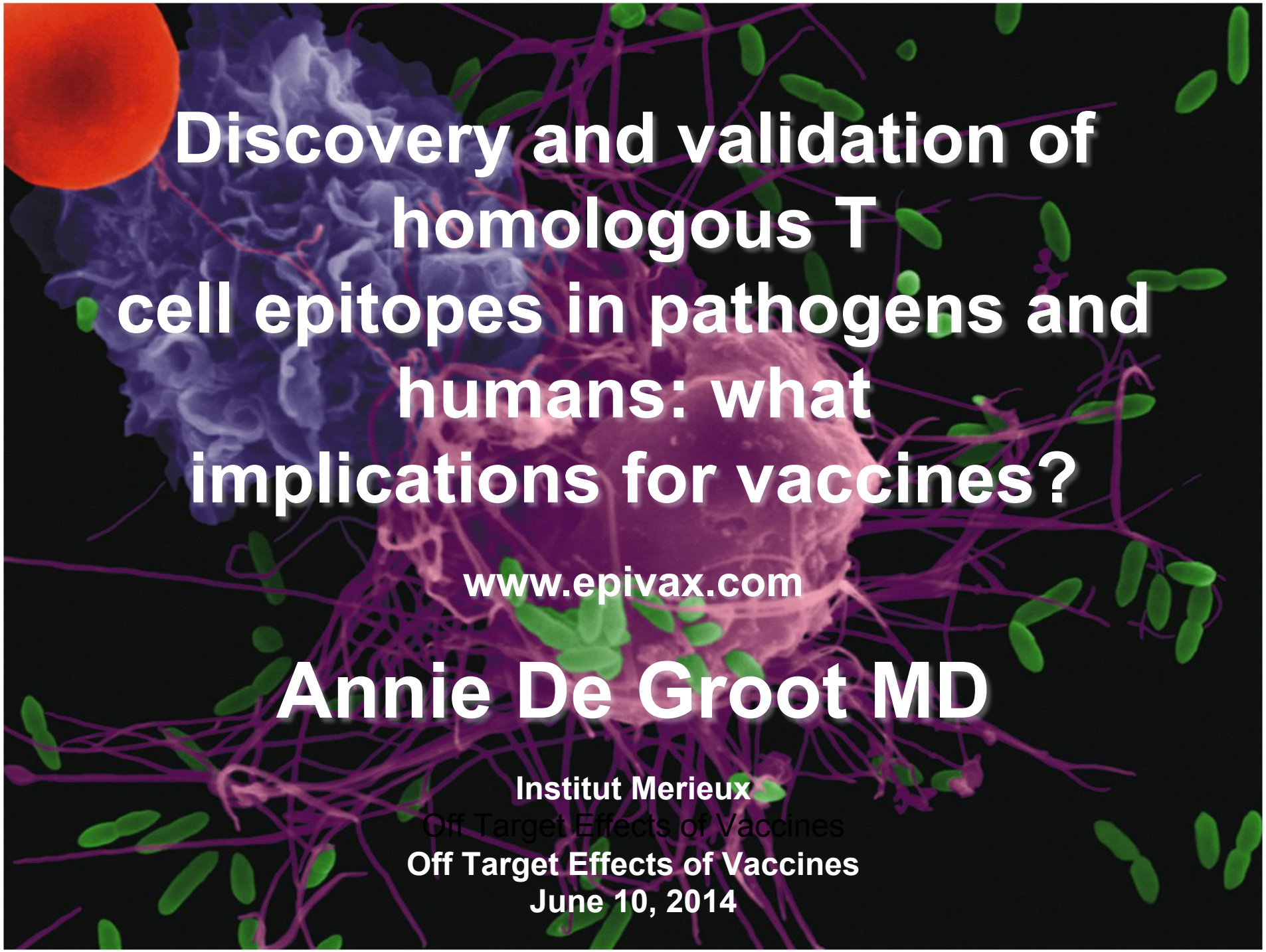

Discovery and validation of homologous T cell epitopes in pathogens and humans: what implications for vaccines?

www.epivax.com

Annie De Groot MD

Institut Merieux

Off Target Effects of Vaccines

Off Target Effects of Vaccines
June 10, 2014

Acknowledgements

Vish Mokashi
Biswajit Biswas

Ted Ross

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Manabu Ato
Yoshi Takahashi

AI058326,
AI058376,
AI078800,
AI082642

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Jinhee Lee
Liisa Selin

Sharon Frey
Mark Buller
Jill Schreiwer

Chris Bailey-Kellogg

Connie Schmaljohn
Lesley C. Dupuy

Drew Hannaman

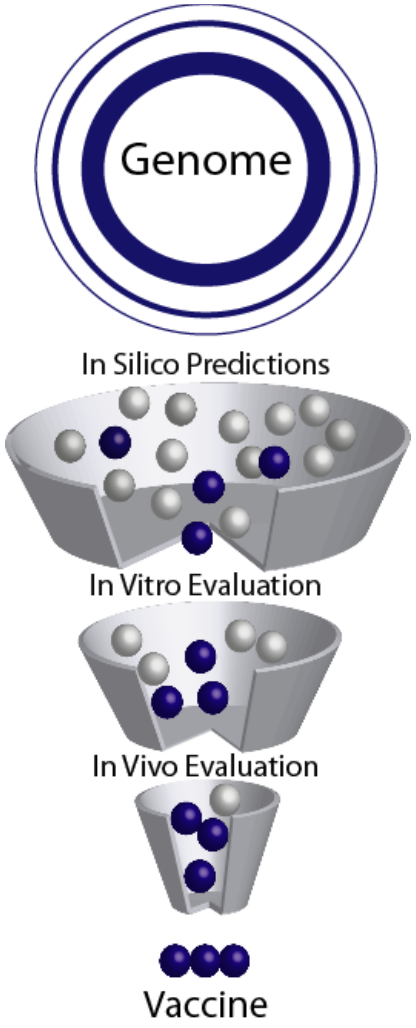
http://bit.ly/EpiPubs

A Public Private Partnership for Global Health

In 2008, URI established a new Institute devoted to applying the EpiVax suite of vaccine design tools to basic vaccine research and development of vaccines for global infectious diseases, primarily neglected tropical diseases.

The Institute operates under an MOU with EpiVax and most projects involve collaborative work. IP that is generated for NTD/other topics is jointly owned.

Outline

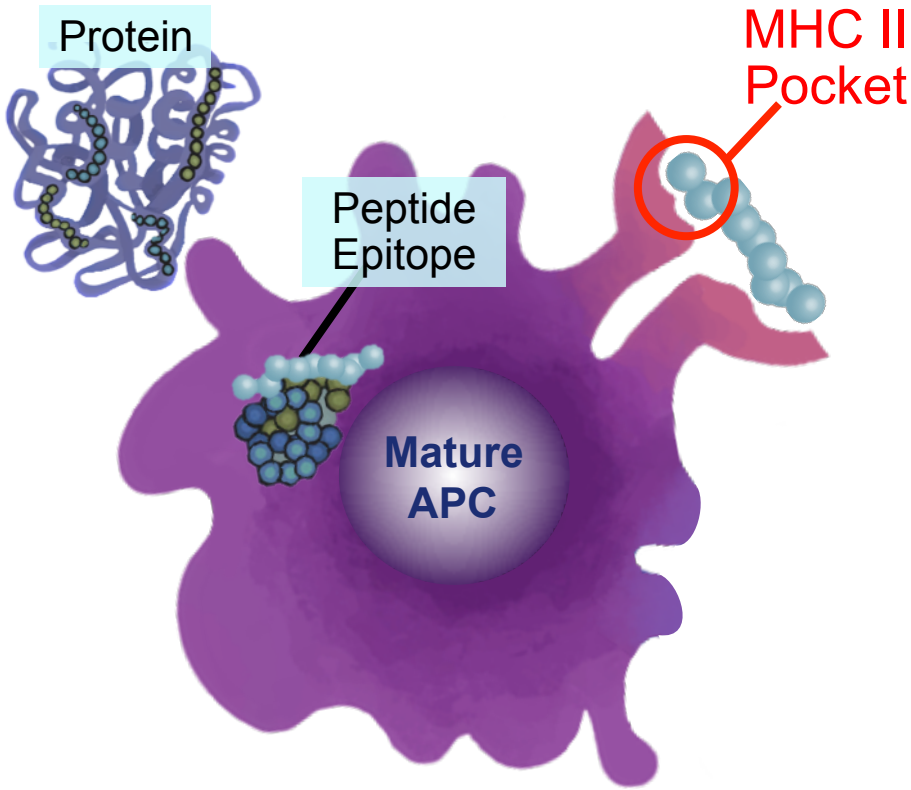


- Epitope mapping basics
- Conserved T cell epitopes may be protective
- Delving deeper – host-pathogen homology
- Engineered antigens for improved efficacy
- Relevance to this topic?

Cloud-based vaccine design

http://bit.ly/EpiPubs

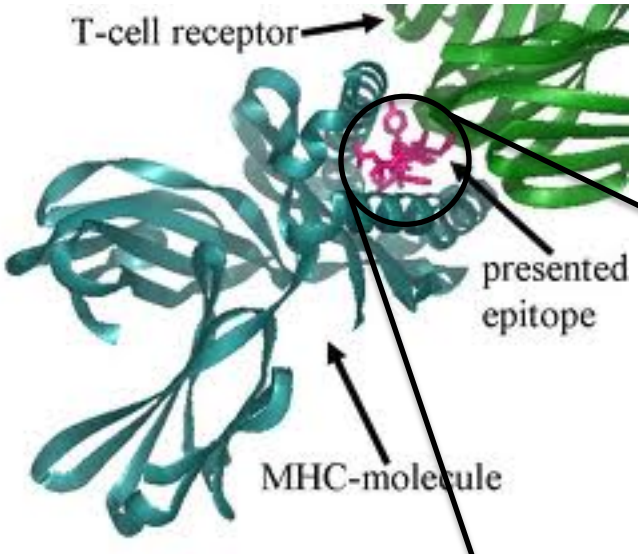
T cell epitopes are linear peptides



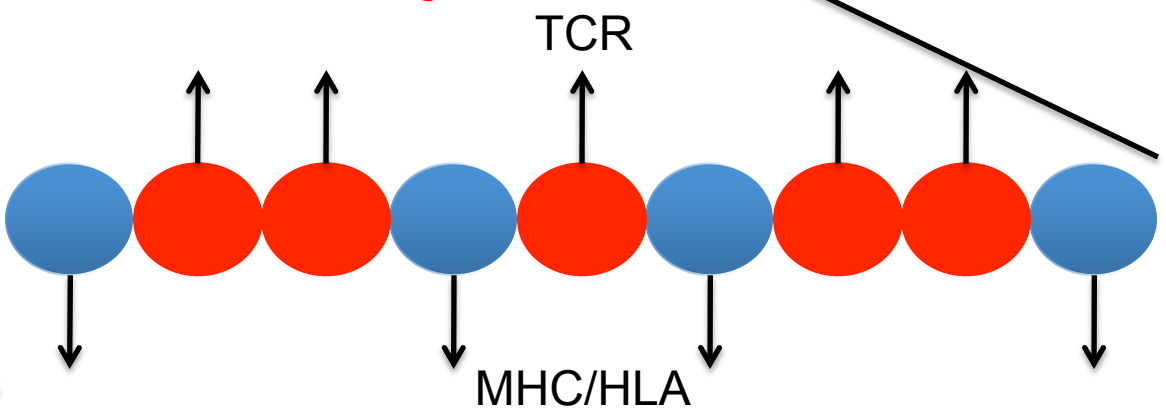
T cell epitopes are linear and directly derived from antigen sequence

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

Predicting T cell epitopes



- TCR facing residues

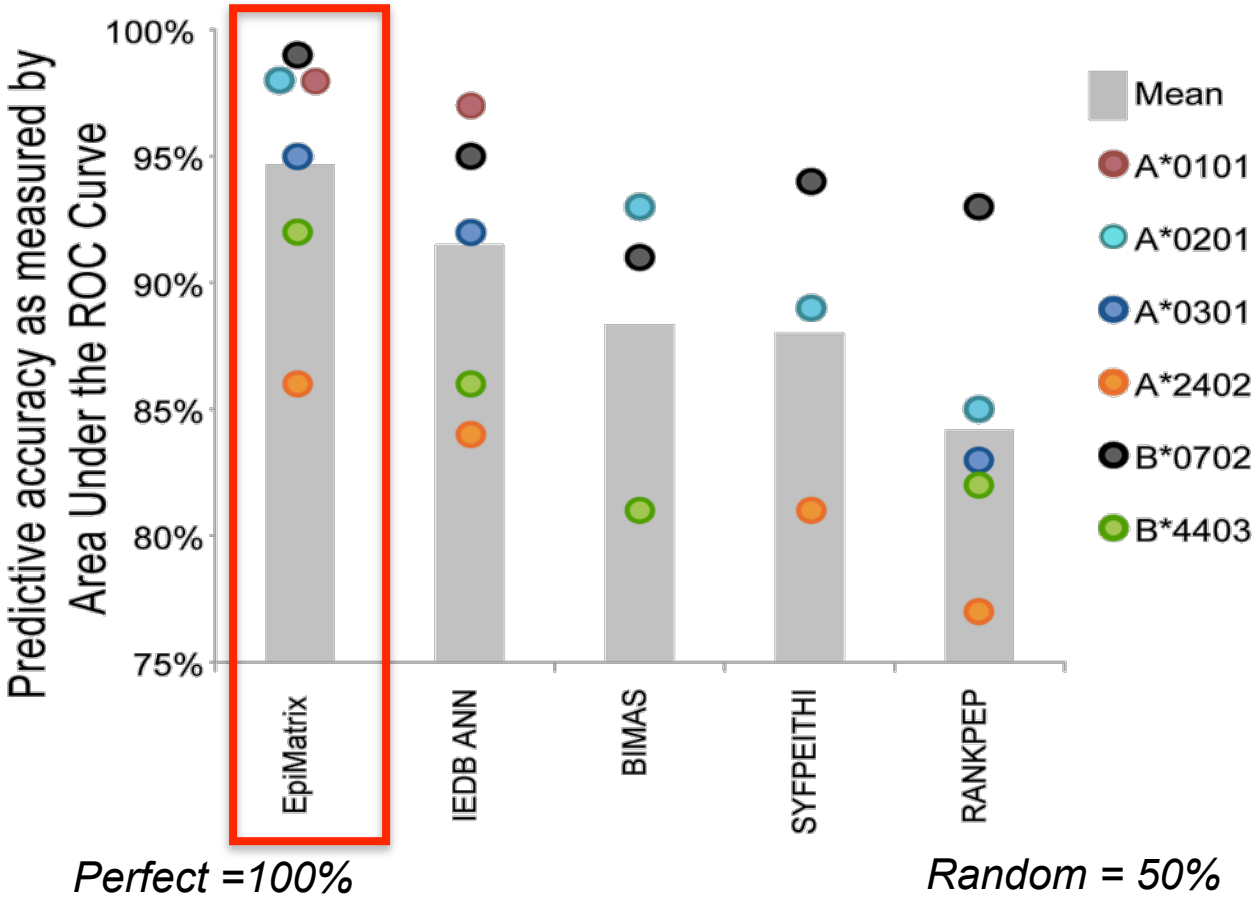


- HLA facing residues

Epitope Identification – "Class I"

Highly Accurate T cell Epitope identification

Accuracy of Class I prediction is nearly 100% for CTL epitopes

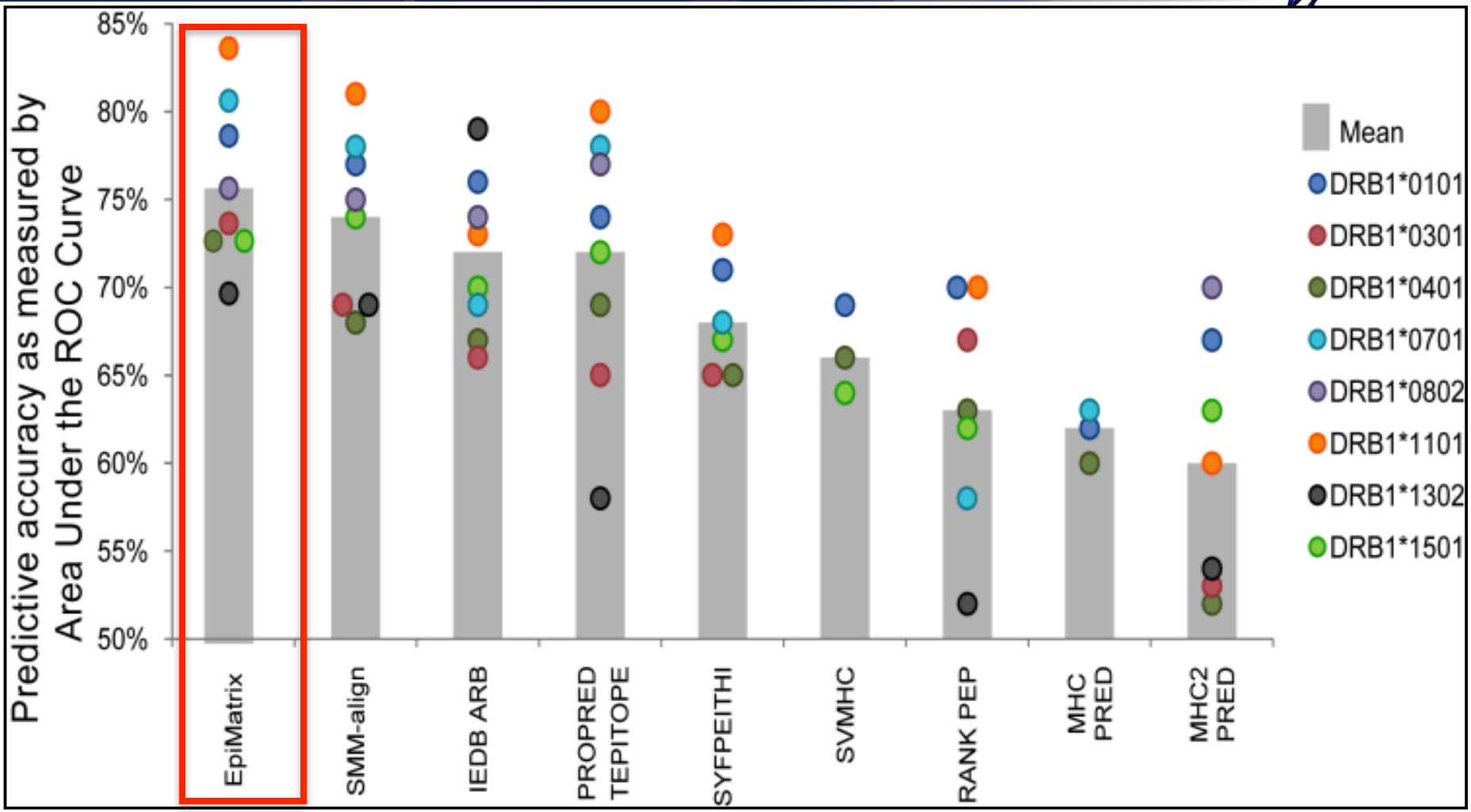


De Groot AS, Martin W. Clin Immunol. 2009 May;131(2): 189-201

Comparison Data Taken From: Wang P, Sidney J, Dow C, Mothé B, Sette A, et al. (2008) PLoS Comput Biol 4(4): e1000048.

