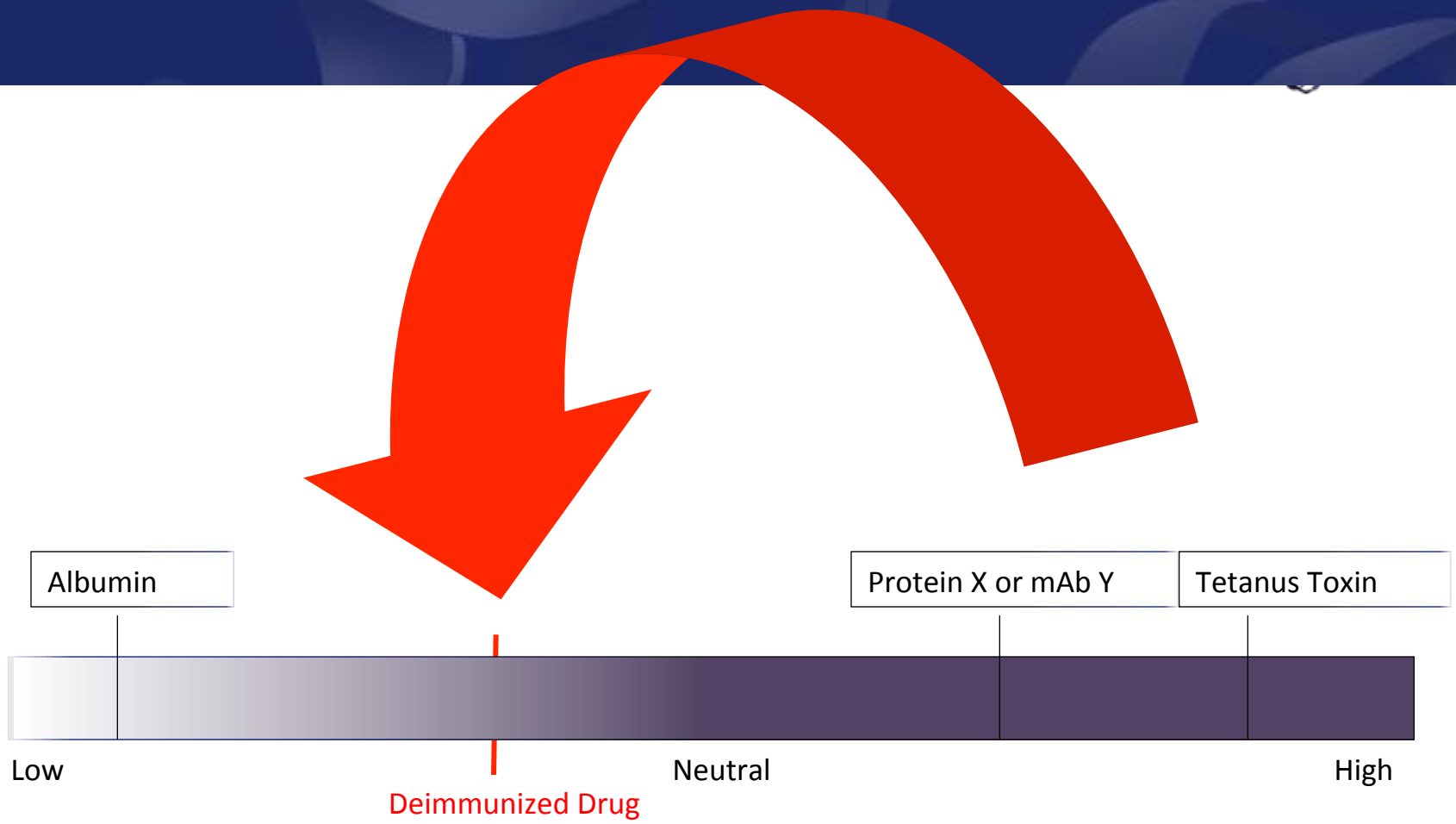


http://bit.ly/Tolerization

Outline

- Who are we?
- What is a Tregitope and . . . What do they do?
- Where are they located?
- How they can be applied to [Non-antibody] protein therapeutics
- Why they are important to immunogenicity

Deimmunization reduces ADA– What's the proof?



In fact, it already happens "Naturally!"

in the context of infectious disease (HIV, HCV etc.)

De-immunization of FVIII

Recent publication

Clinical Immunology (2012) 142, 320–331

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

Currently Partnered with Biotest AG

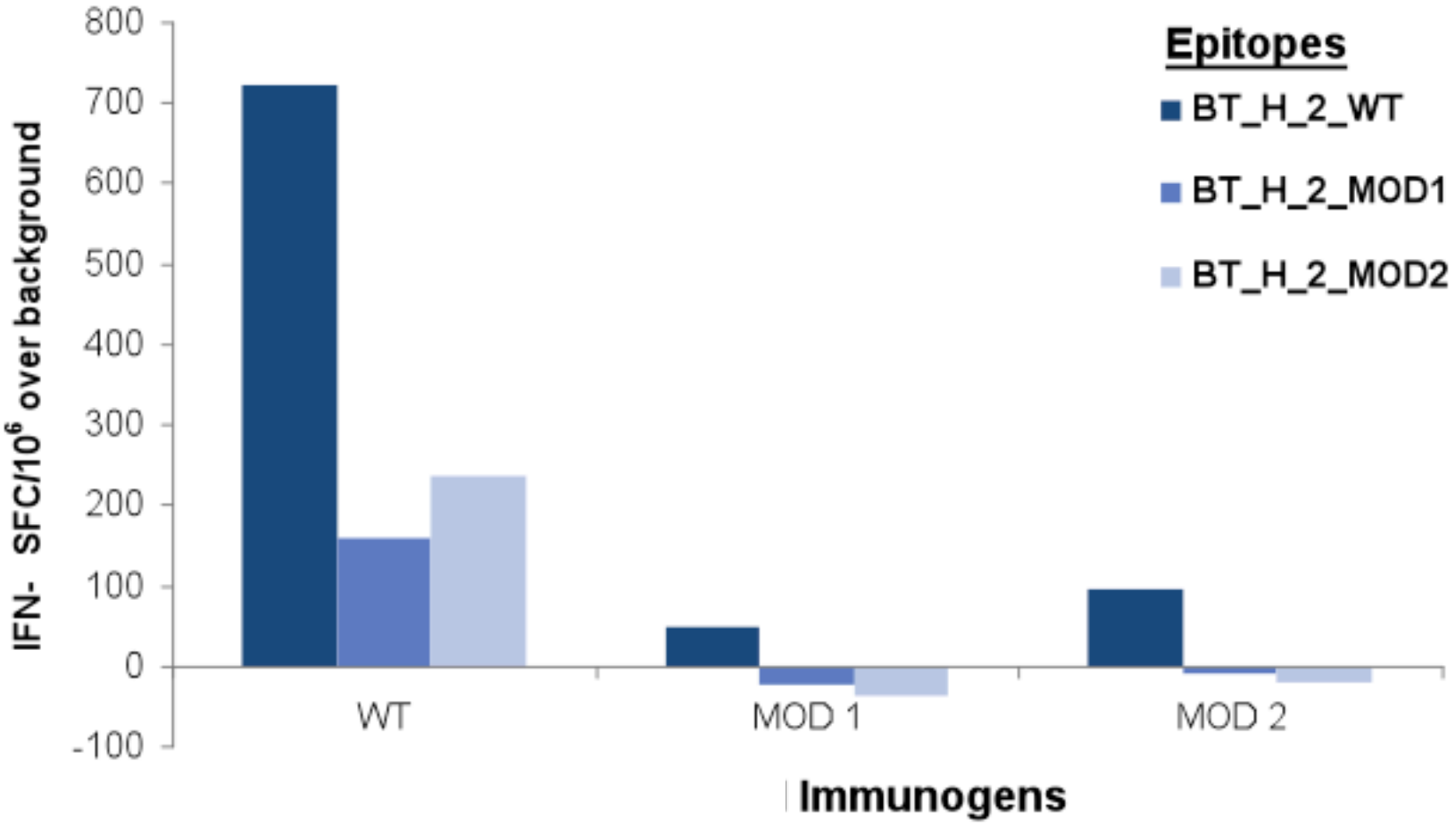
Leonard Moise [a,b,*,1], Chang Song [c,1,2], William D. Martin [a], Ryan Tassone [a], Anne S. De Groot [a,b], David W. Scott [c,d,e,3]

[a] EpiVax, Inc., Providence, RI 02903, USA
[b] Institute for Immunology and Informatics, University of Rhode Island, Providence, RI 02903, USA
[c] Center for Vascular and Inflammatory Diseases, University of Maryland School of Medicine, Baltimore, MD 21201, USA
[d] Department of Surgery, University of Maryland School of Medicine, Baltimore, MD 21201, USA
[e] Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD 21201, USA

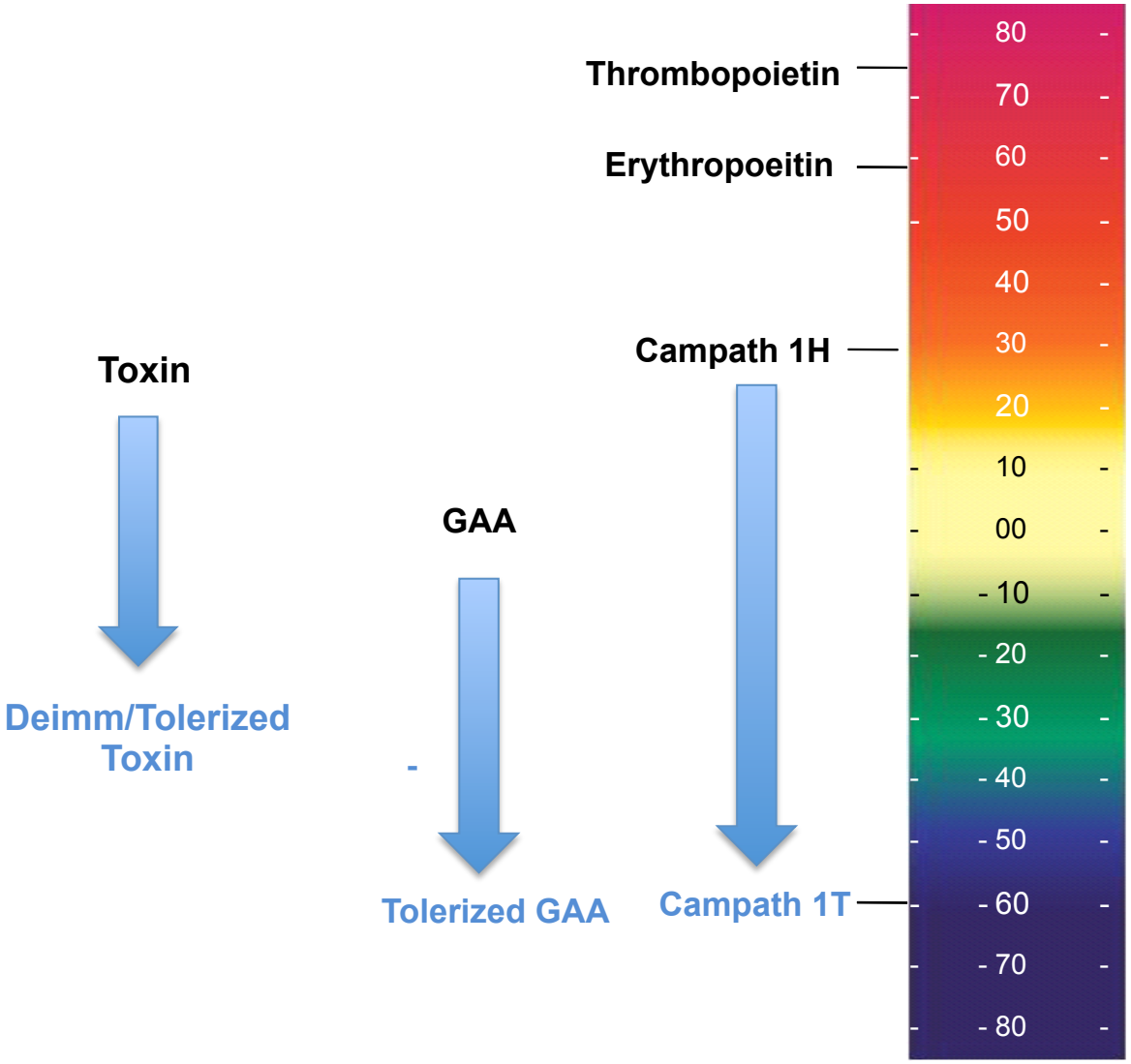
Received 4 September 2011; accepted with revision 24 November 2011
Available online 8 December 2011

De-immunization of Toxin

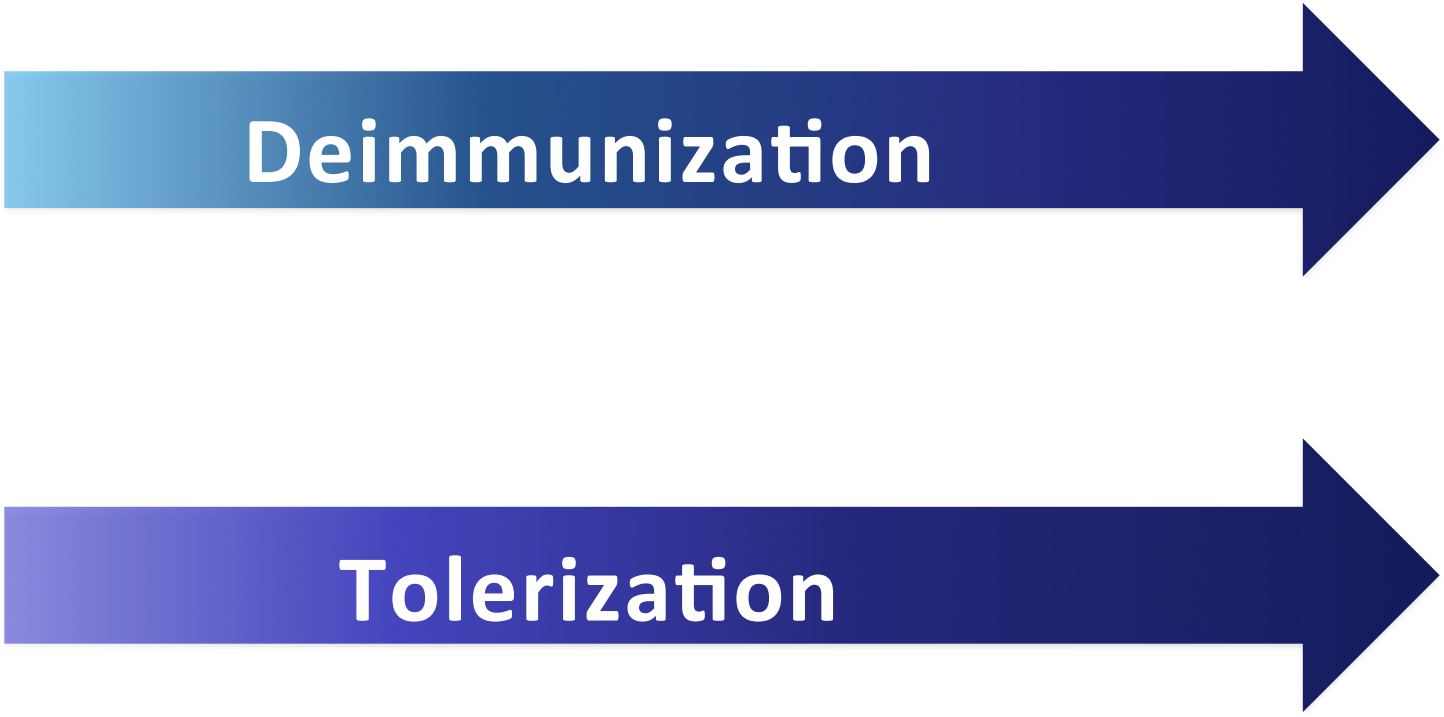
Modifying a single epitope



Lower T cell epitope content?



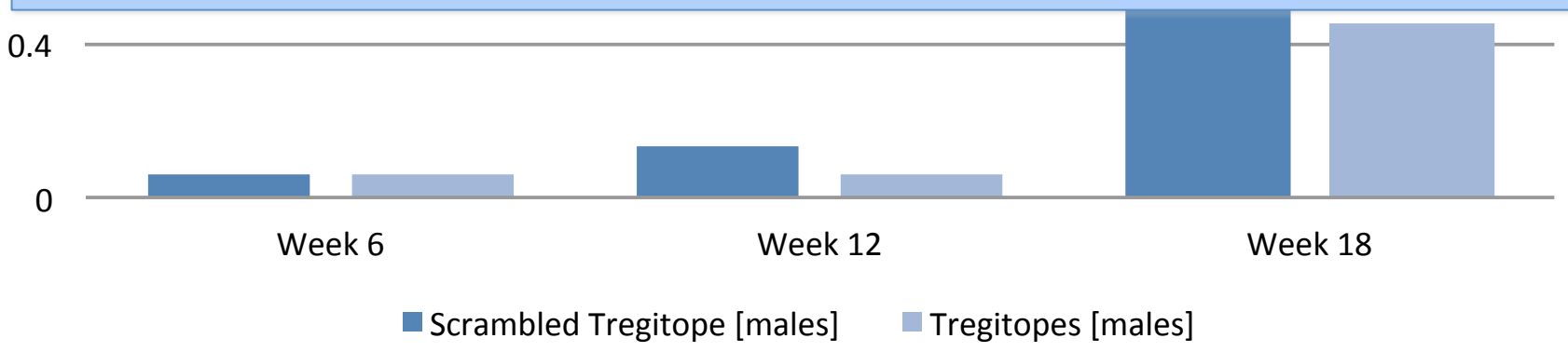
Deimmunization and Tolerization Parallel Paths



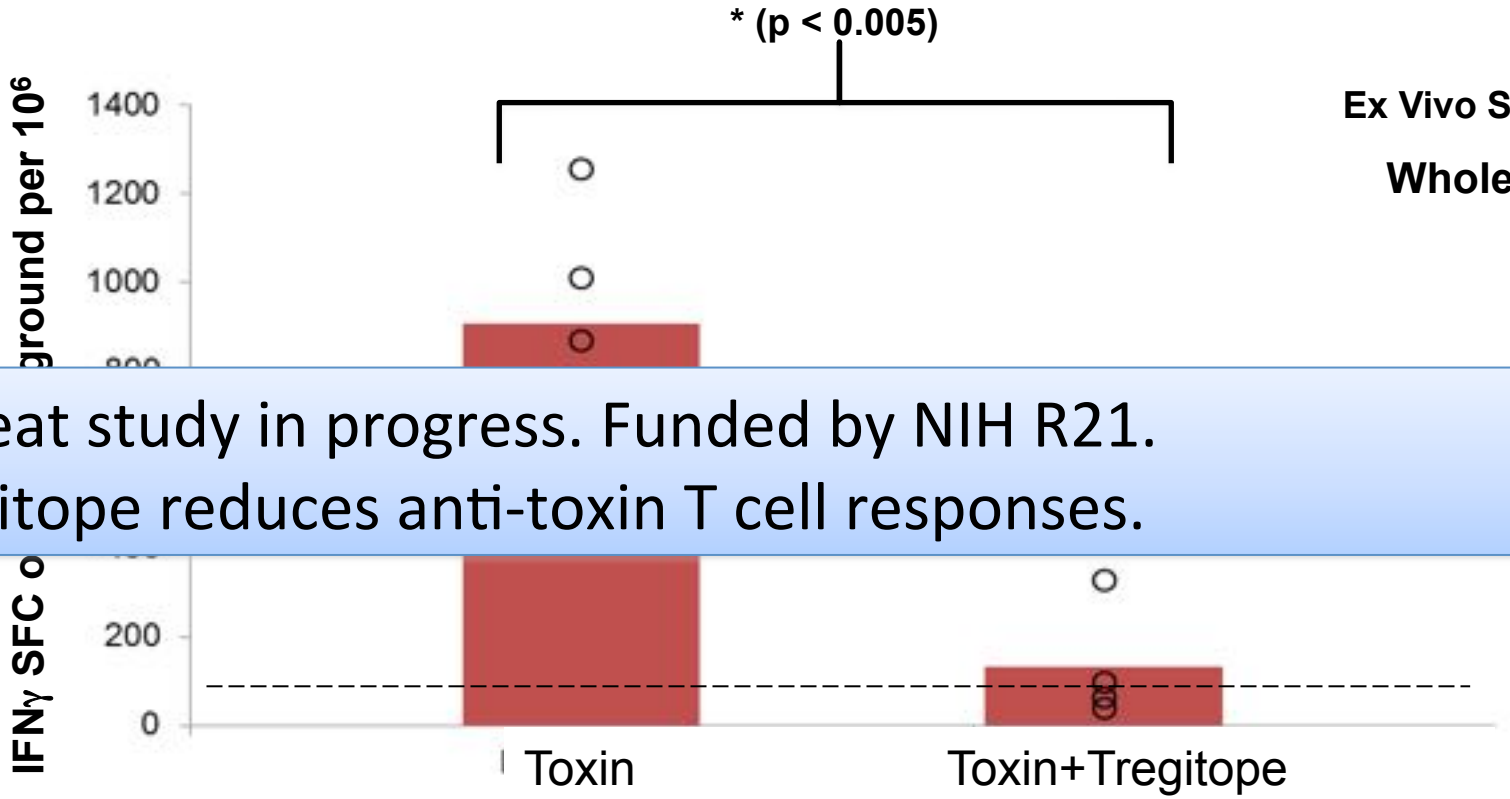
Tregitope + GAA (Myozyme)

Onset anti-GAA IgG (AAV-CBhGAApA at Week 6)

Study in progress. Collaboration with Duke University.
Tregitope reduces anti-GAA antibodies (ADA) at 18 weeks
Data on phenotypic and histochemical effects are pending.