Epitope Identification

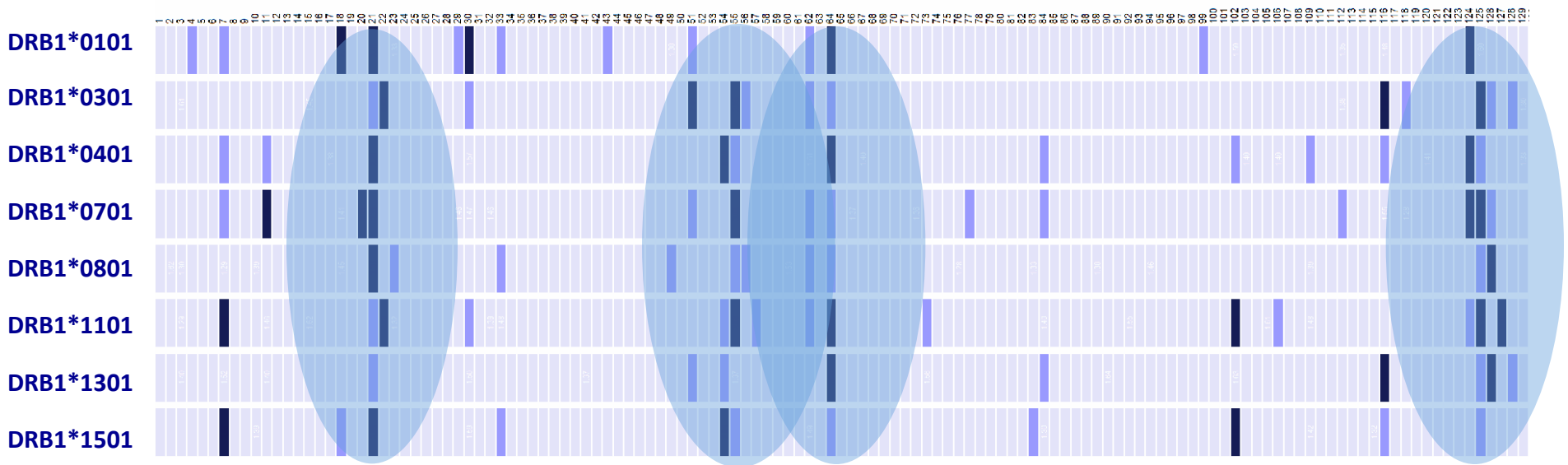
Class II is highly accurate as well – algorithms are improving



De Groot and Martin. Reducing risk, improving outcomes: Bioengineering less immunogenic protein therapeutics. *Clinical Immunology* 2009. 131, 189-201.

Clustered or Promiscuous Epitopes

- T cell epitopes are not randomly distributed but instead tend to **cluster** in specific regions.
  - These clusters can be very powerful, enabling significant immune responses to low scoring proteins.
- T-cell epitope clusters make excellent vaccine candidates:
  - compact; relatively easy to deliver as peptides; highly reactive in-vivo



Epitope Math

Immune Response to a Protein = Sum of Epitopes

Vaccine

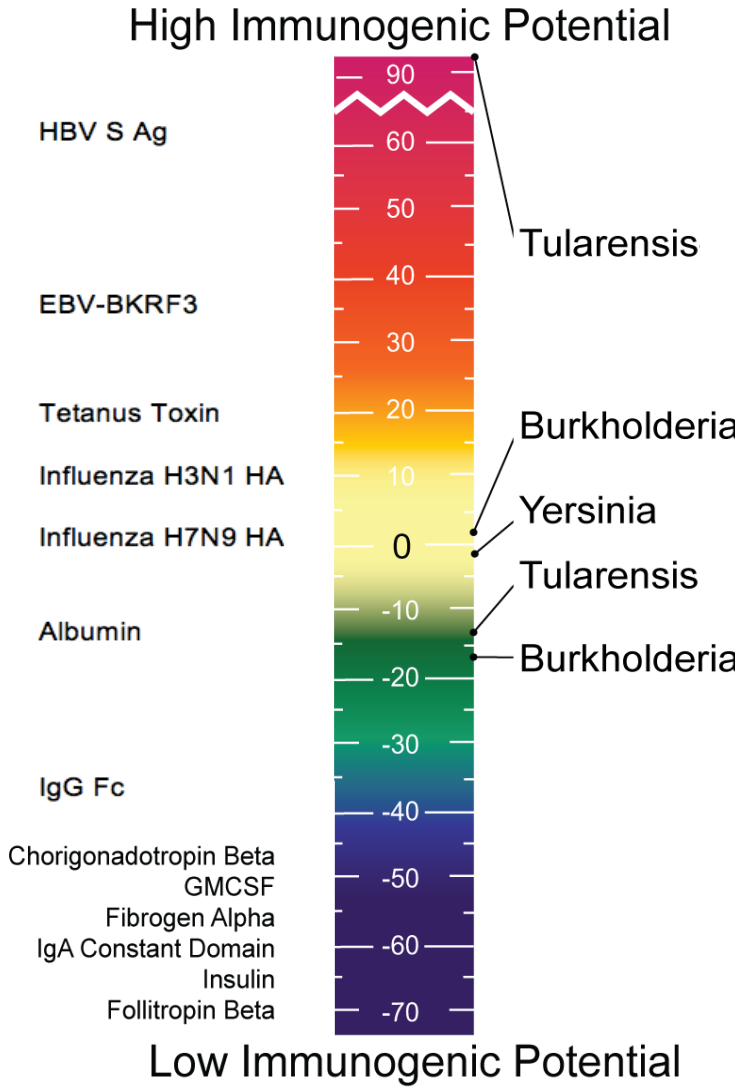
1 + 1 + 1 = Response

Immunogenicity Scale

The number of T cell epitopes contained in the sequence of a protein contributes to its immunogenicity

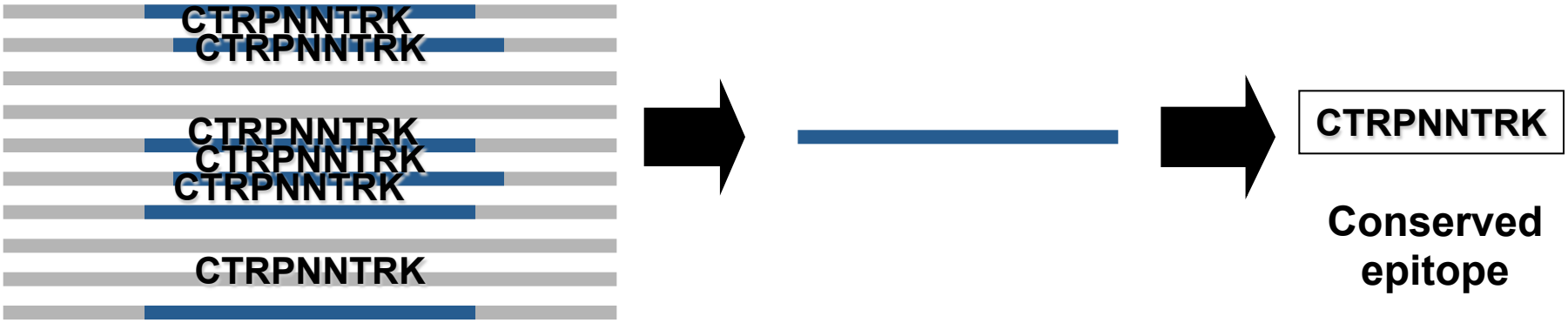
Proteins can be compared based on T cell epitope content per unit sequence.

Use tools to rank antigens for Vaccine candidates.



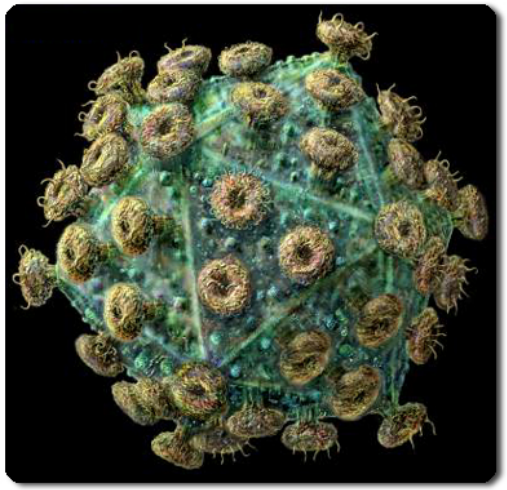
http://bit.ly/EpiPubs

Conservatrix Finds Conserved 9-mers

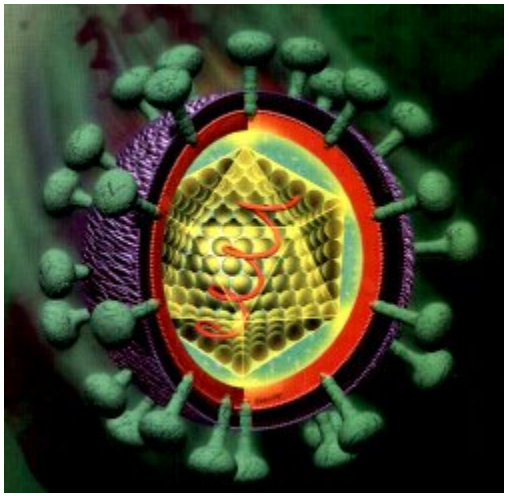


Identifying the most conserved 9-mers allows for protection against more strains with fewer epitopes

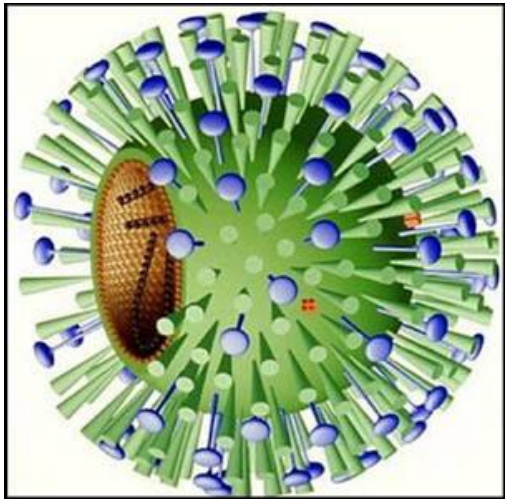
Conservatrix – finds conserved epitopes: Overcome the Challenge of Variability



HIV



HCV



Influenza

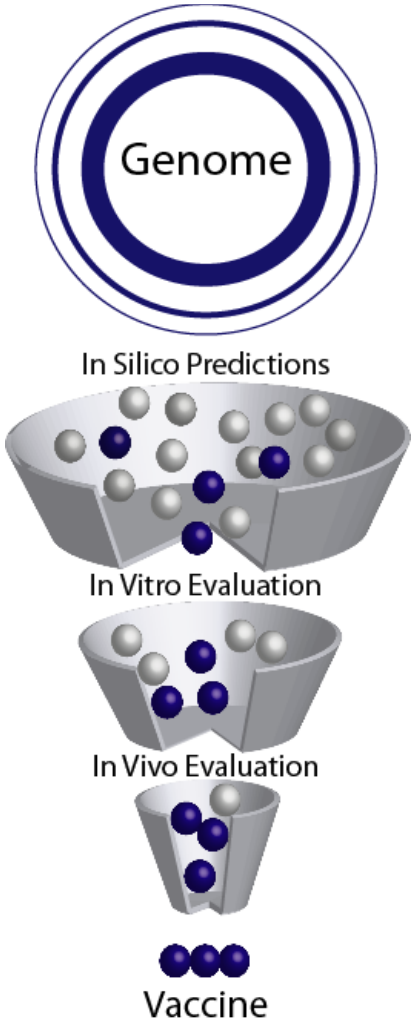
Conserved epitopes may be related to function;

Conserved epitopes may be related to structure;

Conserved epitopes are the Achilles heel of a variable pathogen . . .

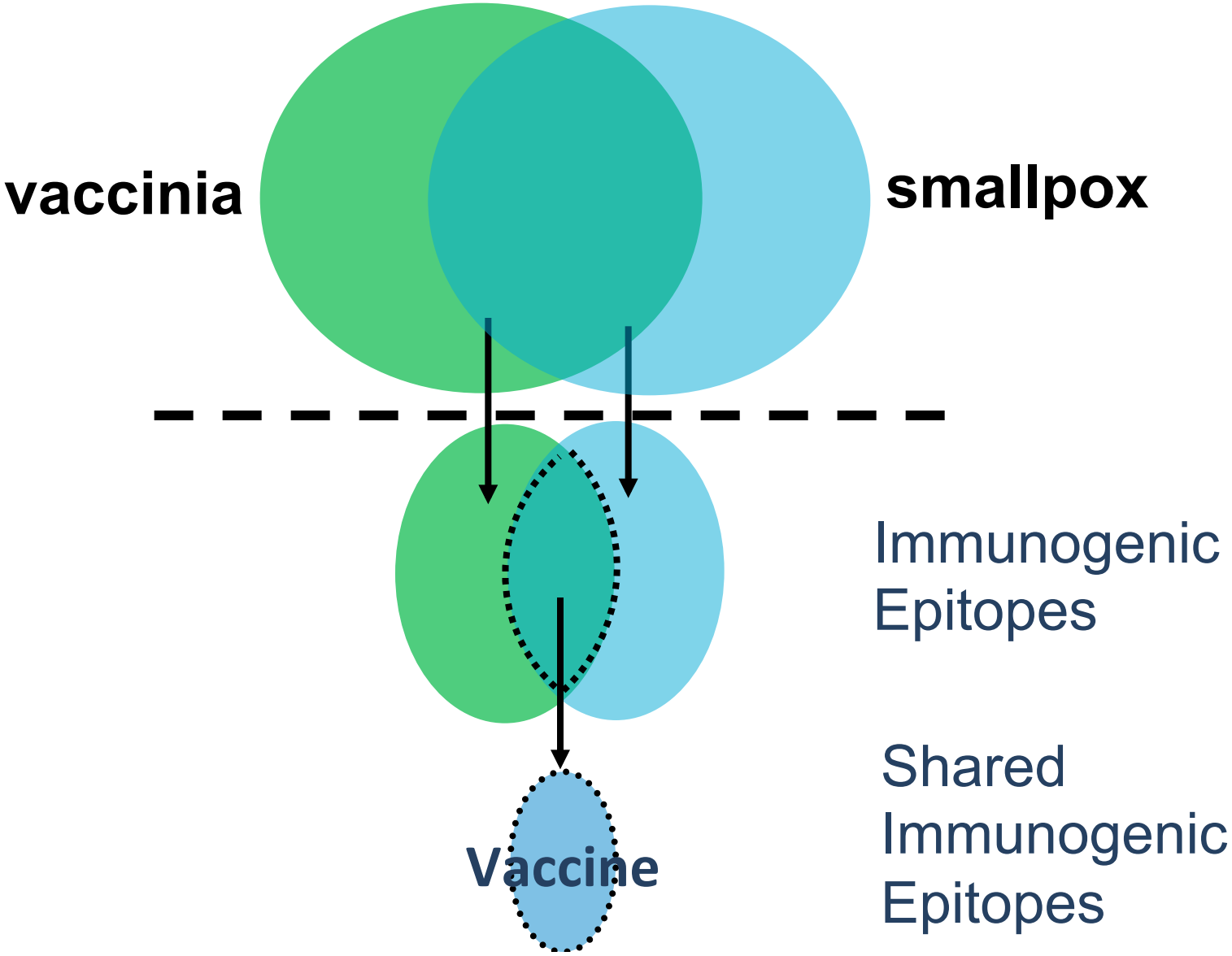
Or . . . . . **Conserved epitopes may engage a response that *helps the pathogen*.**

Outline

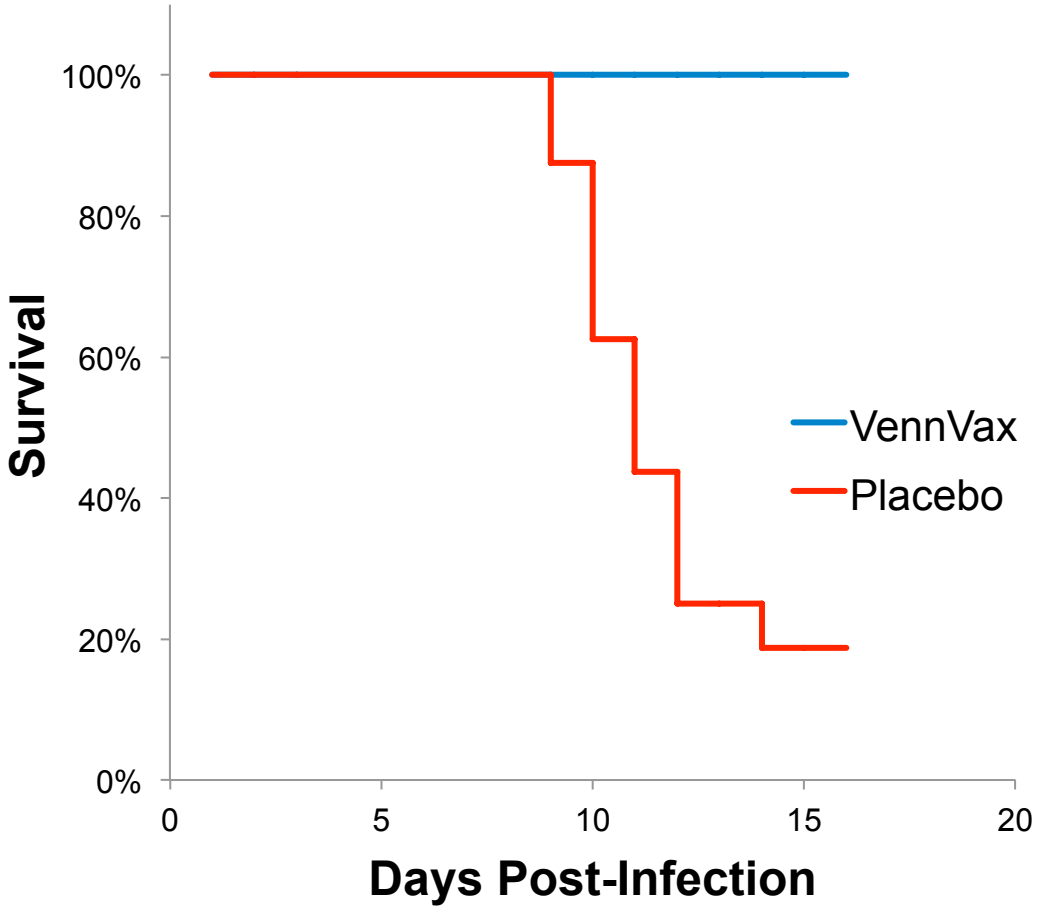


- Epitope mapping basics
- Conserved T cell epitopes may be protective
- Delving deeper – host-pathogen homology
- Engineered antigens for improved efficacy