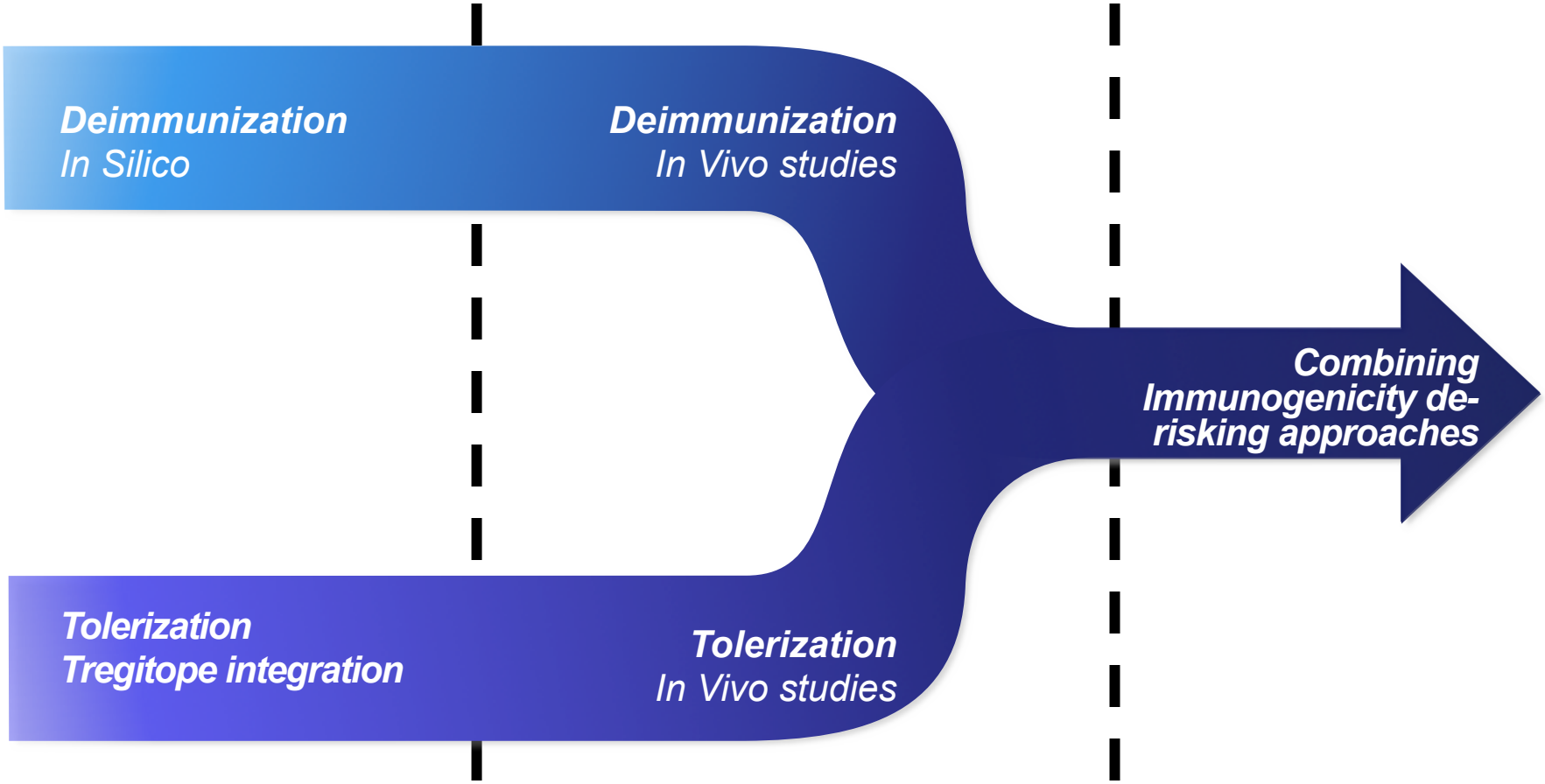
Immunogenic Toxin Co-administer Tregitopes



Repeat study in progress. Funded by NIH R21.
Tregitope reduces anti-toxin T cell responses.

- Tregitope co-administration significantly reduces immune response to toxin

EpiVax – Combined approach



Outline

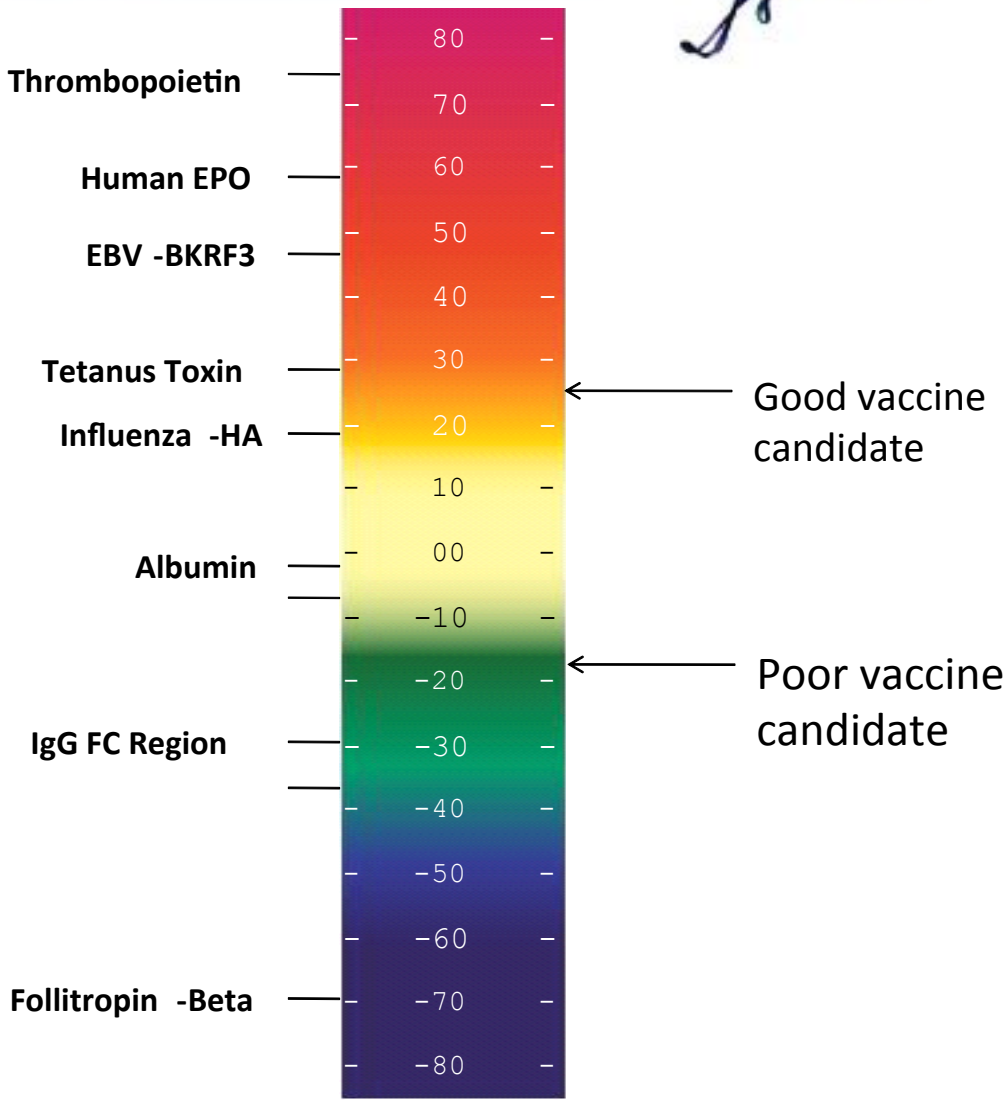
- Who are we?
- What is a Tregitope and . . . What do they do?
- Where are they located?
- Examples from Nature
- Why they are important to immunogenicity

Immunogenicity Scale

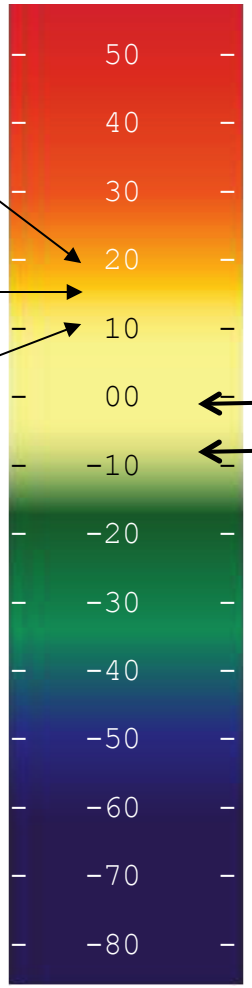
Developed by De Groot and Martin at EpiVax

Proteins ranked by T cell epitope content

Tends to find good B-cell Immunogens as well.