Immunome-Derived Smallpox Vaccine: VennVax



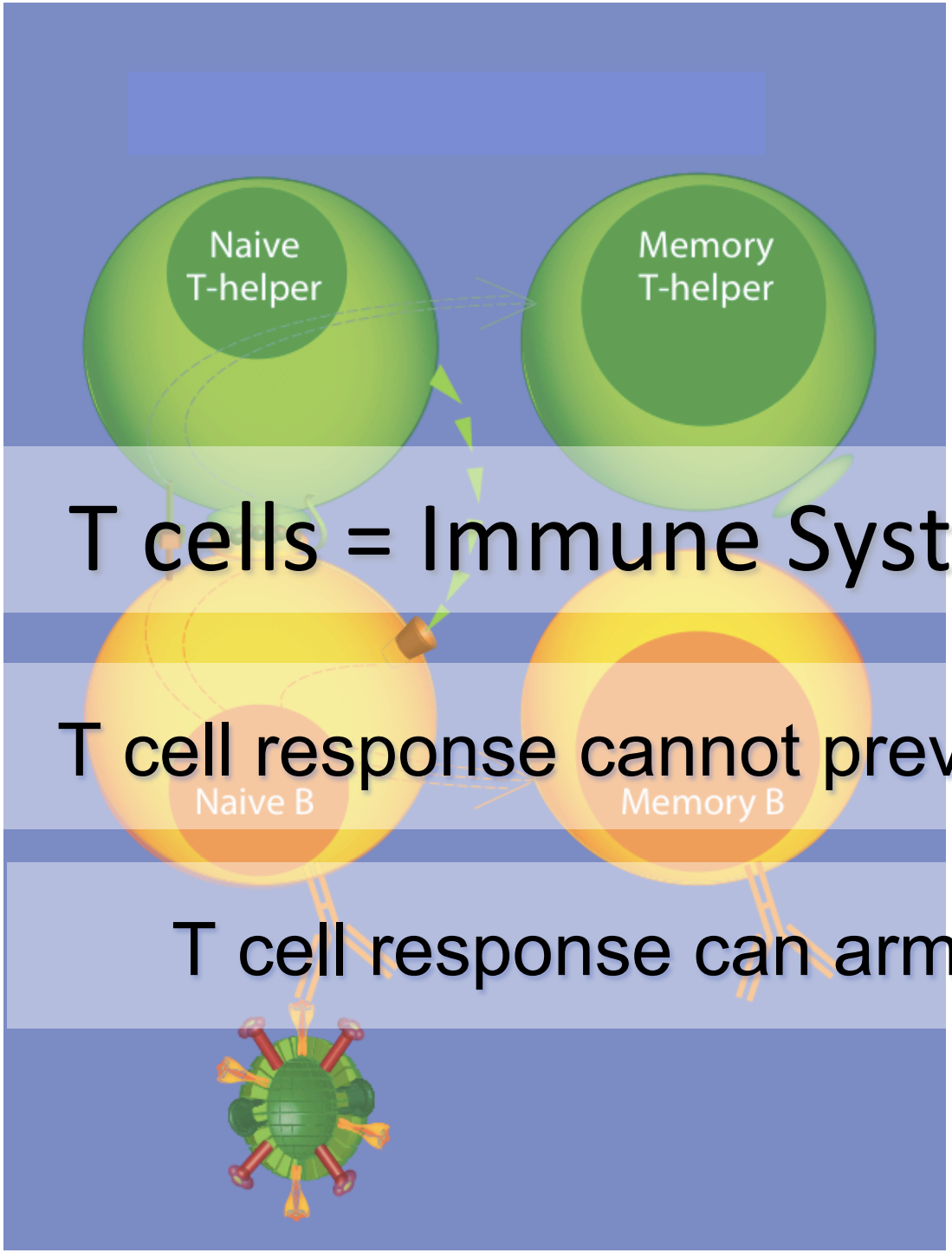
VennVax protects against lethal infection



Infection: intranasal Vaccinia WR 10X LD_{50}

- VennVax stimulates epitope-specific Th1 cytokine responses
- Epitope-specific responses observed in HLA DR3 and DR4 mice
- Complete protection against lethal infection
- Protection *without* vaccine-induced antibody

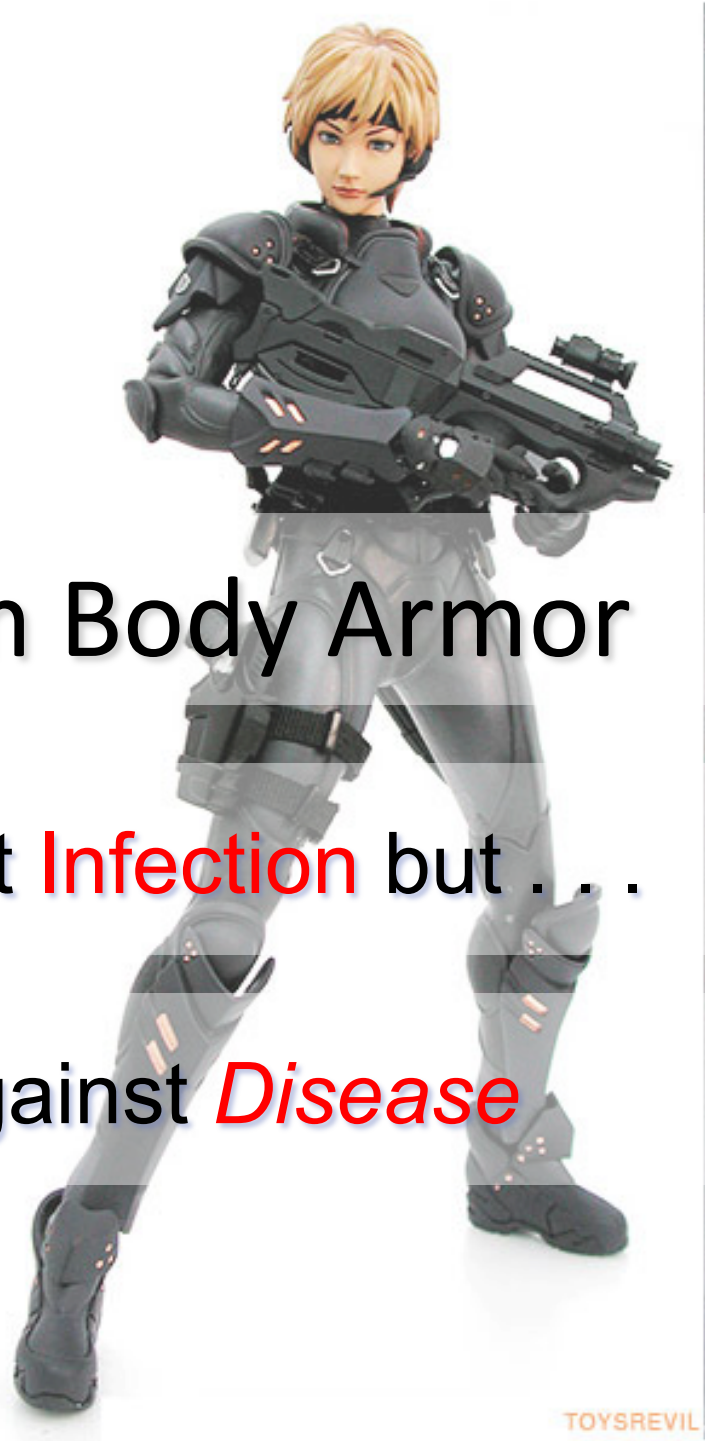
Moise L *et al. Vaccine* 2011;29:501-11



T cells = Immune System Body Armor

T cell response cannot prevent Infection but . . .

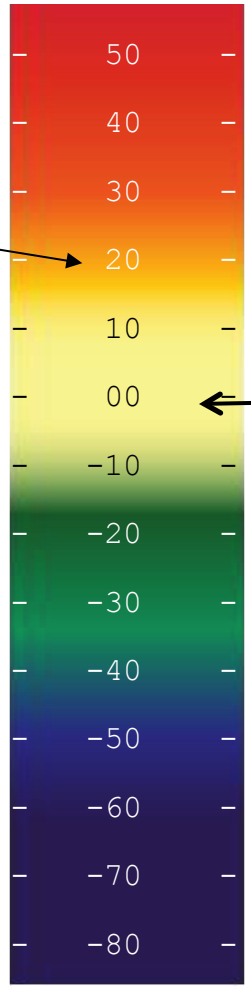
T cell response can arm against *Disease*



Testing the hypothesis: New H1N1 Pandemic is Predicted to be

Highly IMMUNOGENIC

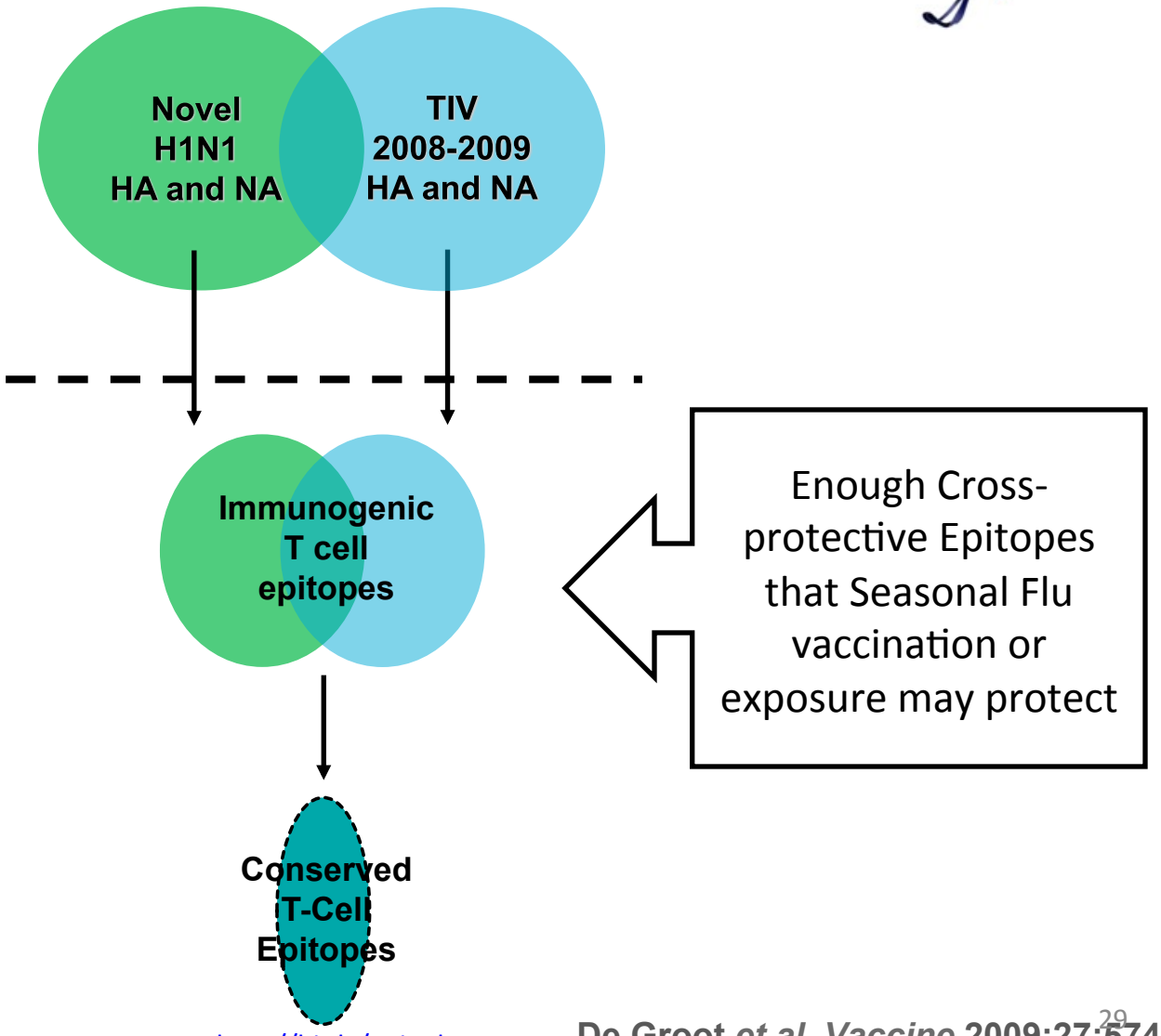
HA A/California/ 07/2009 (H1N1)



- T cell epitope content of H1N1 2009 HA is high
- T cell epitopes in HA are highly cross-conserved with H1N1 Brisbane
- Despite lack of Ab cross-reactivity, we predicted protection against severe disease
- H1N1 HA predicted to be highly immunogenic

http://bit.ly/Pandemic_H1N1

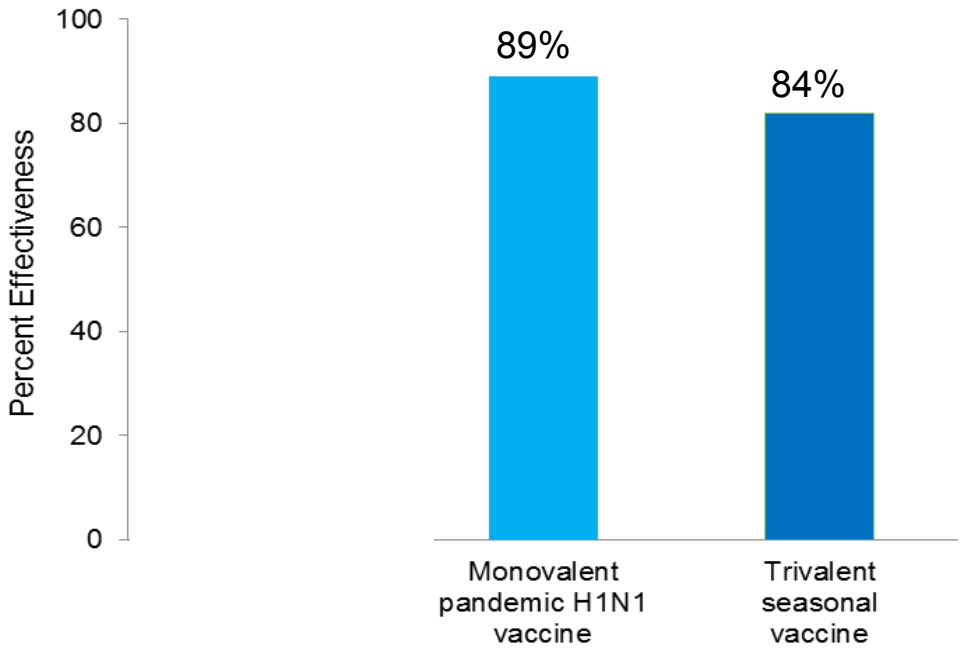
2009 H1N1 contains conserved epitope Sequences – Predicted Cross Protection



http://bit.ly/EpiPubs

De Groot *et al. Vaccine* 2009;27:5740-7

October 2010: New H1N1 Vaccines are CONFIRMED to be Highly IMMUNOGENIC

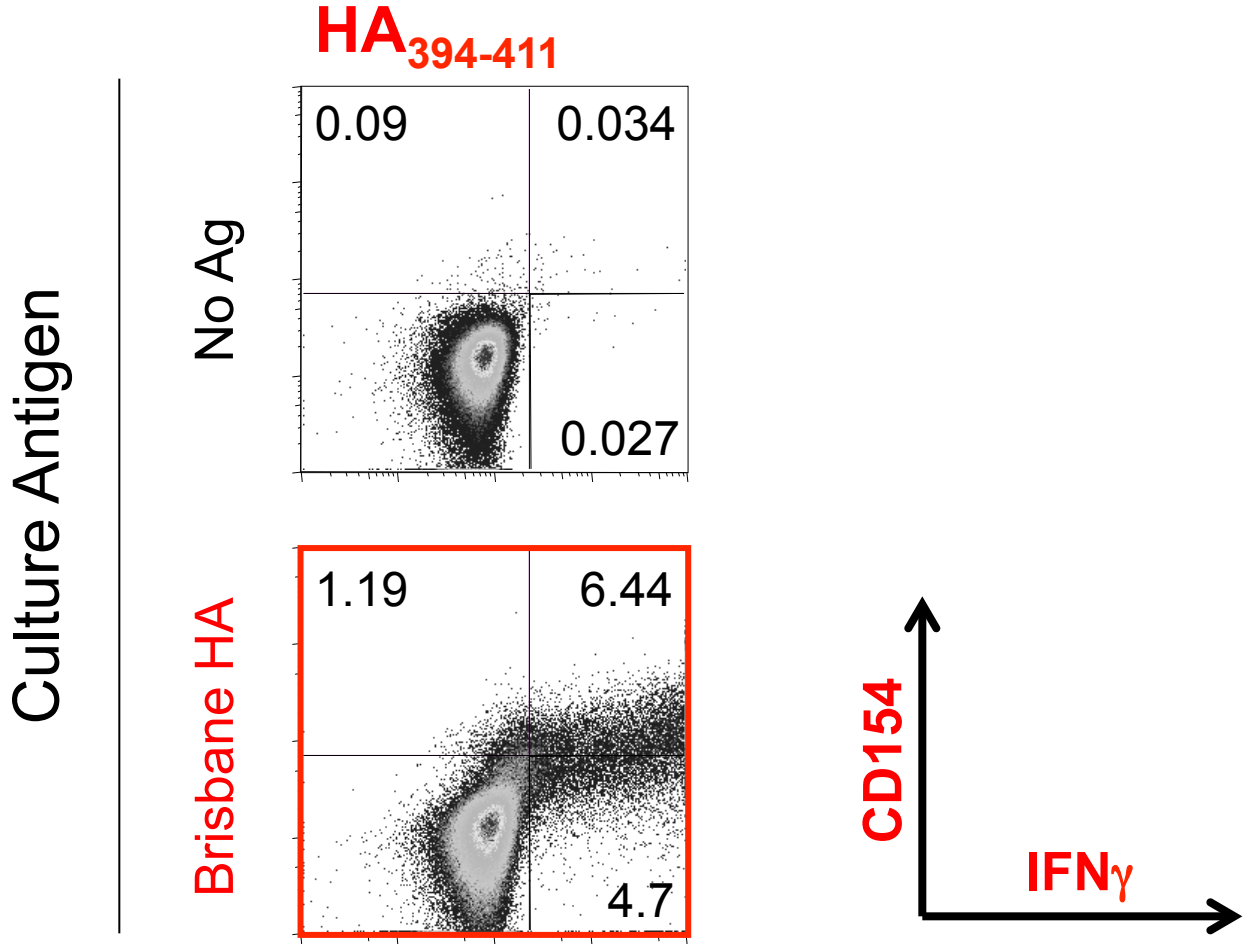


Griffin MR et al. Plos One 2011 Aug 6(8);