For example, new H7N9 Flu is Predicted to be POORLY IMMUNOGENIC



HA A/California/07/2009 (H1N1)

HA A/Victoria/361/2011 (H3N2)

HA A/Texas/50/2012 (H3N2)

Random Expectation

HA A/chicken/Italy/13474/1999 (H7N1) . . . . . -6.23

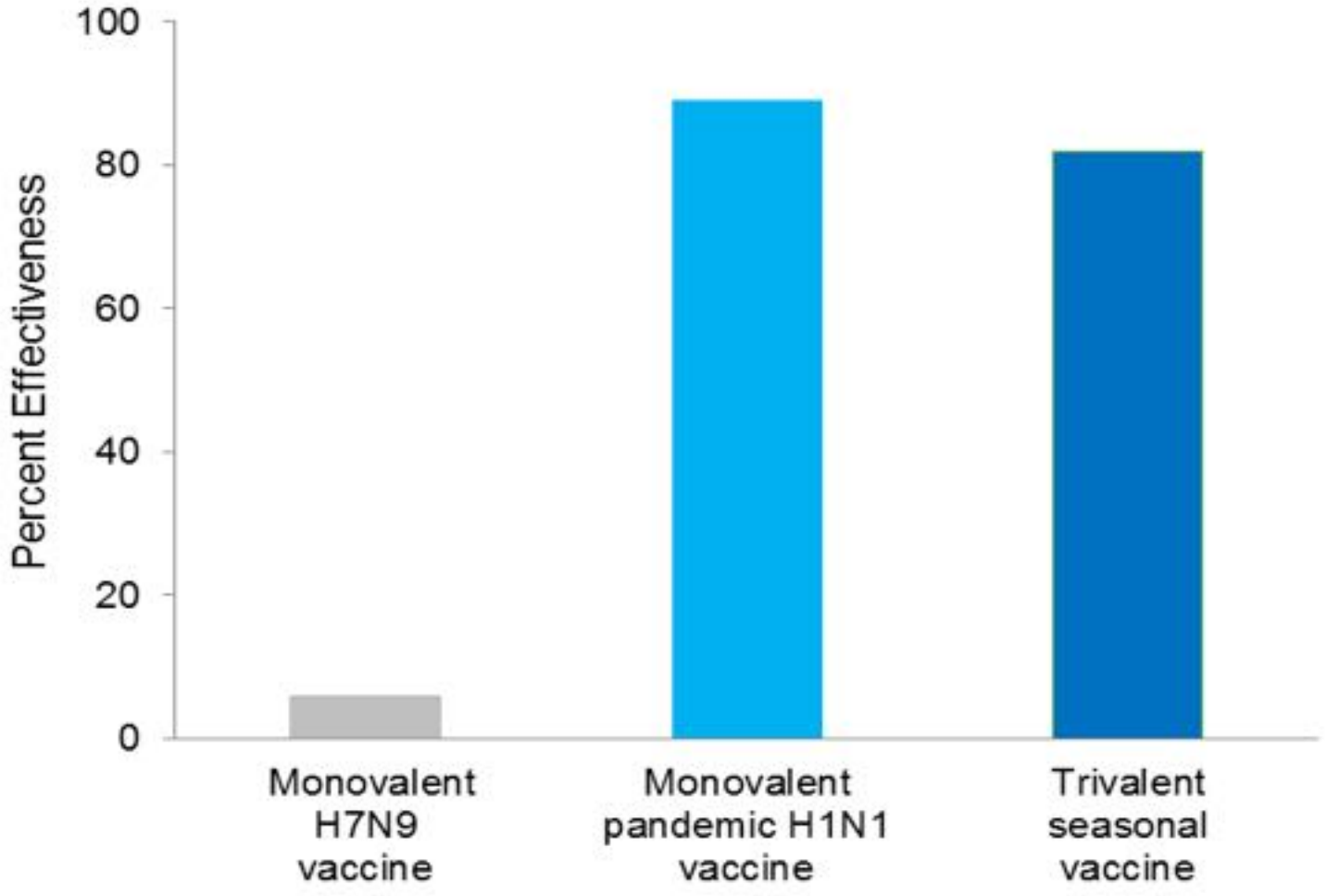
HA A/Shanghai/1/2013 (H7N9) . . . . . -8.11

HA A/mallard/Netherlands/09/2005 (H7N7) . . . . . -8.63

HA A/mallard/Netherlands/12/2000 (H7N3) . . . . . -9.91

And IS POORLY IMMUNOGENIC

Low T cell epitope Content
Low Antibody Titer



Even when other antigens are present!

RESEARCH

. . . Low and Slow . . .

Human Antibody Responses to Avian Influenza A(H7N9) Virus, 2013

Li Guo,[1] Xi Zhang,[1] Lili Ren,[1] Xuelian Yu,[1] Lijuan Chen,[1] Hongli Zhou, Xin Gao, Zheng Teng, Jianguo Li, Jiayu Hu, Chao Wu, Xia Xiao, Yiyi Zhu, Quanyi Wang, Xinghuo Pang, Qi Jin,[2] Fan Wu,[2] and Jianwei Wang[2]

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http://bit.ly/H7N9_Serology

Why are immunoinformatics tools im

Media releases

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November 14, 2013 07:15 CET

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- I
  - **Novartis announces positive clinical trial results for novel H7N9 vaccine**
    - *85% of subjects immunologically protected after receiving second dose of investigational cell culture vaccine when combined with proven MF59® adjuvant*
    - *Vaccine now in large scale production highlighting rapid response capability of novel FDA licensed cell culture technology*
    - *135 confirmed cases and 45 deaths from H7N9 virus since emergence in March according to the World Health Organization[1]*
- \

Basel, Switzerland, November 14, 2013 - Novartis announced today interim results from a Phase 1 clinical trial with its proprietary cell culture vaccine for the H7N9 avian influenza virus involving 400 healthy volunteers (18-64 years of age). The data shows 85% of subjects achieved a protective immune response after two doses of the 15 ug MF59 adjuvanted vaccine. Only 6% of subjects achieved a protective response when given two doses of the 15ug un-adjuvanted vaccine. The full data set from the trial will be submitted to a peer-reviewed journal for publication in the near future.

The vaccine was produced utilizing full-scale cell-culture manufacturing technology, an alternative technology that can significantly accelerate vaccine production versus traditional egg-based methods.[2] Cell-culture technology utilizes a well-characterized mammalian cell line rather than chicken eggs to grow virus strains.[3]

http://bit.ly/H7N9_NovaVax

Implication

- Antibody (ADA) response directly linked to protein
- Presence of other flu proteins did not impact "ADA" response to low immunogenicity protein
- *Lower T cell epitope content – lower ADA*
- *Viral "Tregitopes" – lower ADA*

Application to Biologics?

- Deimmunize (multiple examples)
- Select candidates with lower T cell epitope content (and higher Treg epitope content)
- Add Tregitopes

Clinical Immunology (2013) 149, 534–555

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

REVIEW

T-cell dependent immunogenicity of protein therapeutics: Preclinical assessment and mitigation☆

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http://bit.ly/The_TCWP

Tregitope (negative) EpiMatrix Scores and Immunogenicity in Human Clinical Studies

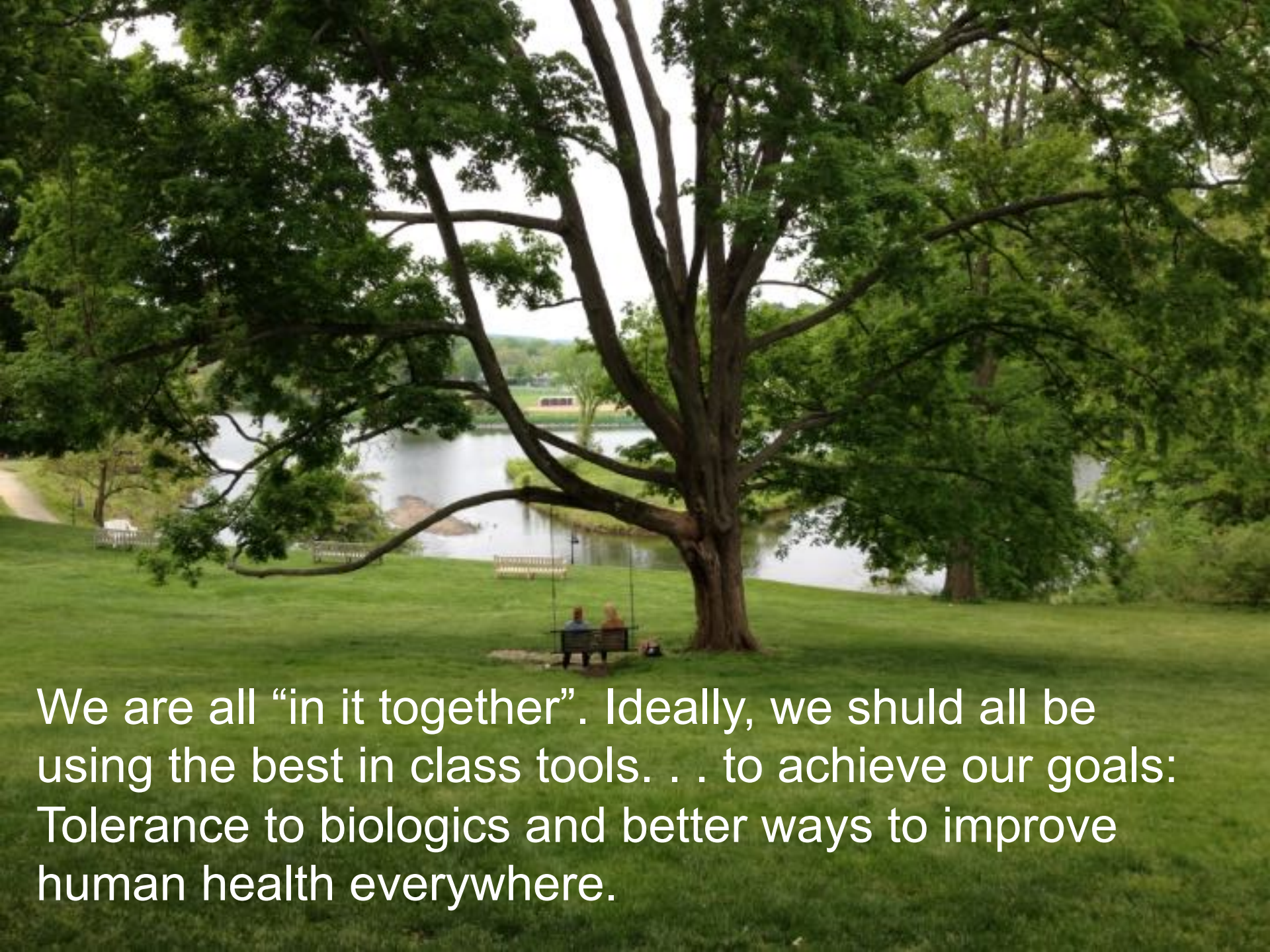
Protein	FPX 1	FPX 2	FPX 3	FPX 4	FPX 5
EpiMatrix score	21.97	34.37	1.62	-1.76	-111.25
Binding Antibodies	37%	53%	7.8%	5.6%	9.3%
Neutralizing Antibodies	40%	12%	0.5%	NA	0%

Client Adjusted Approach – began Prospective immunogenicity screening

http://bit.ly/The_TCWP

Conclusions

- We have much to learn about human immune response
- Treg epitopes – very relevant to biologics
- Context is important
- Is autologous epitope *prevalence* relevant?
- How to use tolerance to address unmet human health needs



We are all “in it together”. Ideally, we shuld all be using the best in class tools. . . to achieve our goals: Tolerance to biologics and better ways to improve human health everywhere.

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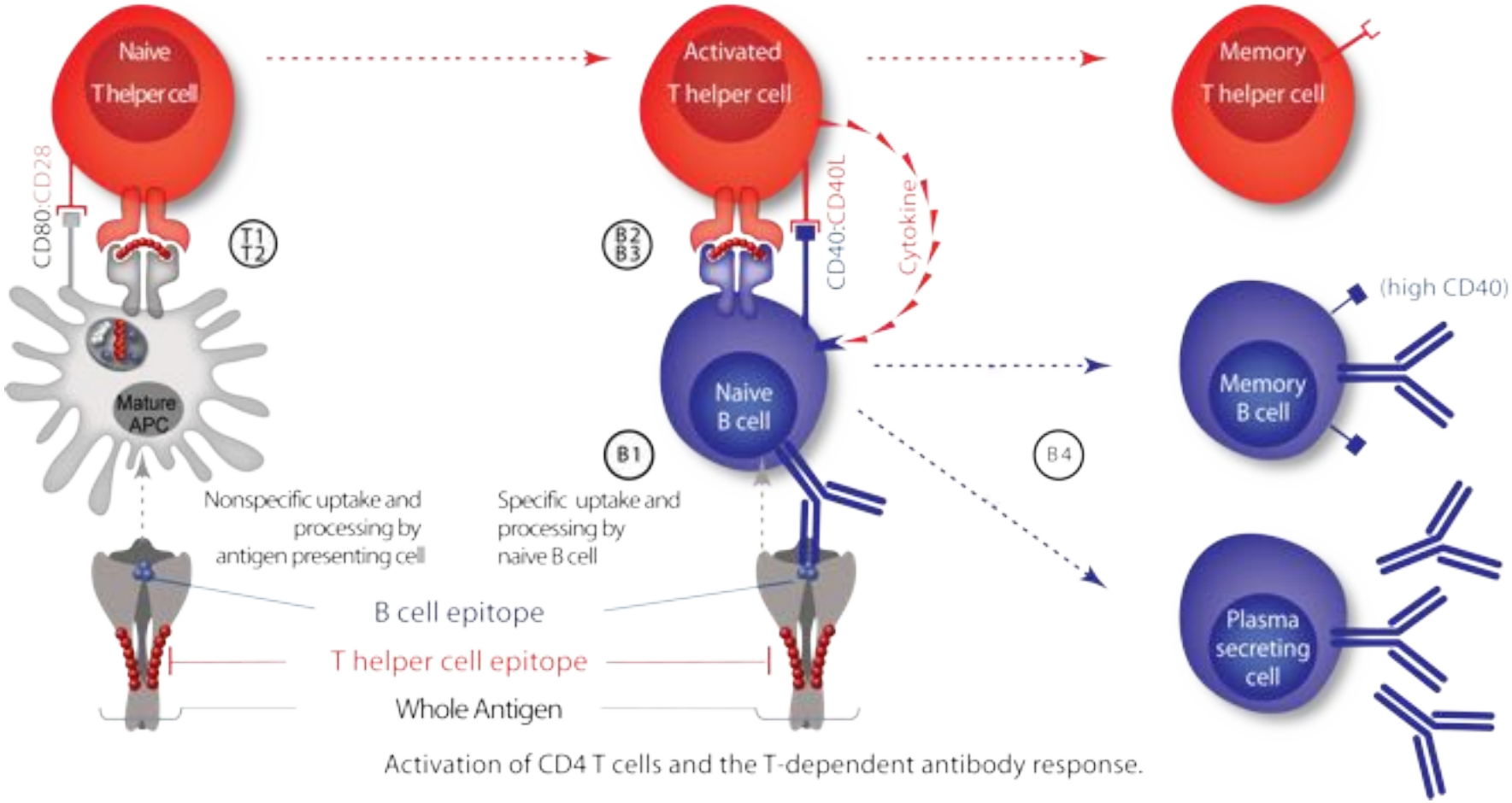
T cell dependent immunogenicity