Goodwin et al. Vaccine 2006(24):1159-1169; CDC MMWR September 20, 2013/ 62(RR07); 1-43

X-cons'd Epitopes are Antigenic

"Immunized" with Brisbane HA whole Flu vaccine - Response to 2009 CA T cell epitopes



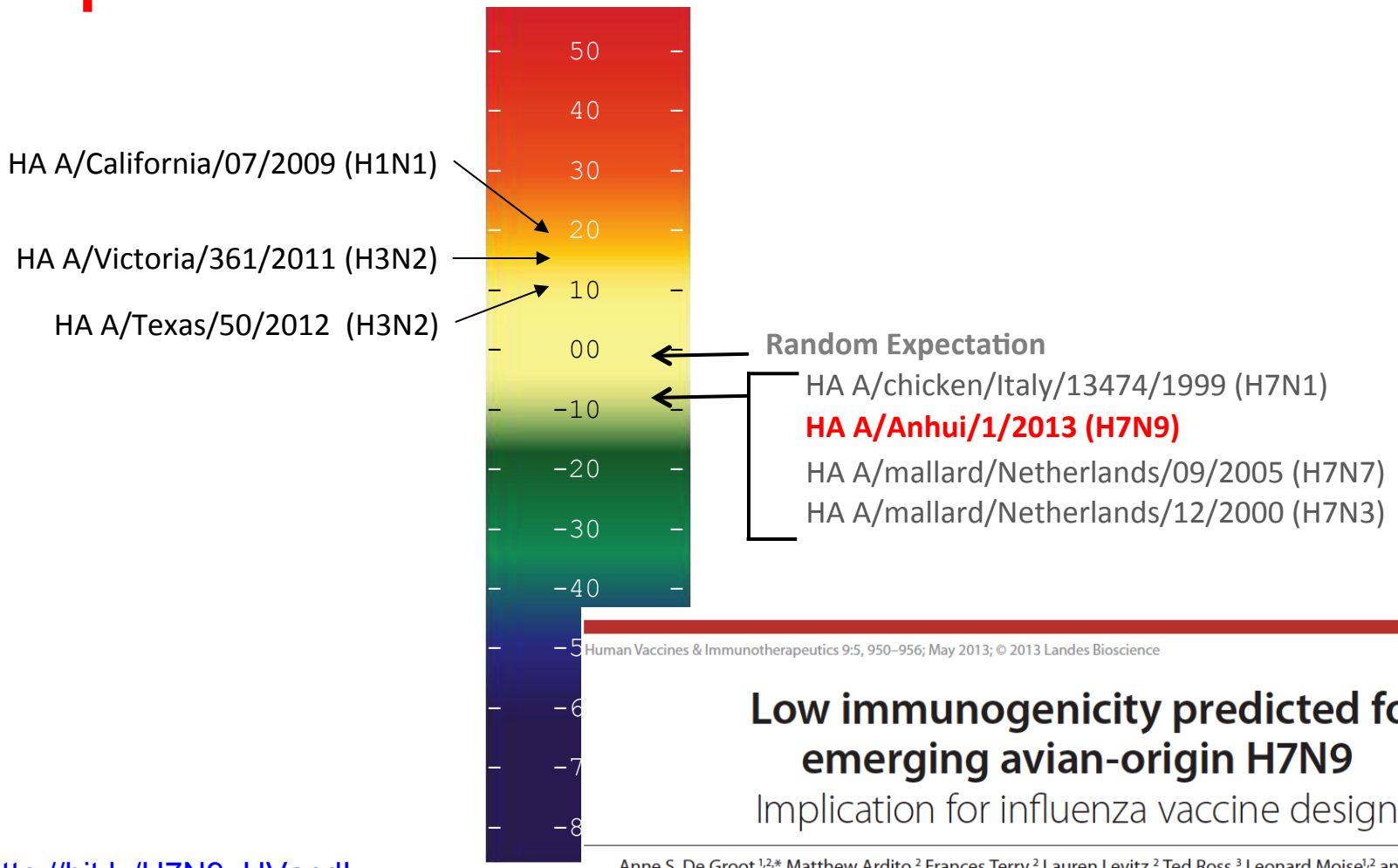
VAXDESIGN

Schanen *et al. Vaccine* 2011;29:3299-309

H1N1 Pandemic Flu
Would exposure to Brisbane be sufficient?

- H1N1 pandemic April- December 2009.
  - CDC announced that antibodies were not cross-protective
  - H1N1 rapidly spreading – significant concern
  - But T cell epitopes were cross-conserved with H1N1 Brisbane
  - Despite lack of Ab cross-reactivity, we predicted protection against severe disease
  - H1N1 HA predicted to be highly immunogenic.
- Was protection due to X-Conserved T epitopes?
  - pH1N1 Highly transmissible (no Ab protection)
  - But Morbidity and Mortality substantially lower than expected (good T cell protection?)
  - Subsequent studies validated this hypothesis in animal models.

In Contrast - - H7N9 Flu Predicted to be POORLY IMMUNOGENIC in April 2013



Human Vaccines & Immunotherapeutics 9:5, 950–956; May 2013; © 2013 Landes Bioscience

Low immunogenicity predicted for emerging avian-origin H7N9

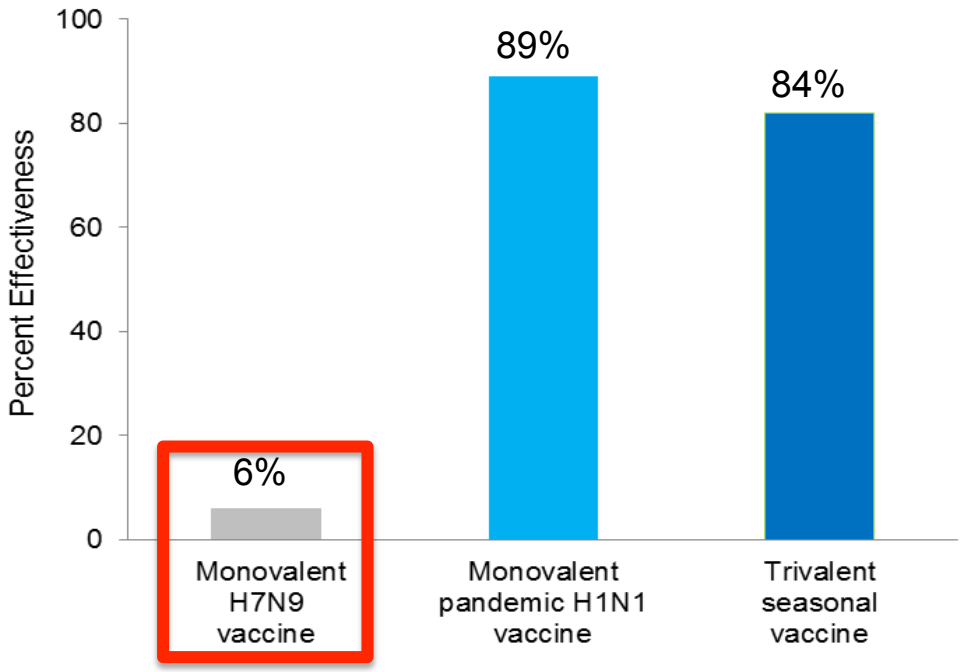
Implication for influenza vaccine design

Anne S. De Groot,[1,2,*] Matthew Ardito,[2] Frances Terry,[2] Lauren Levitz,[2] Ted Ross,[3] Leonard Moise[1,2] and William Martin[2]

[1]Institute for Immunology and Informatics; University of Rhode Island; Providence, RI USA; [2]EpiVax, Inc.; Providence, RI USA; [3]Vaccine and Gene Therapy Institute of Florida; Port St. Lucie, FL USA

http://bit.ly/H7N9_HVandI

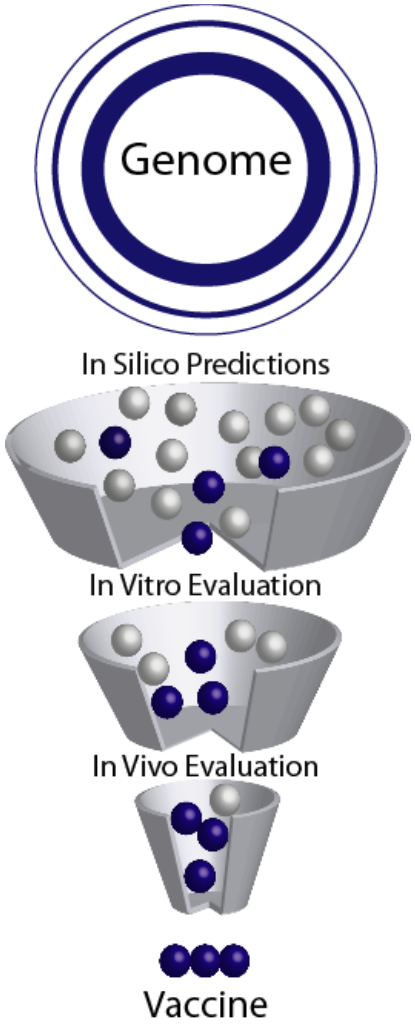
October 2013: New H7N9 Vaccines are CONFIRMED to be POORLY IMMUNOGENIC



Griffin MR et al. Plos One 2011 Aug 6(8);

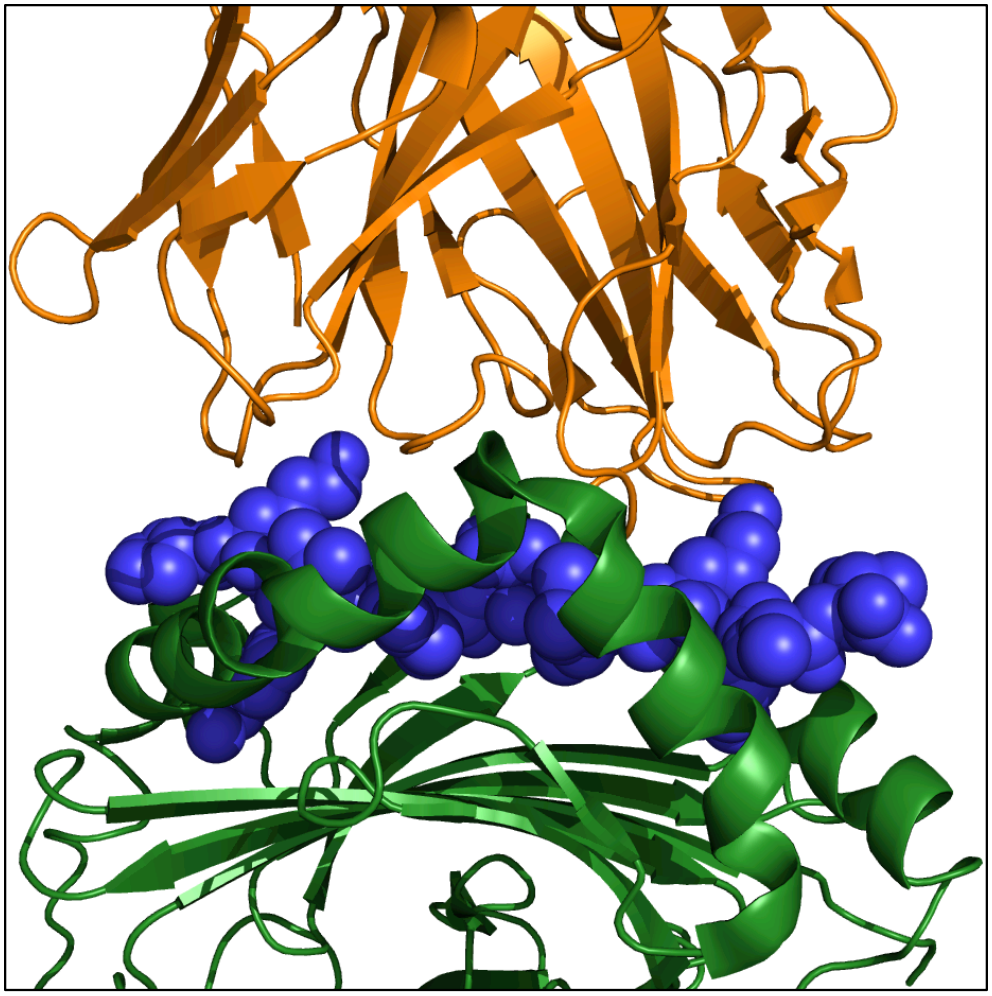
Goodwin et al. Vaccine 2006(24):1159-1169; CDC MMWR September 20, 2013/ 62(RR07); 1-43

Outline



- iVAX platform basics
- Immunoinformatic-driven T cell epitope vaccine illustrated
- Delving deeper – host-pathogen homology
- Engineered antigens for improved efficacy

The Two-Faced Epitope

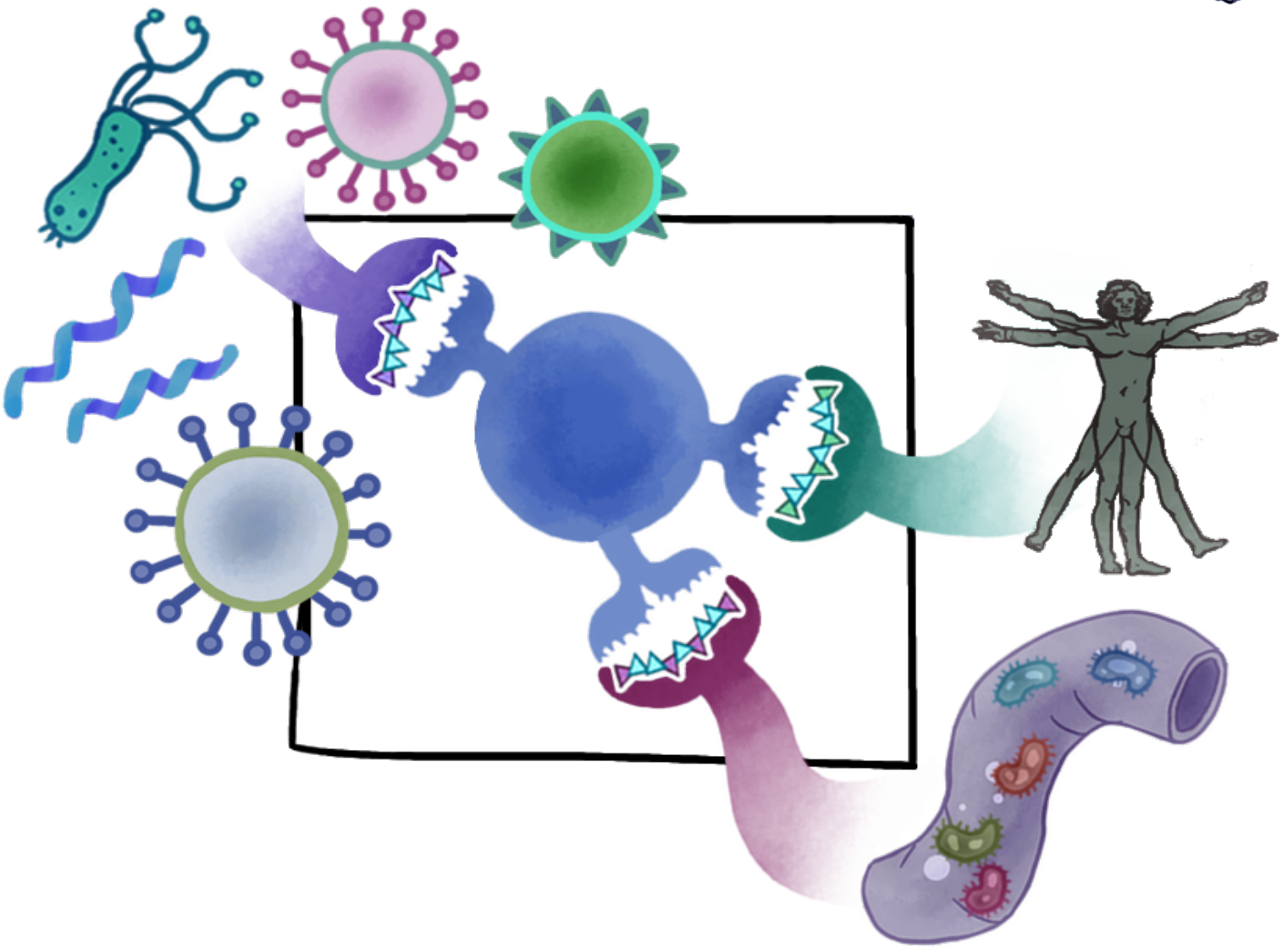


T cell receptor

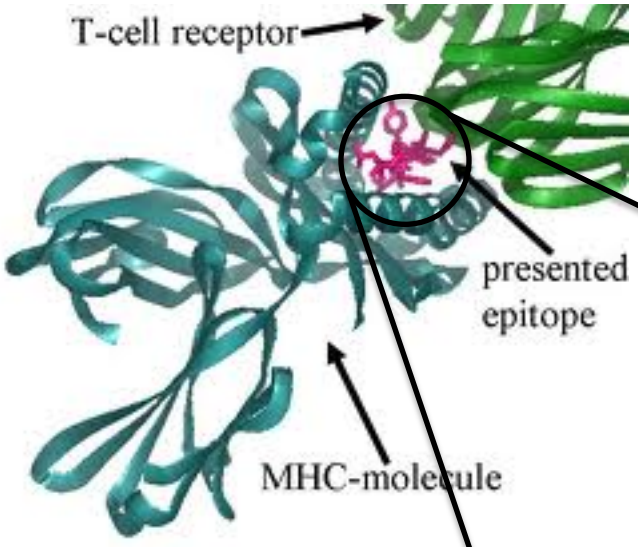
T cell (peptide) epitope

MHC

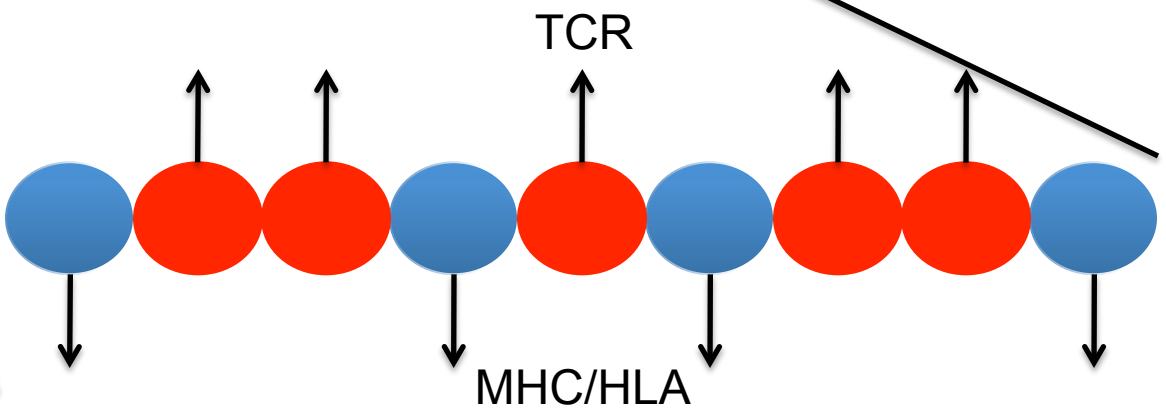
Immune Camouflage?