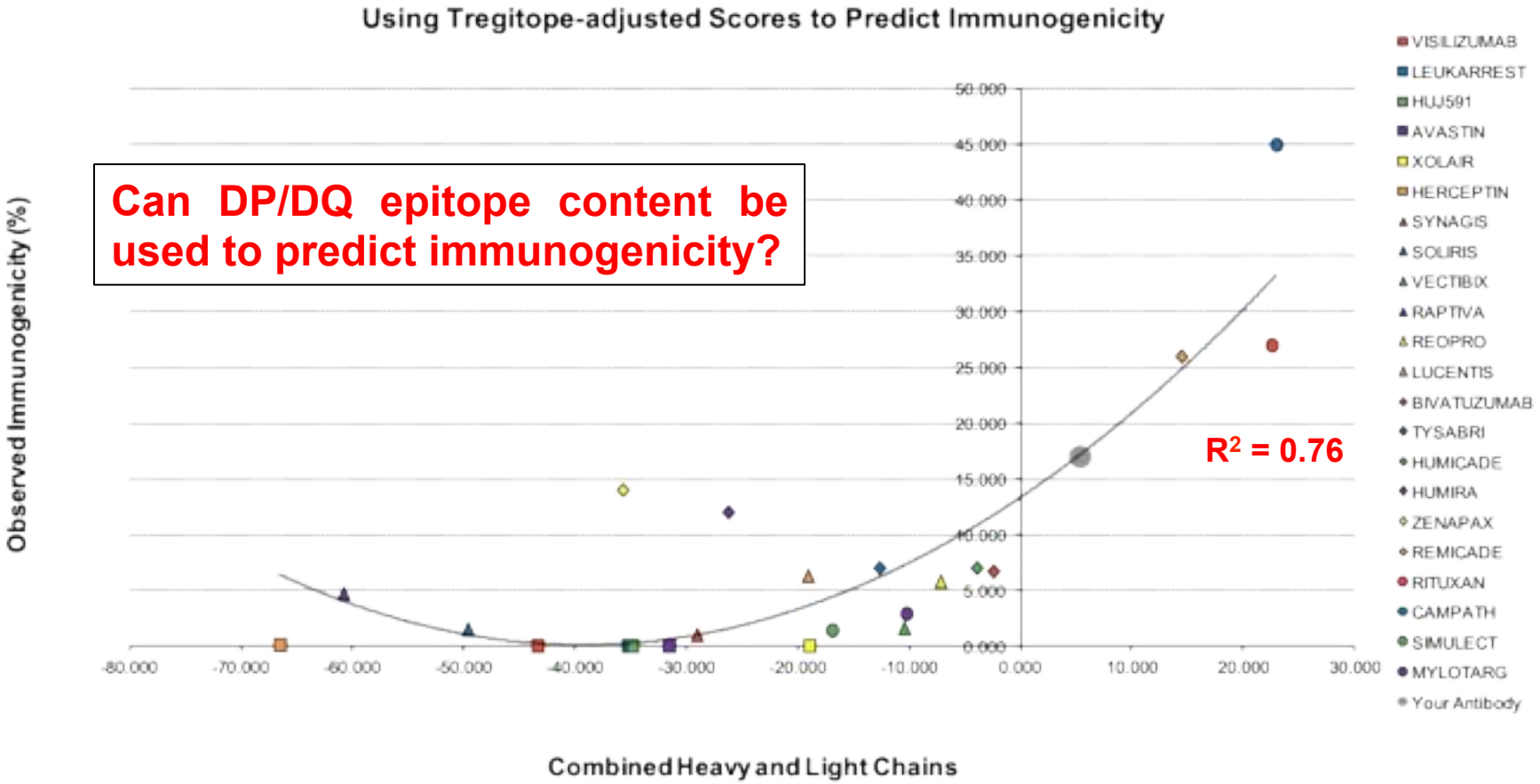
T Cell Activation → B Cell Activation



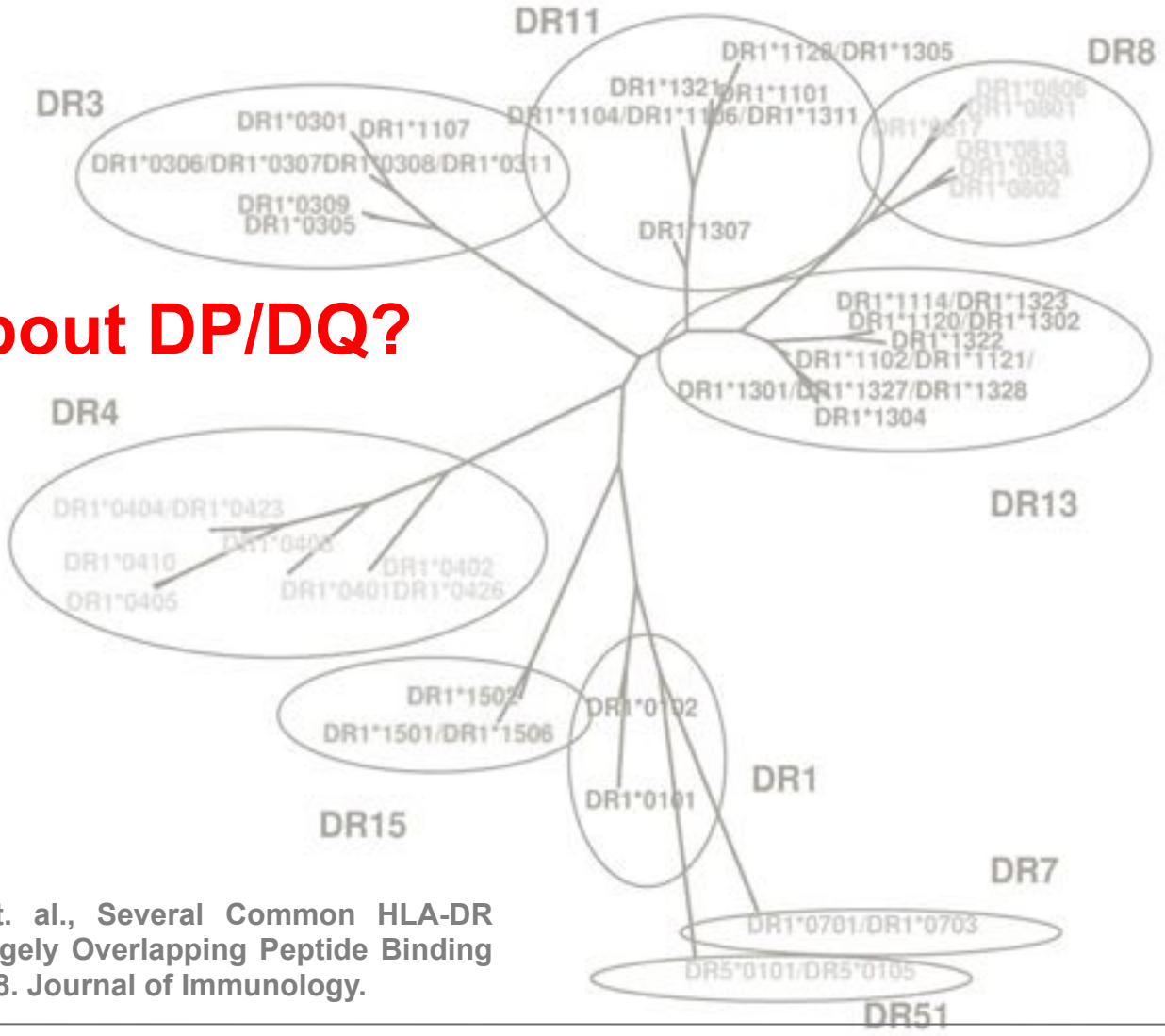
Activation of CD4 T cells and the T-dependent antibody response.

Predicting Immunogenicity

EpiVax finds a high degree of correlation between DR-derived, Tregitope-adjusted EpiMatrix Protein Scores and observed immunogenicity of Mabs.



WHY ONLY DR???



What about DP/DQ?

*** Southwood et. al., Several Common HLA-DR Types Share Largely Overlapping Peptide Binding Repertoires. 1998. Journal of Immunology.**

EpiVax DP/DQ Evaluation

Method

- **Use DP and DQ epitope prediction tools available at IEDB to predict epitope content of 20 licensed monoclonal antibodies for which immunogenicity is known.**
- **Evaluate predictive power of DP/DQ predictions alone or supplemental to DRB1 predictions.**

IEDB DP/DQ Prediction Method

Input = Overlapping 15-mers

May duplicate EpiMatrix 9-mers → upward bias consistent, safely discounted

	Predictive Models			
HLA-DP Haplotype	**SMM**	**ANN**	**COMBLIB**	**Consensus**
DPA+01 DPB1+0401				
DPA+0103 DPB1+0201				
DPA+0201 DPB1+0101				
DPA+0201 DPB1+0501				
DPA+0301 DPB1+0402				
HLA-DQ Haplotype				
DQA1+0101 DQB1+0501				
DQA1+0102 DQB1+0602				
DQA1+0301 DQB1+0302				
DQA1+0401 DQB1+0402				
DQA1+0501 DQB1+0201				
DQA1+0501 DQB1+0301				

Output

EpiMatrix = Z-scores, top 5% are significant

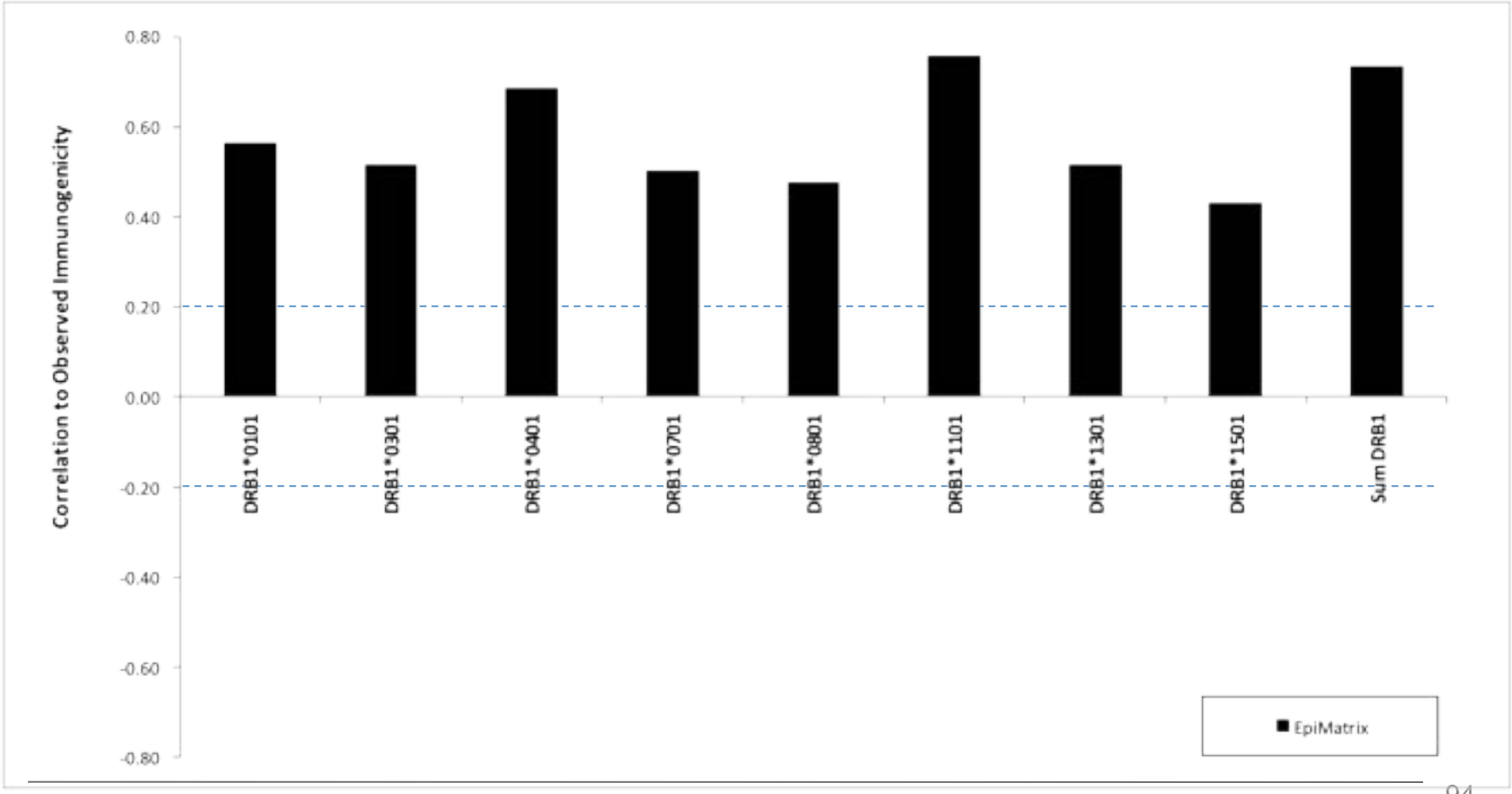
IEDB = Scores as percentile ranks
Ranks below 5% considered significant for comparative analysis

Method

Count number of significant percentile ranks, correlate with Observed Immunogenicity

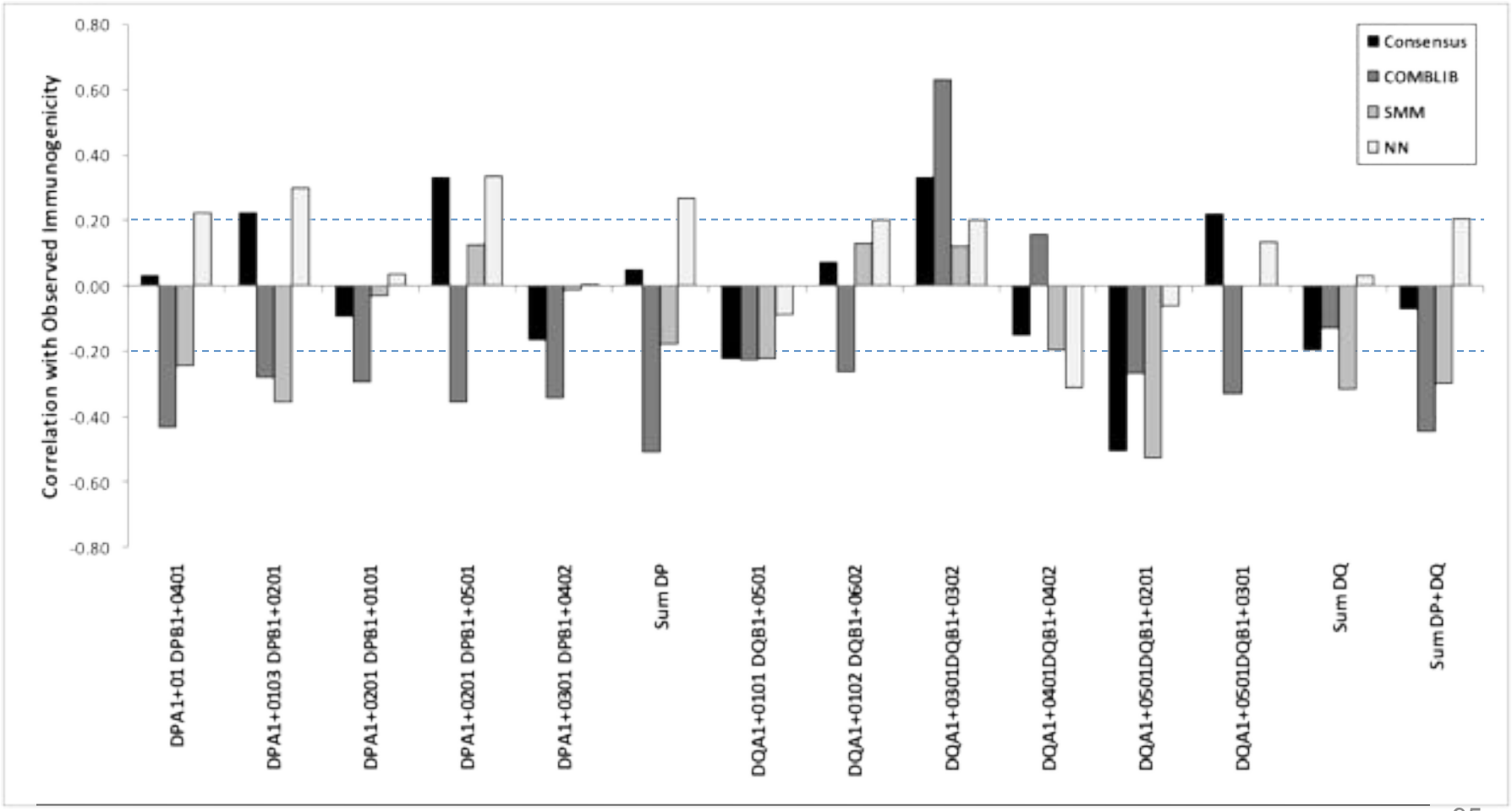
EpiMatrix DR
Consistent Pattern

Counts of significant HLA-DR percentile ranks are consistently positively correlated with observed response, and this correlation is improved by summing across EpiMatrix supertype alleles.