JanusMatrix

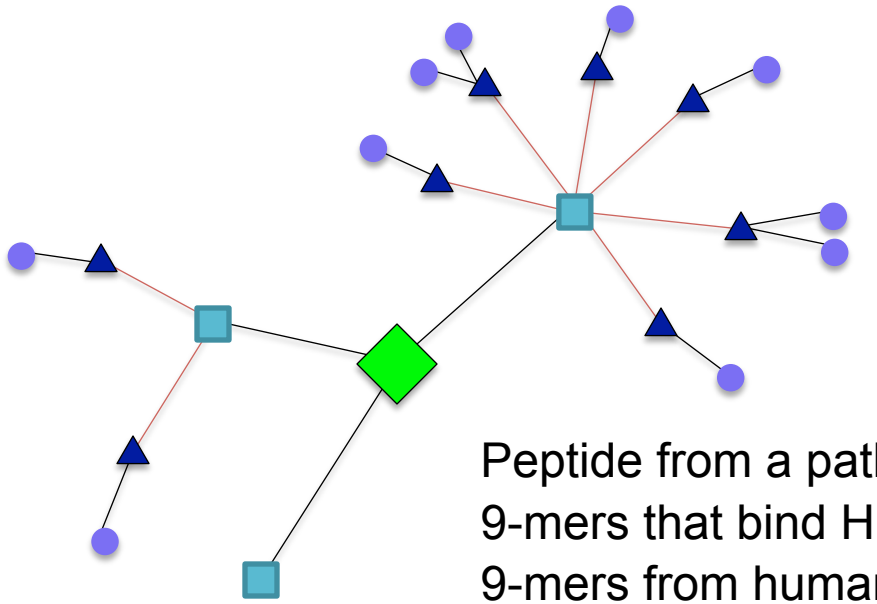


- Identical T cell-facing residues
- Same HLA allele and minimally different MHC-facing residues



Moise et al. **The Two-Faced T cell Epitope: Examining the Host-Microbe Interface with JanusMatrix.** Hum Vaccin Immunother. 2013;9(7):1577-86. http://bit.ly/JanusMatrix.

Networks of cross-conservation



Peptide from a pathogen

9-mers that bind HLA

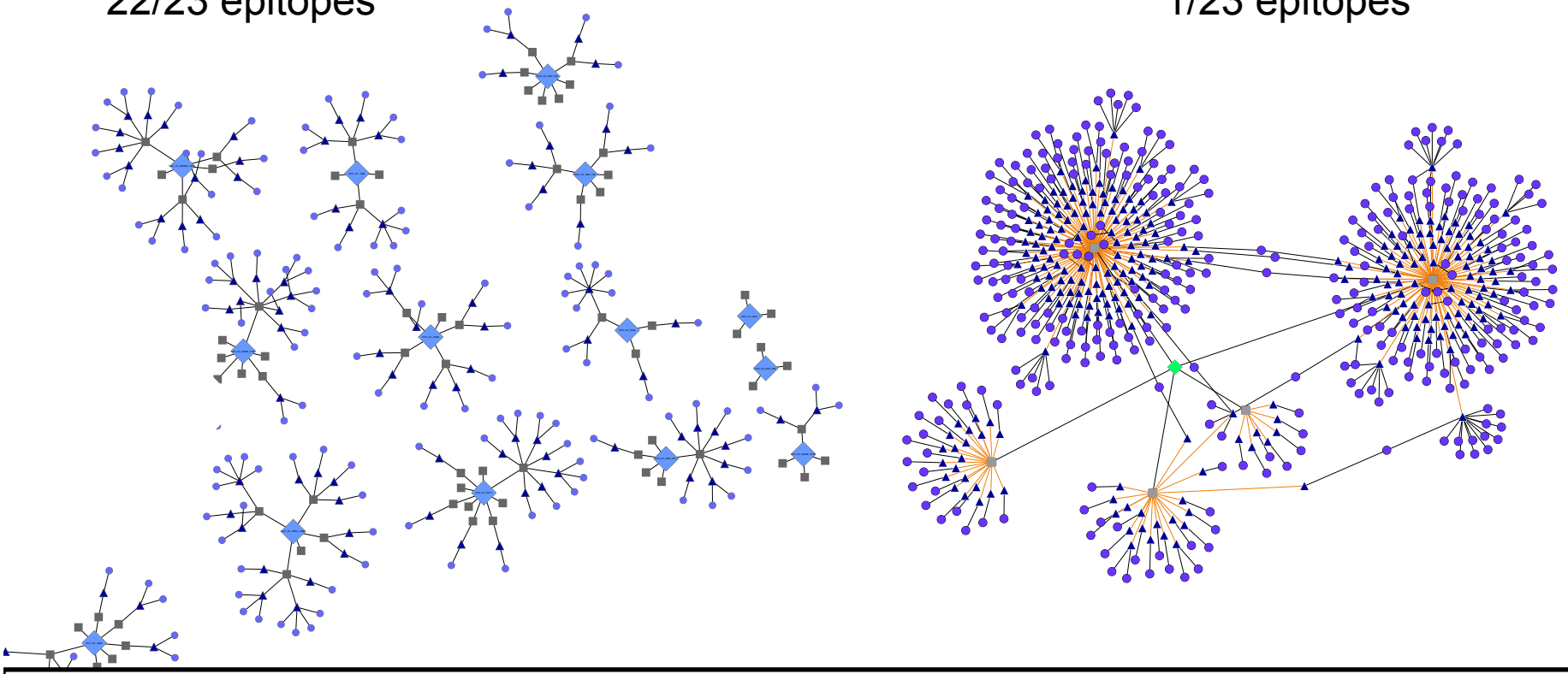
9-mers from human genome that present same TCR face

Source proteins of the human 9-mers

Example: HCV Vaccine Project
Cross-reactivity for 23 epitopes

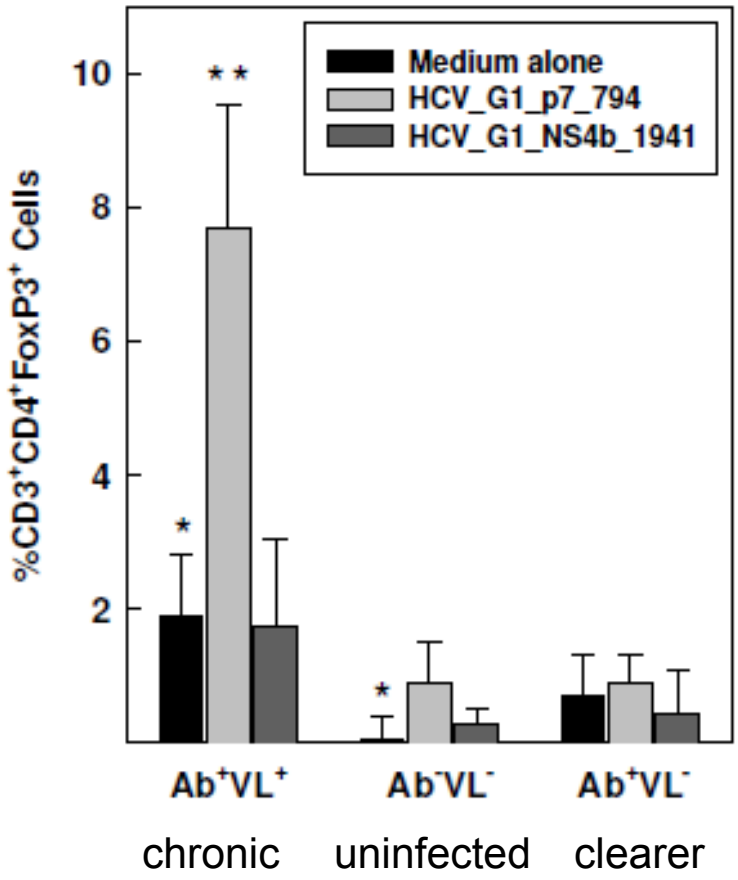
22/23 epitopes

1/23 epitopes



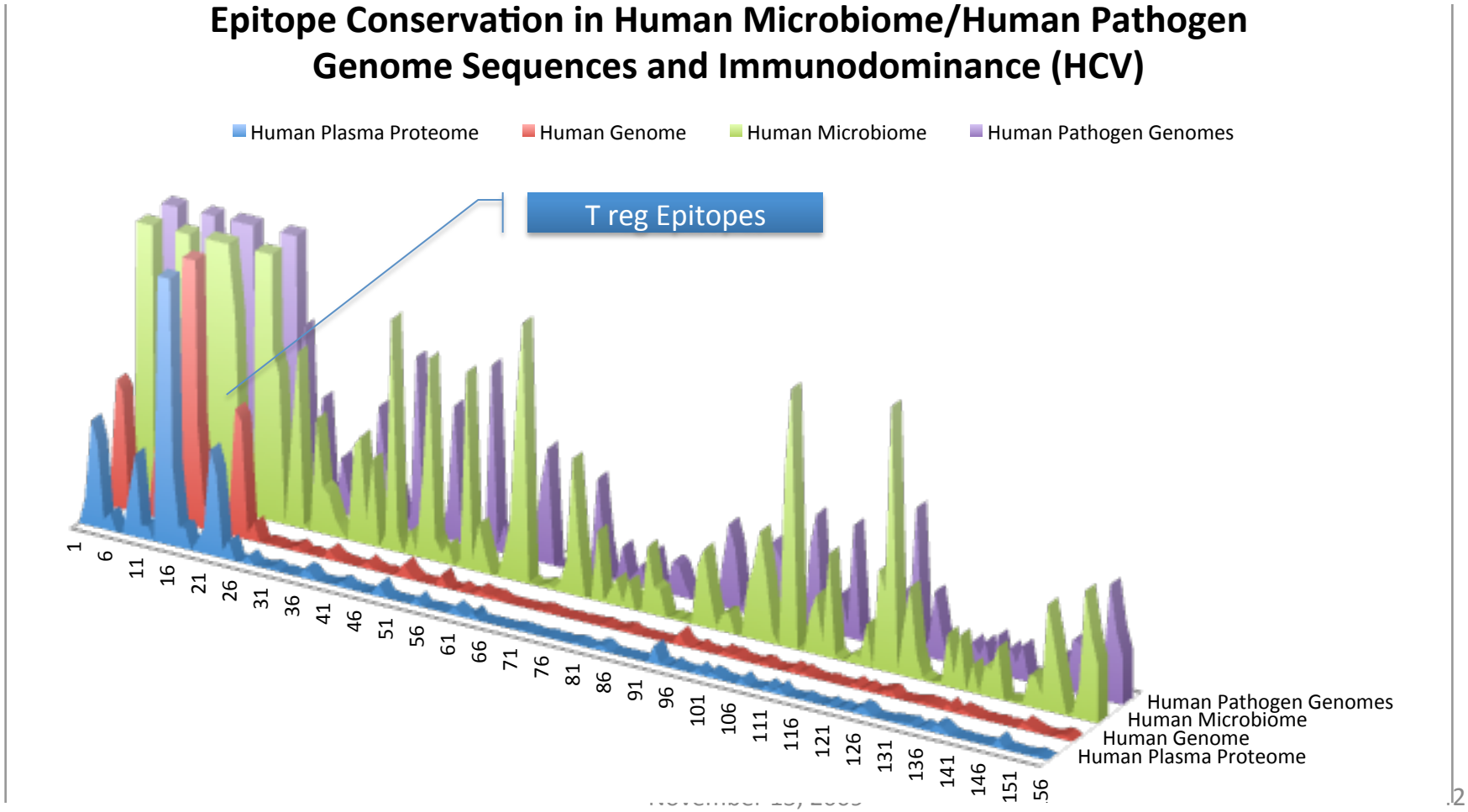
Losikoff PT, Mishra S, Terry F, Gutierrez A, Ardito MT, Fast L, Nevola M, Martin WD, Bailey-Kellogg C, De Groot AS, Gregory SH. **HCV Epitope, Homologous to Multiple Human Protein Sequences, Induces a Regulatory T Cell Response in Infected Patients.** J Hepatol. 2014 Aug 22. pii: S0168-8278(14)00613-8.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

HCV Tregitope stimulates Tregs in chronic infection

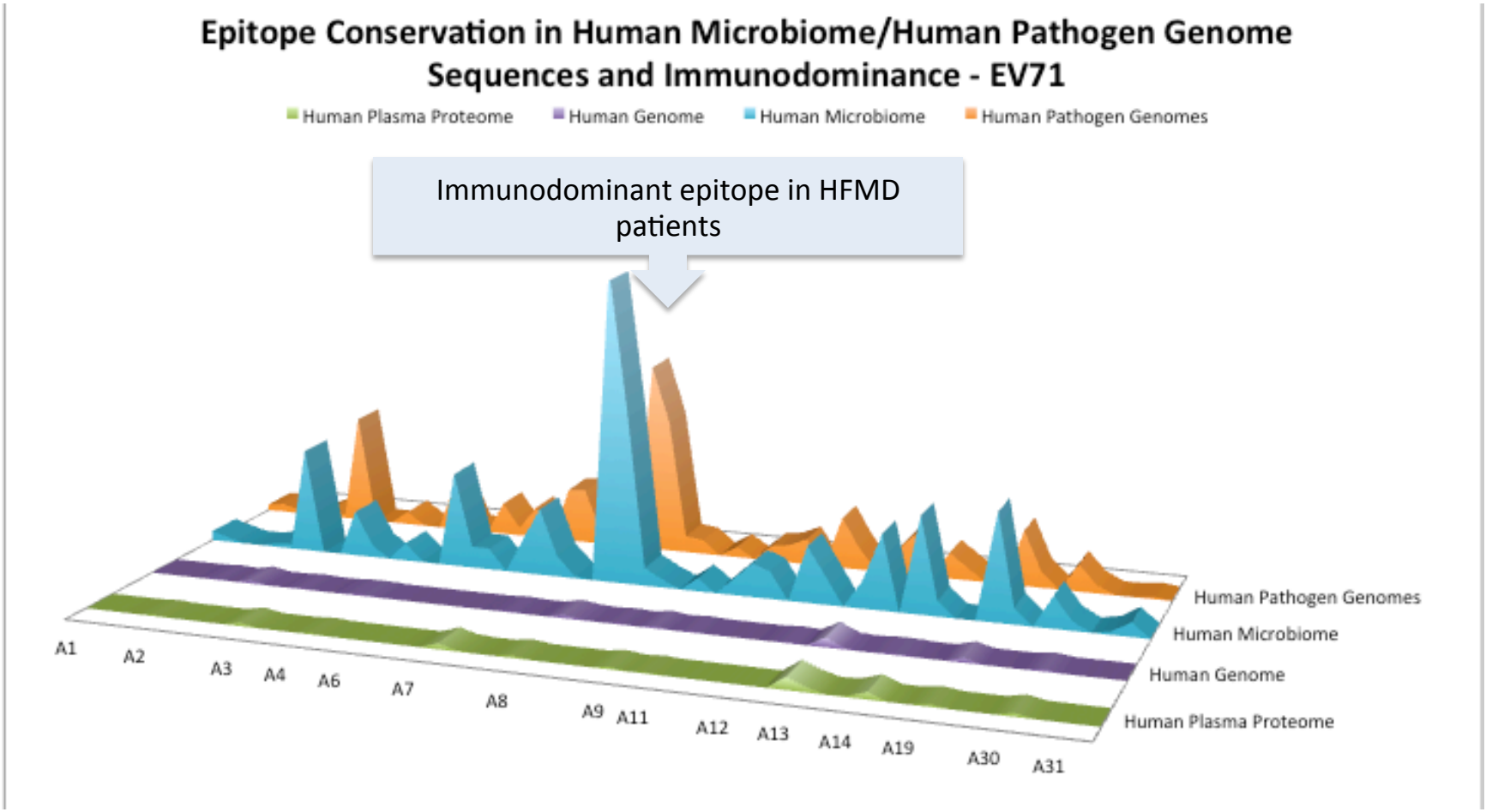


Losikoff *et al. J Hepatol*. 2015 62:48-55

HCV Treg vs T effector: TCR-facing Conservation with Human



EV71 (HFMD) TCR-facing Conservation with Poliovirus (vaccine)



Viral Camouflage

He *et al. BMC Bioinformatics* 2014, **15**(Suppl 4):S1
http://www.biomedcentral.com/1471-2105/15/S4/S1

BMC Bioinformatics

RESEARCH Open Access

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

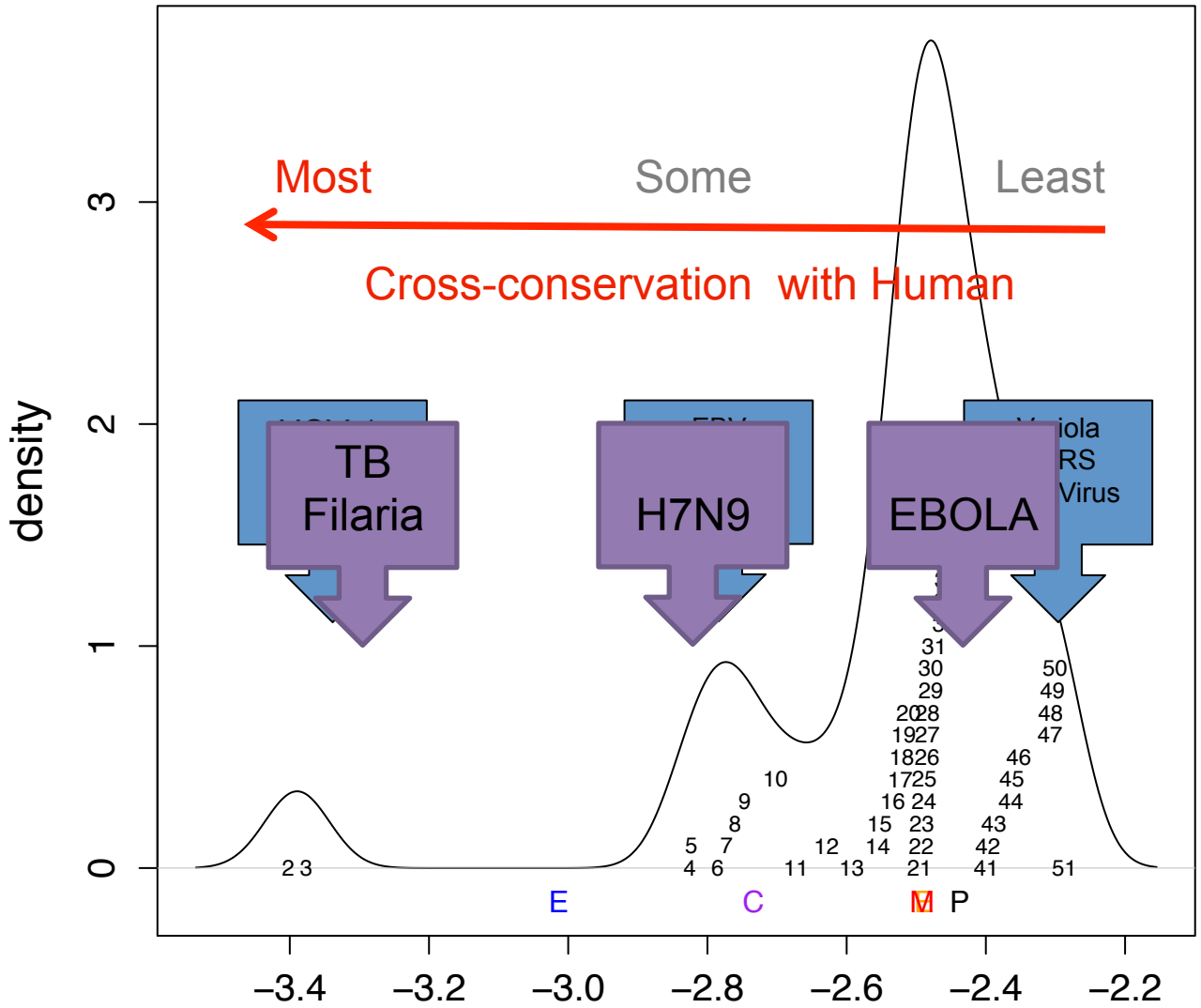
Lu He[1], Anne S De Groot[2,3], Andres H Gutierrez[2], William D Martin[3], Lenny Moise[2,3], Chris Bailey-Kellogg[1*]

From The 3rd ISV Pre-conference Computational Vaccinology Workshop (ICoVax 2013)
Barcelona, Spain. 26 October 2013

http://bit.ly/Viral_Camouflage

Institute for Immunology and Informatics

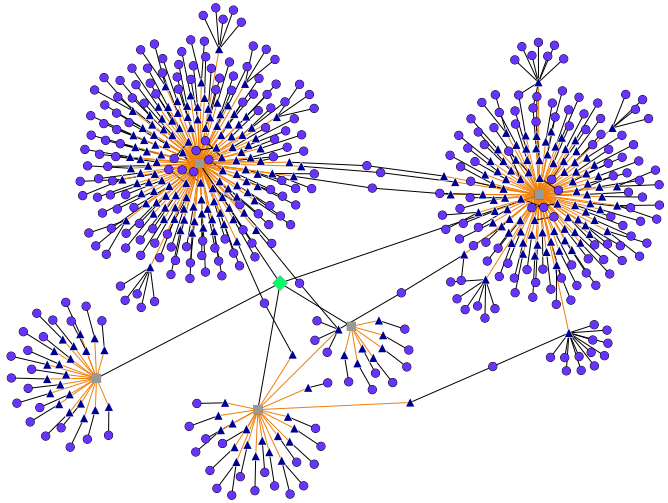
Evaluation of viruses for XR with Human Genome
Human Genome/Human Microbiome/Human Pathogens



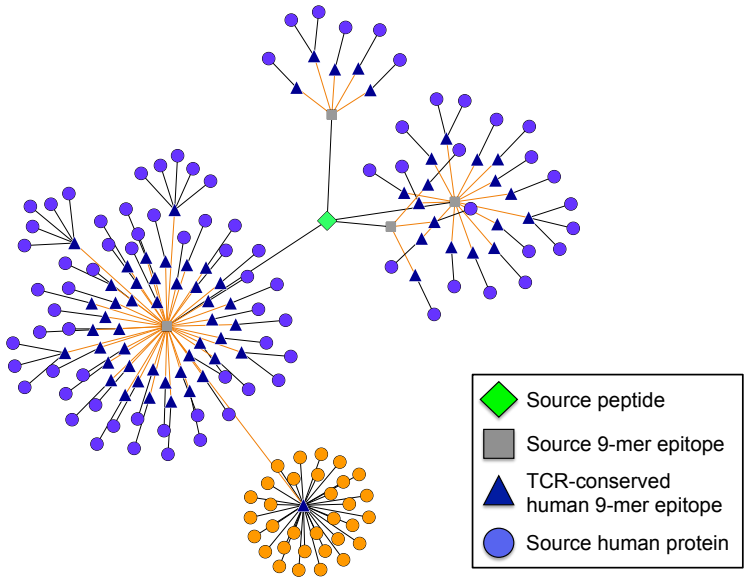
1 Rubella [not shown]
2 Human herpesvirus 2
3 Human herpesvirus 1
4 Human astrovirus
5 Human adenovirus C
6 Human herpesvirus 4 type 2 (EBV)
7 Hepatitis C virus
8 Human papillomavirus type 16
9 Human herpesvirus 5 strain AD169 (CMV)
10 Hepatitis B virus
11 Human T-lymphotropic virus 1
12 Dengue1
13 Human 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

Other Examples?
HIV envelope in RV144 vaccine

HCV peptide

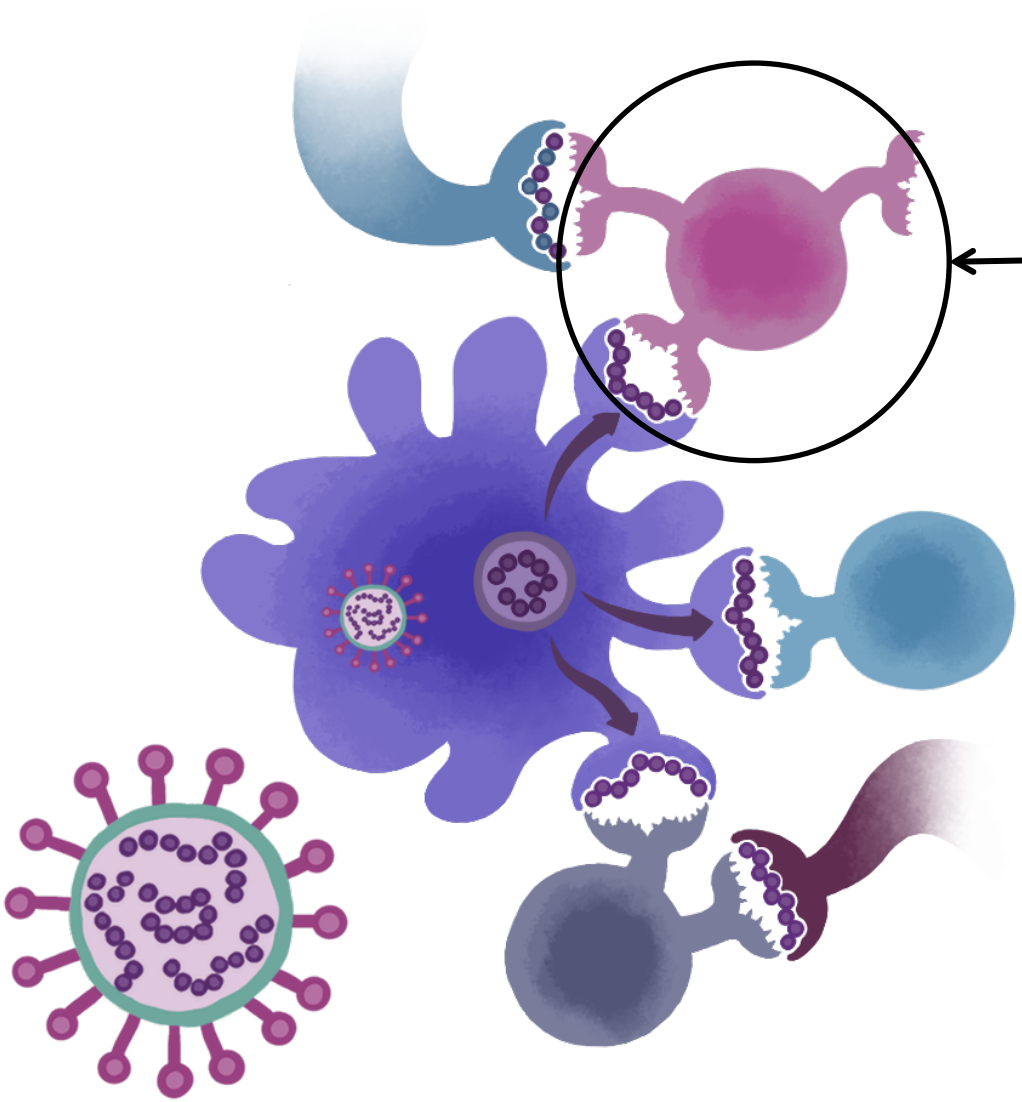


HIV peptide



Moise L, Terry F, Gutierrez AH, Tassone R, Losikoff P, Gregory SH, Martin WD, De Groot AS. Smarter vaccine design will circumvent regulatory T cell-mediated evasion in chronic HIV and HCV infection. In: Why vaccines to HIV, HCV and Malaria have so far failed - challenges to developing vaccines against immunoregulating pathogens. Frontiers in Microbiology. 2014. Editor (Gowans). http://bit.ly/Smarter_Vaccines_2014. (Open Access)

The Two –faced T cell epitope



Pathogens may be able to evolve T cell epitopes that trigger Tregs instead of T effectors . .

Institute for Global Health and Infectious Diseases at UNC Malaria Vaccine Study

University of North Carolina (UNC)
John Juliano and Steve Meshnick
Graduate Student Lauren Levitz

UNC GILLINGS SCHOOL OF GLOBAL PUBLIC HEALTH

Institute for Global Health and Infectious Diseases at UNC Malaria Vaccine Study

Use of Massively Parallel Pyrosequencing to Evaluate the Diversity of and Selection on *Plasmodium falciparum csp* T-Cell Epitopes in Lilongwe, Malawi

Jeffrey A. Bailey,[1] Tisungane Mvalo,[2] Nagesh Aragam,[3] Matthew Weiser,[4] Seth Congdon,[3] Debbie Kamwendo,[2] Francis Martinson,[2] Irving Hoffman,[3] Steven R. Meshnick,[5] and Jonathan J. Juliano[3]

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580 • JID 2012:206 (15 August) • Bailey et al

- Cross-sectional study of 50 children and 50 adults in Lilongwe, Malawi

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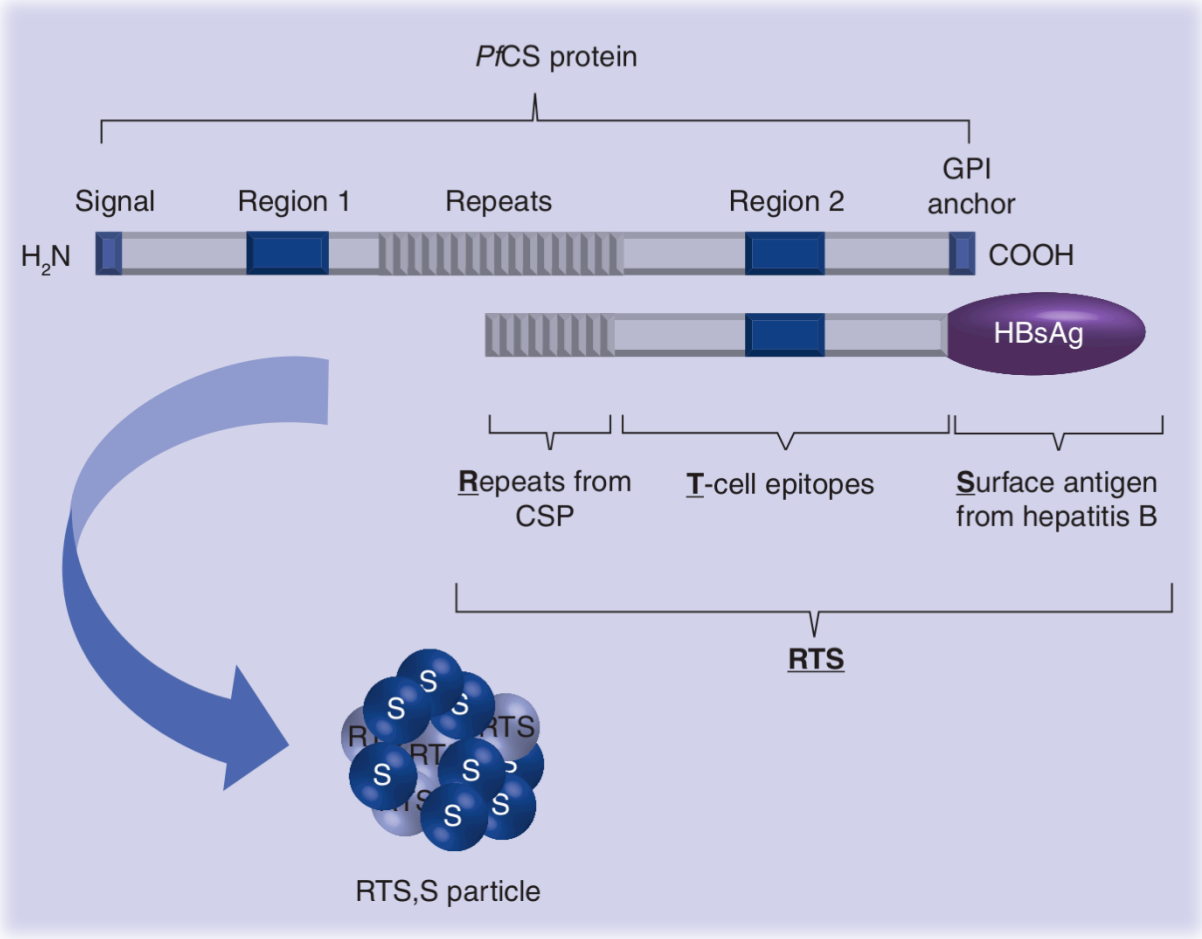
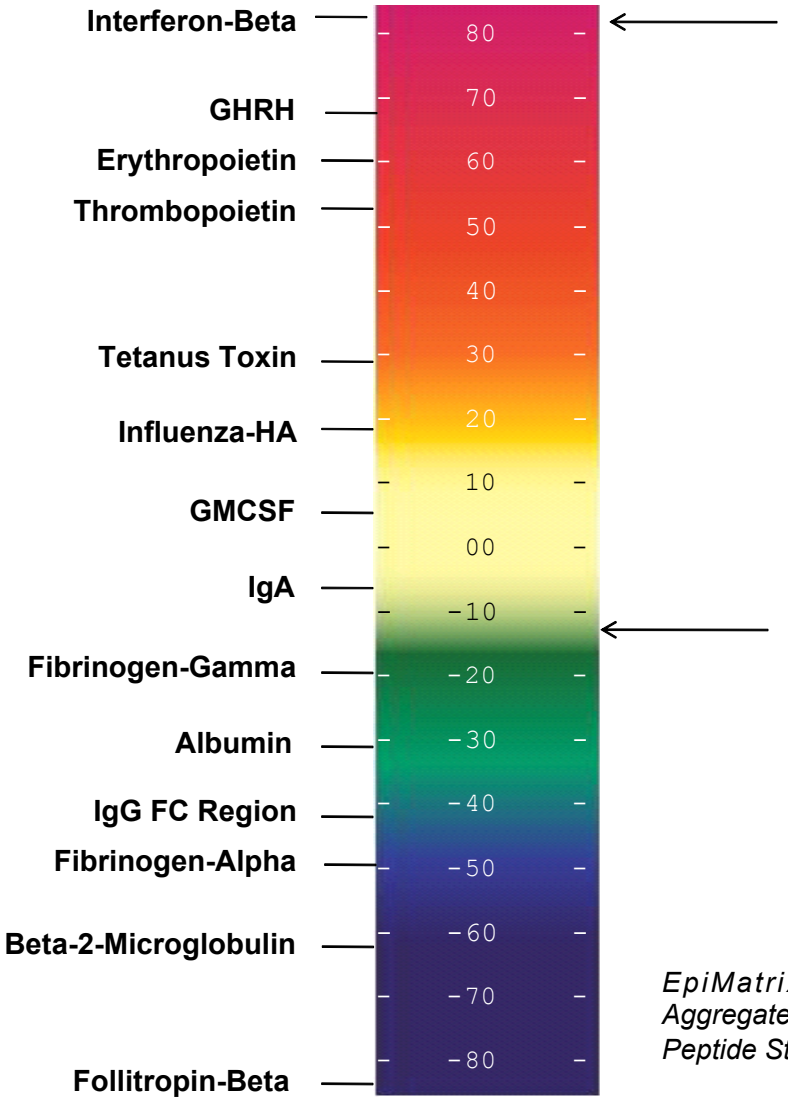
RTS,S Vaccine Components



RTS,S Vaccine Components



Hepatitis B S Protein (226AA)

EpiMatrix Protein Score: 81.69

P. Falciparum CSP (189 AA - NF54 strain)

EpiMatrix Protein Score: - 13.72

EpiMatrix Predicted Excess/Shortfall in Aggregate Immunogenicity Relative to a Random Peptide Standard

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TRIAD

RTS,S Vaccine Components: NPNA Repeats ("R")