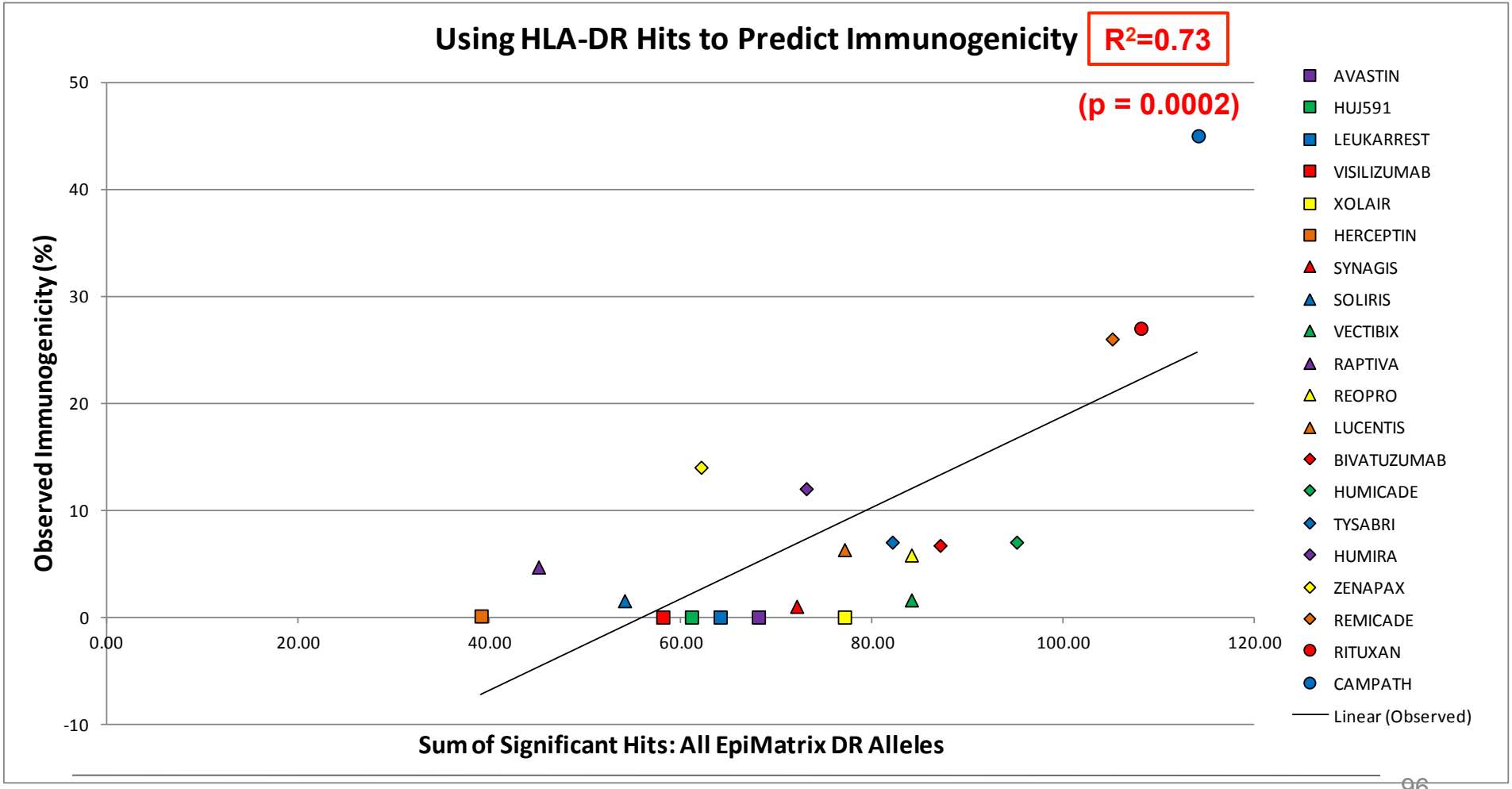
IEDB DP/DQ – Inconsistent Pattern

Counts of significant HLA-DP or DQ percentile ranks are not consistently correlated with observed response and summing counts across alleles does not improve correlation, with the exception of the COMBLIB method predictions for HLA-DP alleles (which gave a negative correlation).



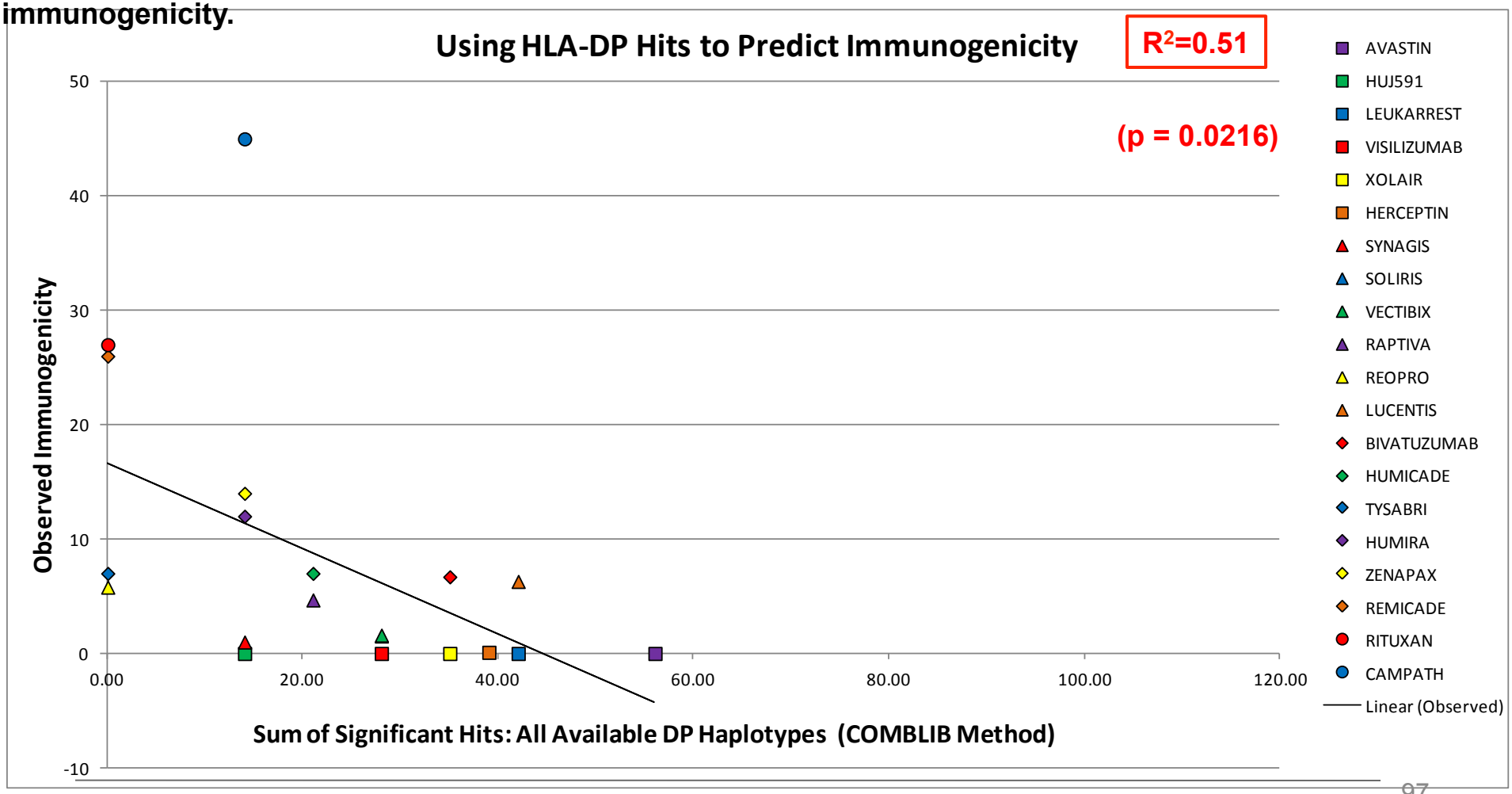
Comparison EpiMatrix Results
Linear Regression: Sum DR

The sum of EpiMatrix HLA-DR Scores is positively correlated with observed immunogenicity with a high degree of significance



IEDB DP/DQ Best Example Results
Linear Regression: Sum DP (COMBLIB)

Sum of HLA-DP Scores using the COMBLIB model at IEDB is negatively correlated with observed response, suggesting that lack of DP content could lead to immunogenicity.