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	MMAPNPNAN	9	0.66	0.72	0.91	0.94	0.81	0.59	0.60	1.18	0
2	MAPNPNANP	10	-0.03	0.07	0.62	-0.69	0.09	0.58	0.29	-0.43	0
3	APNPNANPN	11	-0.91	-1.15	-0.58	-0.67	-0.96	-1.14	-1.24	-0.43	0
4	PNPNANPNA	12	0.26	0.01	0.57	-0.79	0.13	0.72	0.12	0.17	0
5	NPNANPNAN	13	-0.01	-0.41	0.03	0.27	0.02	-0.33	-1.18	-0.04	0
6	PNANPNANP	14	-0.11	-0.28	0.55	-0.76	0.01	0.50	-0.05	-0.76	0
7	NANPNANPN	15	-0.28	-0.70	0.03	-0.05	-0.30	-0.50	-0.80	0.00	0
8	ANPNANPNA	16	0.31	0.17	0.63	-0.74	0.19	0.77	0.28	0.32	0
9	NPNANPNAN	17	-0.01	-0.41	0.03	0.27	0.02	-0.33	-1.18	-0.04	0
10	PNANPNANP	18	-0.11	-0.28	0.55	-0.76	0.01	0.50	-0.05	-0.76	0
11	NANPNANPN	19	-0.28	-0.70	0.03	-0.05	-0.30	-0.50	-0.80	0.00	0
12	ANPNANPNA	20	0.31	0.17	0.63	-0.74	0.19	0.77	0.28	0.32	0
13	NPNANPNAN	21	-0.01	-0.41	0.03	0.27	0.02	-0.33	-1.18	-0.04	0
14	PNANPNANP	22	-0.11	-0.28	0.55	-0.76	0.01	0.50	-0.05	-0.76	0
15	NANPNANPN	23	-0.28	-0.70	0.03	-0.05	-0.30	-0.50	-0.80	0.00	0
16	ANPNANPNA	24	0.31	0.17	0.63	-0.74	0.19	0.77	0.28	0.32	0
17	NPNANPNAN	25	-0.01	-0.41	0.03	0.27	0.02	-0.33	-1.18	-0.04	0
18	PNANPNANP	26	-0.11	-0.28	0.55	-0.76	0.01	0.50	-0.05	-0.76	0
19	NANPNANPN	27	-0.28	-0.70	0.03	-0.05	-0.30	-0.50	-0.80	0.00	0
⋮											
59	NANPNANPN	67	-0.28	-0.70	0.03	-0.05	-0.30	-0.50	-0.80	0.00	0
60	ANPNANPNA	68	0.31	0.17	0.63	-0.74	0.19	0.77	0.28	0.32	0
61	NPNANPNAN	69	-0.01	-0.41	0.03	0.27	0.02	-0.33	-1.18	-0.04	0
62	PNANPNANP	70	-0.11	-0.28	0.55	-0.76	0.01	0.50	-0.05	-0.76	0
63	NANPNANPN	71	-0.28	-0.70	0.03	-0.05	-0.30	-0.50	-0.80	0.00	0
64	ANPNANPNK	72	-0.02	-0.21	0.48	-1.11	0.37	0.62	-0.10	-0.00	0
65	NPNANPNKN	73	0.12	-0.28	0.14	0.39	0.14	-0.20	-1.06	0.07	0

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RTS,S Vaccine Components: T cell Epitopes ("T")

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	RNVDENANA	9	-0.02	1.18	1.09	-1.37	-0.43	-0.31	0.30	-0.38	0
2	NVDENANAN	10	0.33	-0.54	0.37	0.13	-0.82	-0.25	-1.51	-0.81	0
3	VDENANANS	11	0.99	0.96	1.44	-0.40	0.87	1.44	1.16	0.52	0
4	DENANANSA	12	0.79	0.97	1.09	0.92	0.30	0.64	0.16	0.92	0
5	ENANANSAV	13	1.15	0.65	1.04	1.18	0.35	0.72	0.55	0.71	0
6	NANANSAVK	14	0.27	-0.14	0.84	-0.19	0.24	0.28	0.07	0.04	0
7	ANANSAVKN	15	1.00	0.28	0.78	0.45	0.84	0.16	0.28	0.38	0
8	NANSAVKNN	16	-0.20	-1.12	-0.36	0.38	-0.55	-0.49	-1.22	-1.34	0
9	ANSAVKNNN	17	0.09	0.96	-0.05	-0.04	1.24	0.86	0.16	1.00	0
10	NSAVKNNNN	18	0.62	-0.10	0.67	0.79	0.42	0.70	-0.66	0.77	0
11	SAVKNNNNE	19	-1.17	0.30	-0.91	-0.49	0.85	-0.58	-0.02	-1.08	0
12	AVKNNNNEE	20	0.18	1.20	0.64	0.25	1.28	0.54	0.41	0.12	0
13	VKNNNNEEP	21	1.05	1.39	1.65	0.89	1.29	1.41	0.91	0.62	1
14	KNNNNEEPS	22	0.04	0.71	0.40	-0.07	0.63	0.86	0.24	-0.30	0
15	NNNNEEPSD	23	-0.94	-0.66	-1.03	-0.87	0.83	-0.21	-0.54	-0.82	0
16	NNNEEPSDK	24	0.17	-0.26	0.47	-0.29	-0.92	-0.22	-0.57	-1.12	0
17	NNEEPSDKH	25	0.22	-0.58	0.61	-0.75	-1.21	-0.36	-1.30	-1.52	0
18	NEEPSDKHI	26	-1.63	-1.51	-1.16	-0.75	-1.63	-1.96	-0.61	-0.66	0
19	EEPSDKHIK	27	-1.20	-0.35	-1.16	-1.44	-1.11	-0.26	-0.64	-1.61	0
20	EPSDKHIKE	28	-1.63	0.41	-0.54	-1.80	0.07	-1.59	-0.70	-1.46	0
21	PSDKHIKEY	29	-2.61	-1.59	-2.70	-1.41	-1.84	-2.03	-0.90	-2.83	0
22	SDKHIKEYL	30	-0.99	-0.20	-1.33	-0.12	-0.76	-0.81	0.86	-0.55	0
23	DKHIKEYLN	31	0.15	-0.64	0.30	0.55	0.18	0.74	-0.41	0.63	0
24	KHIKEYLNK	32	-0.25	-0.55	-0.58	-0.04	0.24	0.00	0.06	-0.35	0
25	HIKEYLNKI	33	0.47	1.32	0.25	0.85	-0.10	0.57	0.31	0.27	0
26	IKEYLNKIQ	34	1.10	0.36	1.59	1.77	2.20	1.64	1.89	1.86	5
27	KEYLNKIQN	35	0.13	-0.20	-0.37	-0.64	1.17	0.83	-0.00	0.51	0
28	EYLNKIQNS	36	-0.00	0.53	0.53	0.25	0.52	0.59	-0.67	-0.15	0
29	YLNKIQNSL	37	1.56	1.60	0.51	2.58	2.08	1.64	1.25	0.50	2
30	LNKIQNSLS	38	2.59	2.02	2.92	1.80	1.58	2.52	2.23	2.62	7
31	NKIQNSLST	39	1.83	0.32	1.91	1.65	1.07	1.65	1.18	1.86	5
32	KIQNSLSTE	40	-0.96	0.78	-0.66	-0.70	0.97	0.00	0.67	-0.33	0
33	IQNSLSTEW	41	1.37	1.61	1.14	2.54	0.21	0.13	1.25	1.27	1
34	QNSLSTEWS	42	1.17	0.25	1.67	0.26	1.04	1.24	0.01	0.01	1
35	NSLSTEWSP	43	-1.25	-0.79	-1.29	-0.22	-1.85	-0.10	-1.64	-1.08	0
36	SLSTEWSPC	44	-0.43	-1.49	-0.69	0.76	-0.70	-1.53	-0.74	0.48	0

"Th2" region

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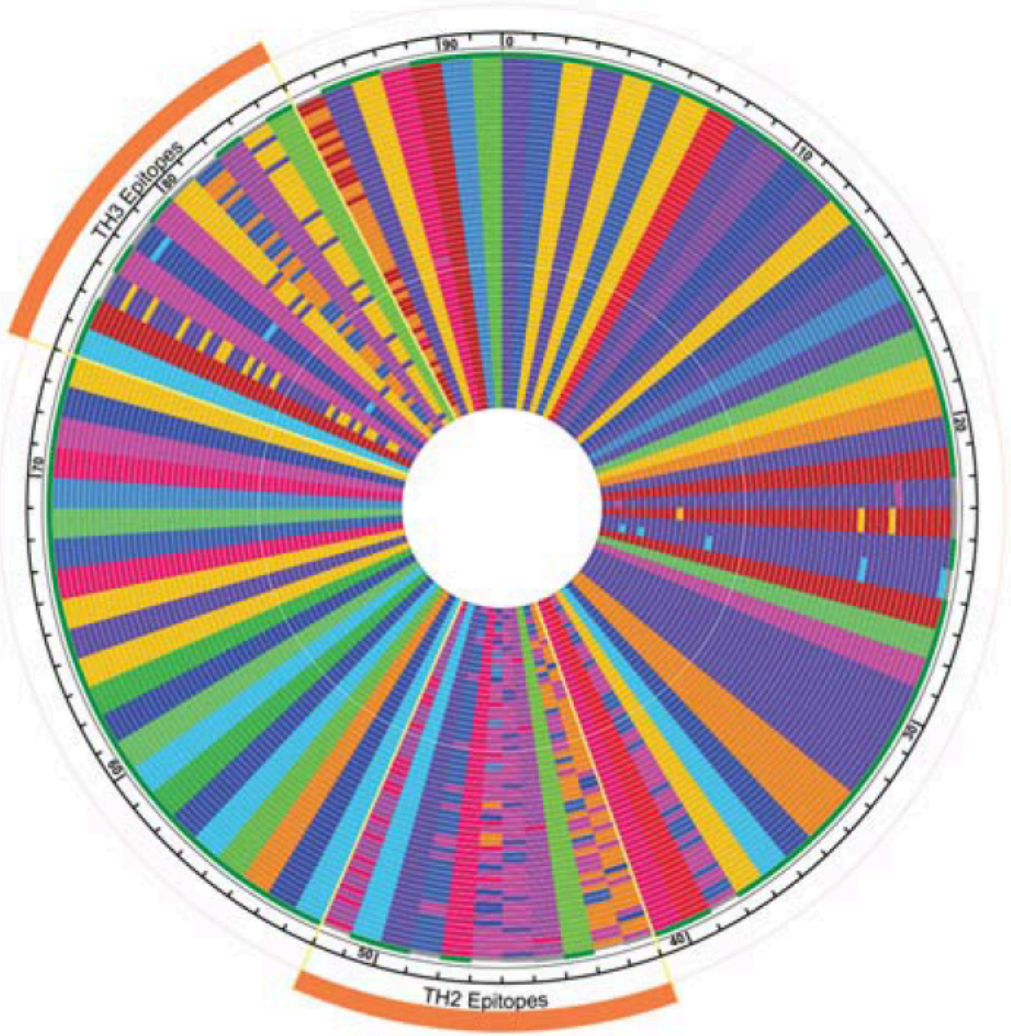
Analysis of Circulating Strains



- 1,568,200 sequences were used to construct variants in the parasite population
- 57 were genetically unique
- 30 unique Th2 epitopes
- 15 unique Th3 epitopes
- Only 2 variants contained the same Th2 and Th3 epitopes as the vaccine strain

Analysis of Circulating Strains

Allele	Average z-scores of:				# of participants with this allele	% of study population (N=117) with this allele
	All circulating malaria strains (high to low)	**Vaccine strain + HBsAg**	**Vaccine strain alone**	**HBsAg alone**		
DRB1*0801	**2.24 (HIGH)**	2.03	1.04	3.02	10	**8.5%**
DRB1*0701	0.65	**22.98**	**3.11**	**42.84***	20	17.1%
DRB1*0401	0.53	7.61	0.91	14.31	6	5.1%
DRB1*1101	0.23	12.78	0.73	24.82	40	34.2%
DRB1*1301	-0.61	4.48	-0.89	9.85	30	24.8%
DRB1*0101	-2.02	8.08	-2.81	18.97	13	11.1%
DRB1*1501	**-2.54 (Low)**	15.89	-0.89	32.66	36	**30.8%**
DRB1*0301	-3.26	-5.16	-5.21	-5.11	32	25.6%
Other	-	-	-	-	31	26.5%

*HLA DR7-subjects less responsive to HBV vaccination…HBV contains a highly human-homologous T cell epitope restricted by HLA DR7

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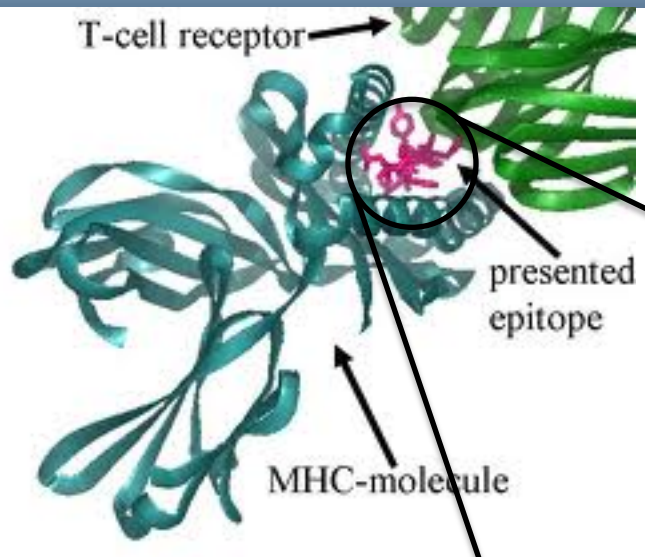
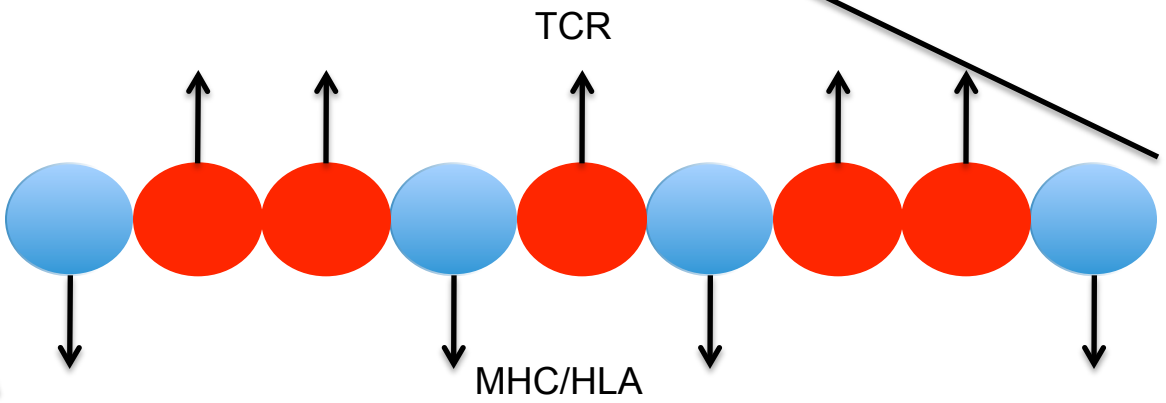
JanusMatrix



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



Moise, et al. **The Two-Faced T cell Epitope: Examining the Host-Microbe Interface with JanusMatrix.** Hum Vaccin Immunother. 2013 Apr 12;9(7). http://bit.ly/JanusMatrix.

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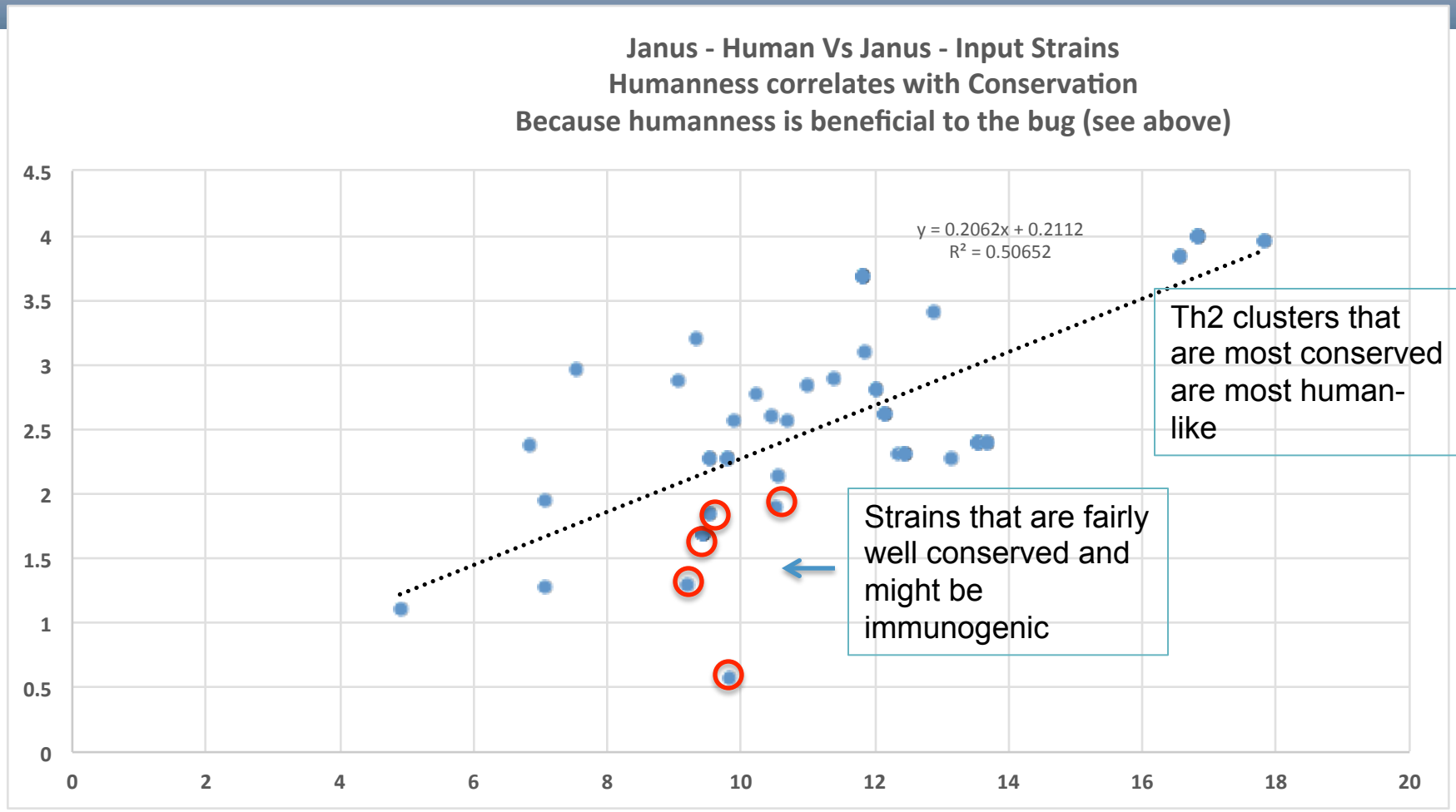
Human vs. CSP Cross-conservation (of Th2 epitope)