IEDB DP/DQ Prediction Methods

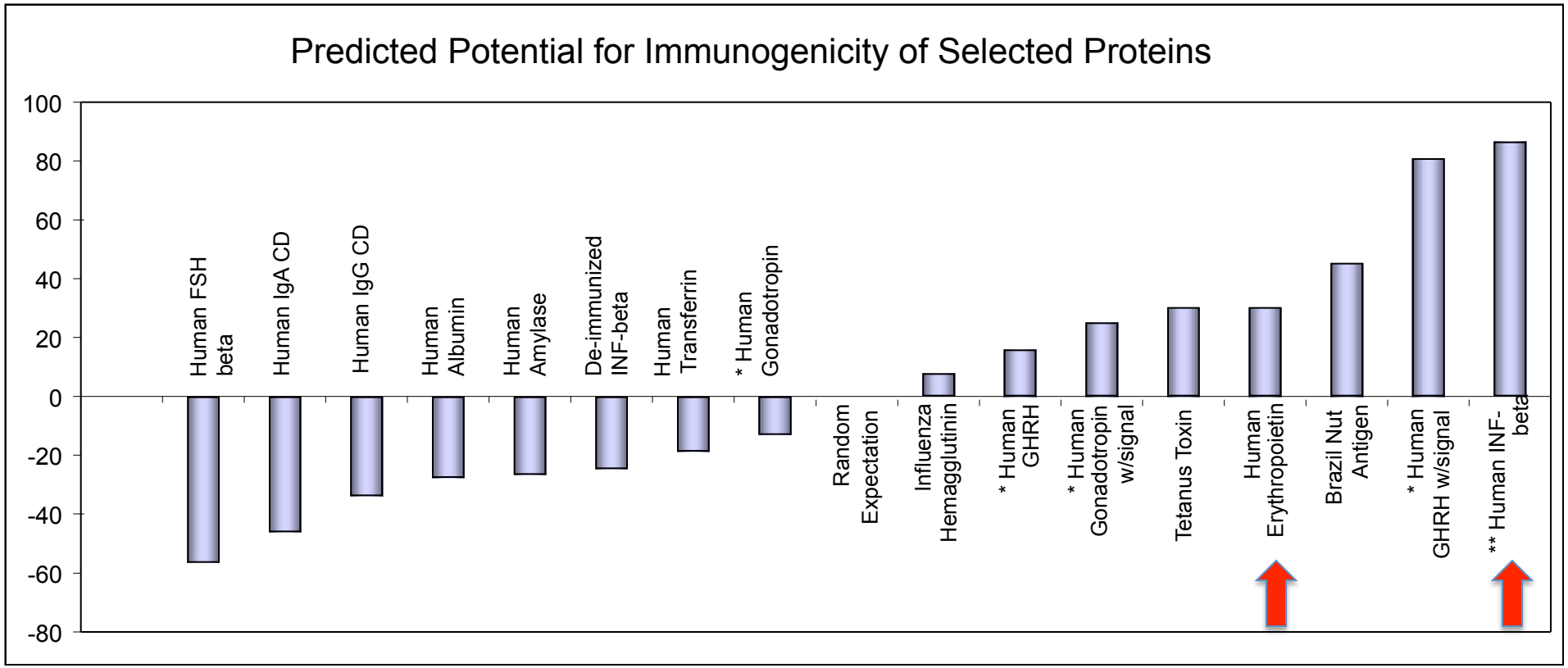
Counts of significant HLA-DP or DQ percentile ranks are not consistently correlated with observed response and summing counts across alleles does not improve correlation, with the exception of the COMBLIB method predictions for HLA-DP alleles.

HLA allele	Predictive Model									
	EpiMatrix		Consensus		CombLib		SSM		NN	
	Raw	Adjusted	Raw	Adjusted	Raw	Adjusted	Raw	Adjusted	Raw	Adjusted

In Silico Prediction of HLA-DP and -DQ Epitope Content is Poorly Correlated with Clinical Immunogenicity of Therapeutic Proteins

DRB1*1	**0.58**	**0.76**	0.34	**0.58**	-	-	**0.47**	**0.76**	0.15	0.15
DRB1*1	0.12	**0.52**	0.36	0.35	-	-	0.17	0.17	**0.53**	**0.50**
DRB1*1	0.05	0.43	-0.15	-0.01	-	-	-0.13	-0.04	-0.15	-0.11
DPA1+01 DPB1+0401	-	-	0.03	0.19	-0.43	0.21	-0.25	0.02	0.22	0.30
DPA1+0103 DPB1+0201	-	-	0.23	0.39	-0.28	-0.02	-0.36	0.17	0.3	0.32
DPA1+0201 DPB1+0101	-	-	-0.09	0.05	-0.30	-0.08	-0.03	**0.59**	0.04	0.13
DPA1+0201 DPB1+0501	-	-	0.33	0.57	-0.36	-0.04	0.13	0.41	0.33	0.35
DPA1+0301 DPB1+0402	-	-	-0.17	0.29	-0.34	0.27	-0.01	0.38	0	0.28
DQA1+0101 DQB1+0501	-	-	-0.22	0.26	-0.23	-0.05	-0.22	0.34	-0.09	0.34
DQA1+0102 DQB1+0602	-	-	0.07	-0.25	-0.26	0.31	0.13	-0.27	0.2	-0.09
DQA1+0301 DQB1+0302	-	-	0.33	-0.02	**0.63**	0.07	0.12	-0.19	0.2	-0.15
DQA1+0401 DQB1+0402	-	-	-0.15	0.11	0.16	0.25	-0.2	-0.17	-0.31	-0.06
DQA1+0501 DQB1+0201	-	-	**-0.51**	-0.08	-0.27	0.05	**-0.53**	-0.16	-0.06	-0.16
DQA1+0501 DQB1+0301	-	-	0.22	-0.15	-0.33	0.04	0	0.00	0.14	-0.23
All DR	**0.45**	**0.73**	0.36	**0.73**	0.03	0.03	0.32	**0.64**	0.07	0.28
All DP	-	-	0.05	0.41	**-0.51**	0.13	-0.18	0.41	0.27	0.43
All DQ	-	-	-0.2	0.08	-0.13	0.19	-0.32	-0.04	0.03	-0.05
All DP/DQ	-	-	-0.07	0.35	**-0.45**	0.26	-0.3	0.31	0.21	0.30
All DR/DP/DQ	-	-	0.24	**0.67**	-	-	0.09	**0.54**	0.18	0.39

Early Observation: Range of T cell epitope Content In Autologous Proteins



We observed: serum proteins have fewer T cell epitopes

De Groot AS, Goldberg M, Moise L, Martin W. **Evolutionary deimmunization: An ancillary mechanism for self-tolerance.**
Cell Immunol. 2007 Apr 17; Pages 148-153. http://dx.doi.org/10.1016/j.cellimm.2007.02.006

More *Common* Proteins are Naturally Deimmunized

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Evolutionary deimmunization: An ancillary mechanism for self-tolerance?

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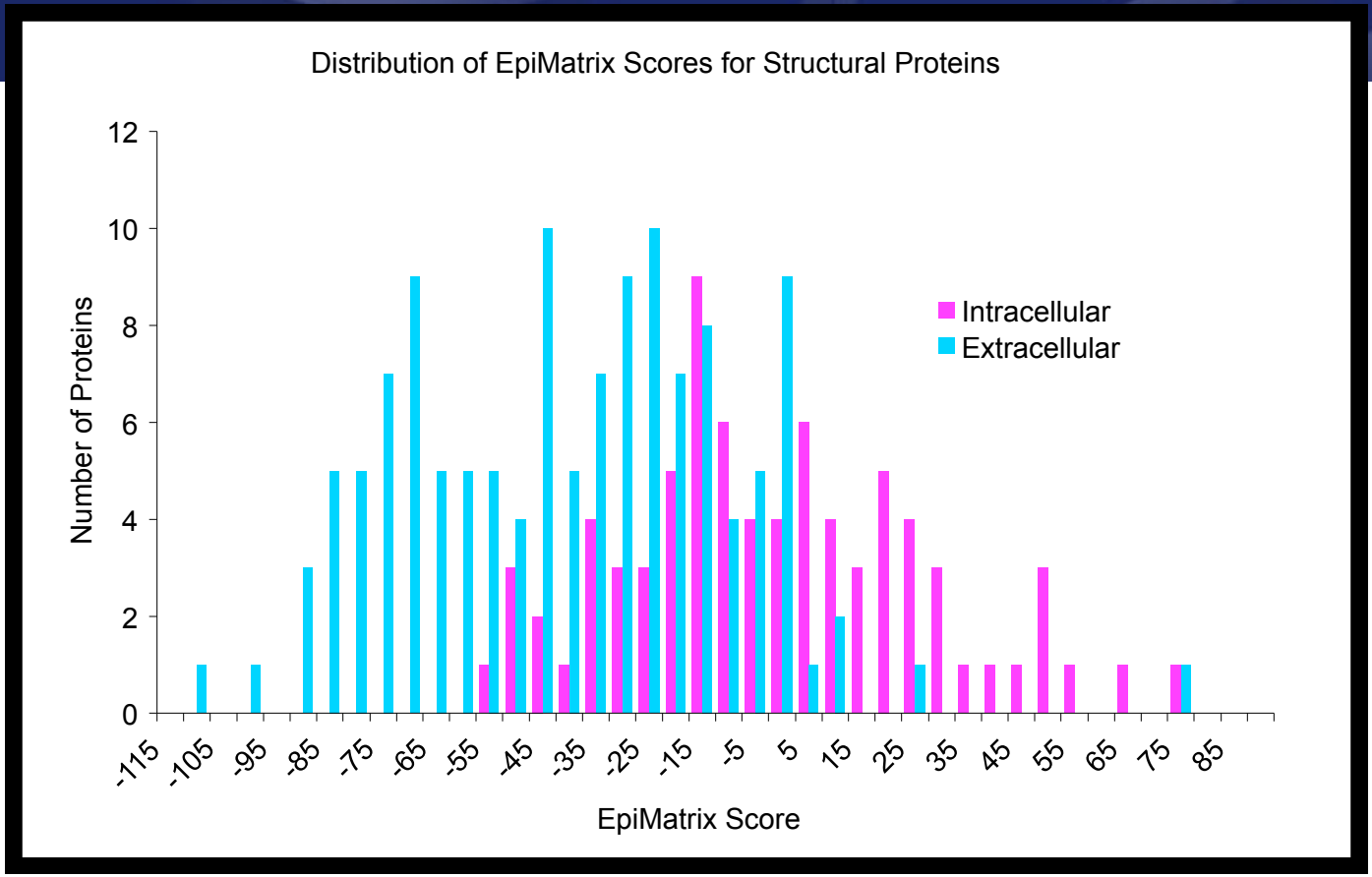
Extracellular (low immunogenicity) proteins have fewer T cell epitopes



EPX scores of 1,100 intracellular and 1,135 extracellular proteins graphed by percentile. EPX Score is calculated by aggregating the EpiMatrix scores of all predicted T-cell epitopes contained within a given protein sequence and adjusting for expected T-cell epitope content and protein length.

The difference between the two curves is statistically significant (Kolmogorov-Simirnoff test, D=.094, P<.003).