Best correlation is between
Higher Conservation – Higher Human-like TCR Face

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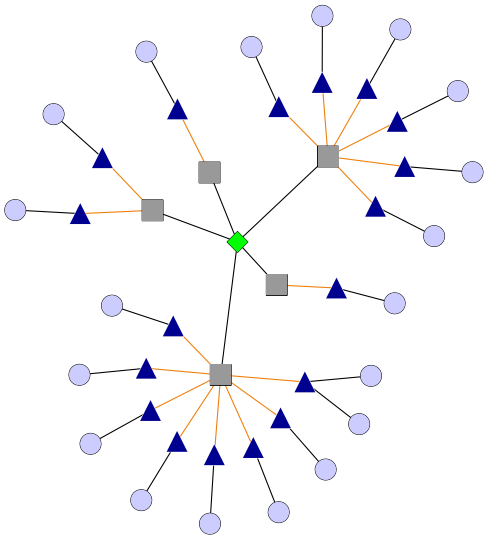
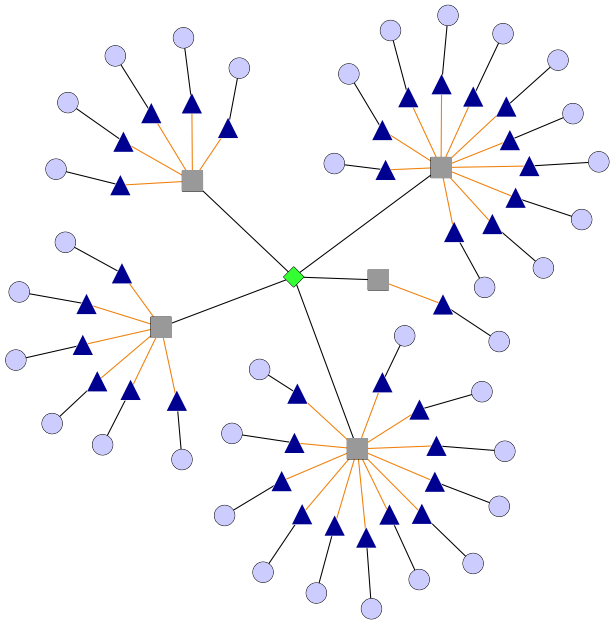
TRIAD

Highly Pf-conserved Th2 epitopes
From strains that have highest “human-like” score

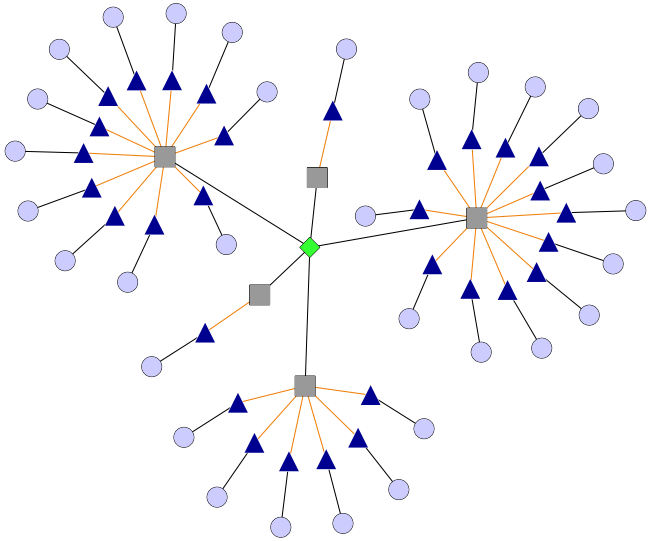
From RTS,S (pUID08)



Homology with other Th2 epitopes: 67%
Cluster score: 32.3
Delta: 23.7
Janus Human: 2.14



Homology with other Th2 epitopes: **82%**
Cluster score: **38.54**
Delta: *42.08*
Janus Human: ***4.00***

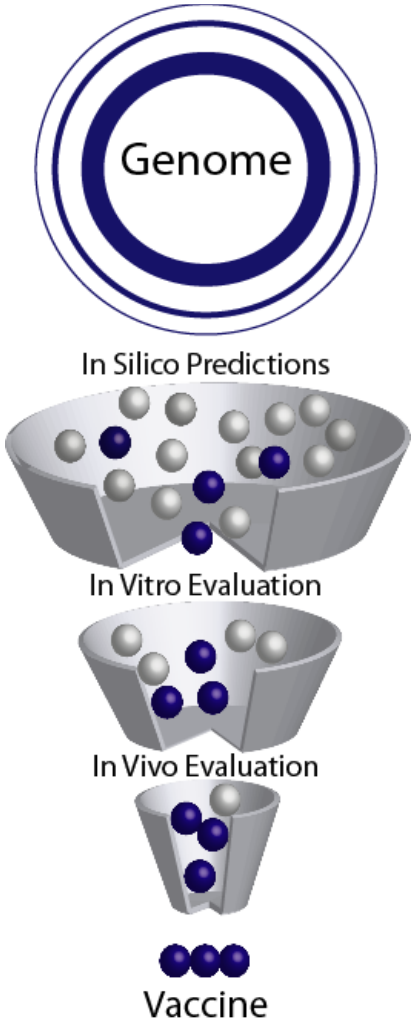


Homology with other Th2 epitopes: **95%**
Cluster score: **36.88**
Delta: *36.25*
Janus Human: ***3.95***

Implications for off target effects

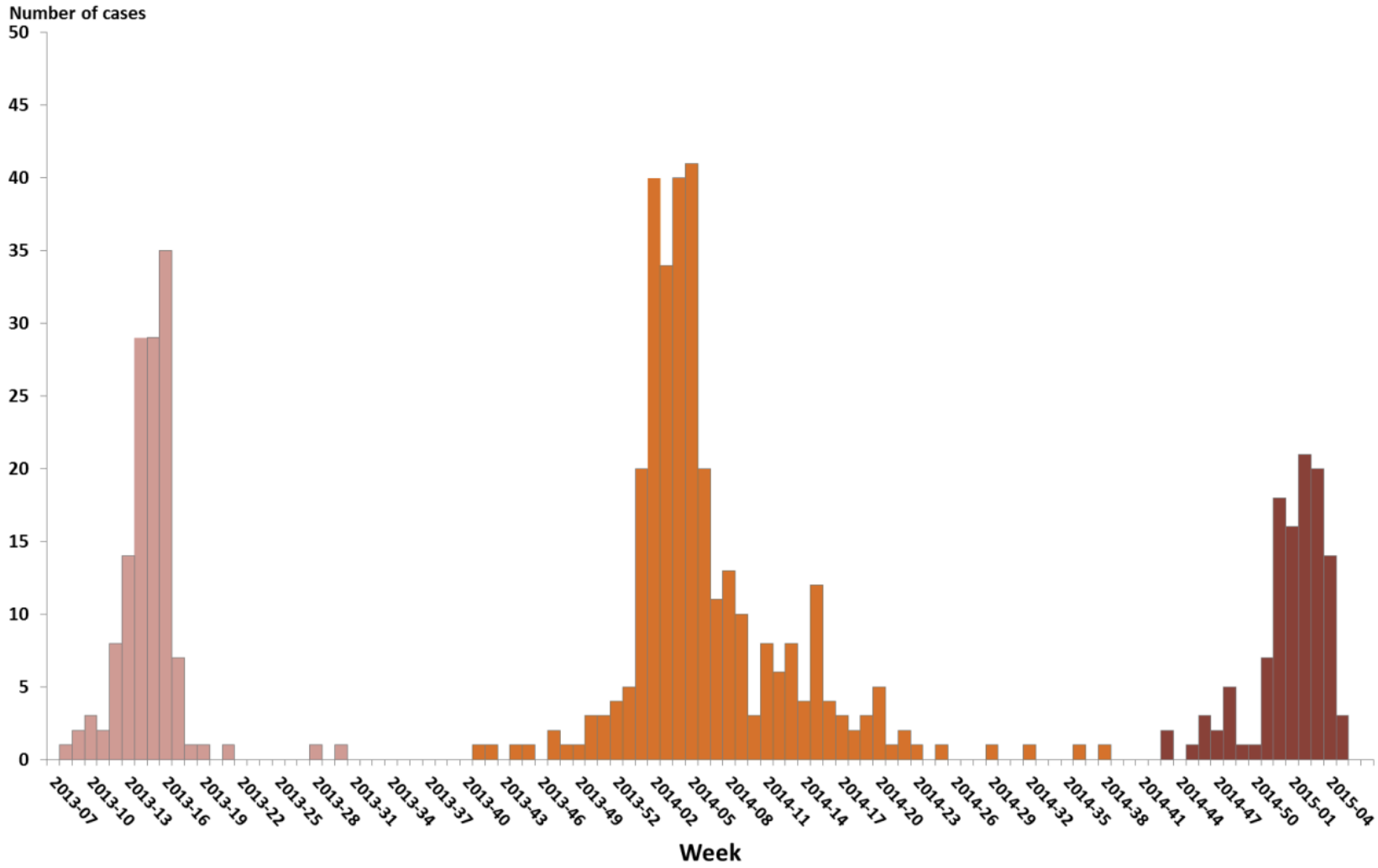
- Vaccines for chronic infections and must circumvent potential Treg activation that can handicap efficacy.
- Epitope-driven approaches to vaccine design that involve careful consideration of the T cell subsets primed during immunization will advance vaccine development.
- Are there epitopes in pathogens that are conserved with deleterious epitopes, associated with harmful effects?

Outline



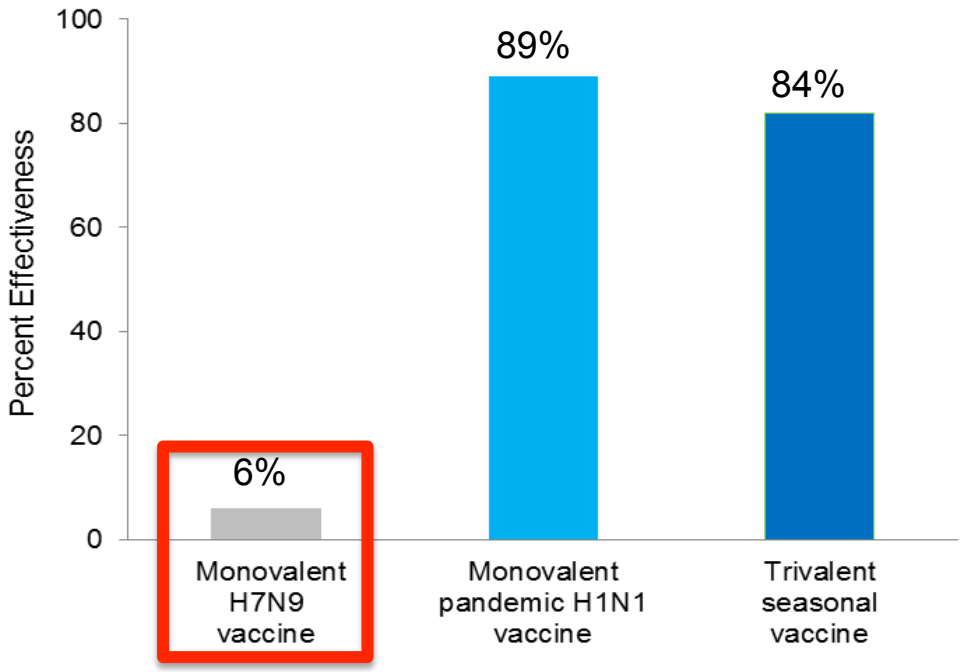
- Epitope mapping basics
- Immunoinformatic-driven T cell epitope vaccine illustrated
- Delving deeper – host-pathogen homology
- Engineered antigens for improved efficacy

A recurring “Epidemic” – Leap to Human to Human Transmission?



Source: WHO Disease Outbreak News

October 2013: New H7N9 Vaccines are CONFIRMED to be POORLY IMMUNOGENIC



Griffin MR et al. Plos One 2011 Aug 6(8);

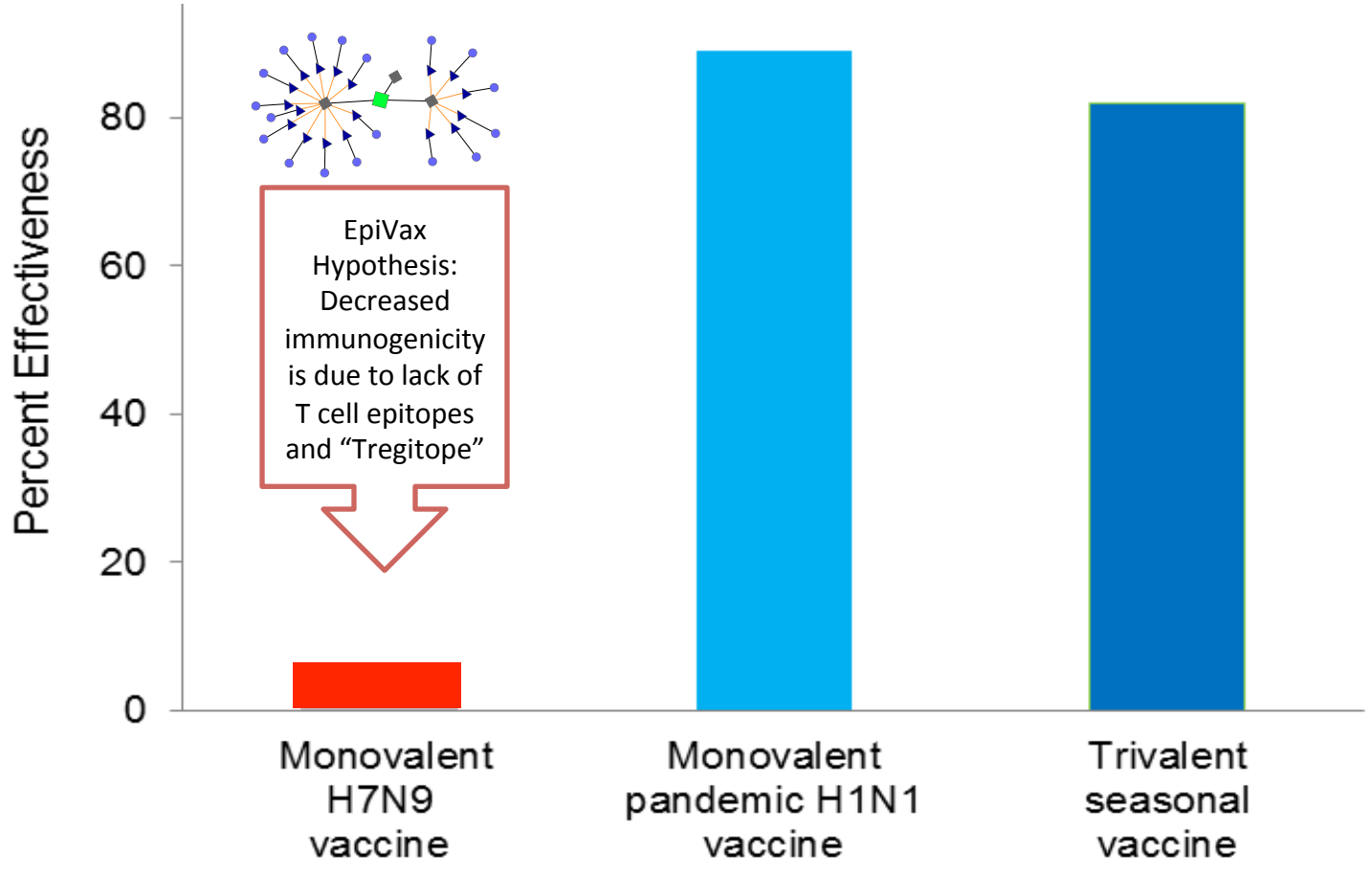
Goodwin et al. Vaccine 2006(24):1159-1169; CDC MMWR September 20, 2013/ 62(RR07); 1-43

Human Antibody Responses to Avian Influenza A(H7N9) Virus

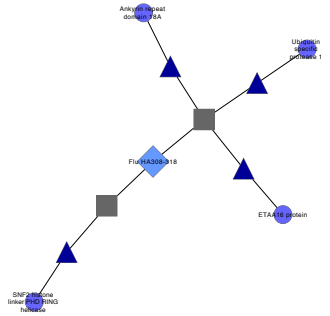
- Delayed nAb development
  - Anti-H7 IgG absent in acute-phase sera
  - Anti-H7 IgG present in convalescent-phase sera
  - HAI detected 30 days after disease onset
- Low anti-H7 antibody affinity and avidity

Guo et al. 2014 EID

H7N9 contains a regulatory T cell epitope

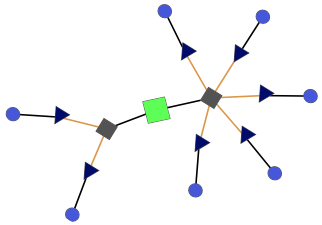


Key H7N9 Flu Epitope
Is Altered – More Cross-reactive with HG

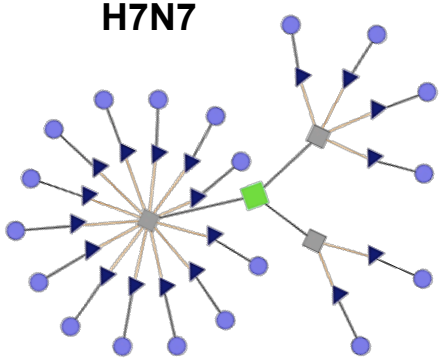


H1N1

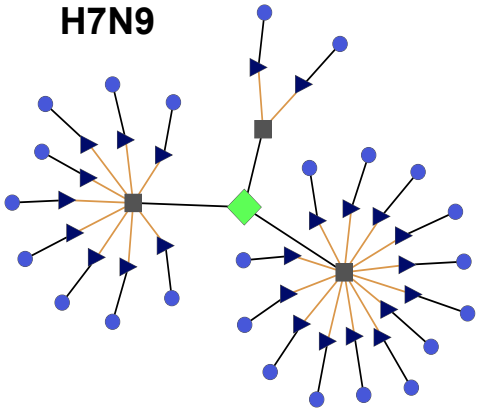
H3N2



H7N7

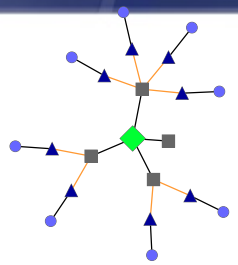


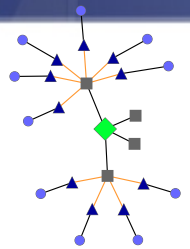
H7N9

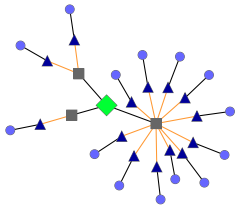


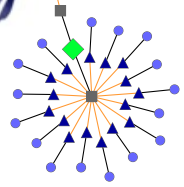
3 Selected HG-XCON H7N9 Peptides Evaluated in detailed studies

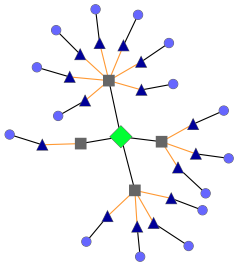

4_H7N9_HA

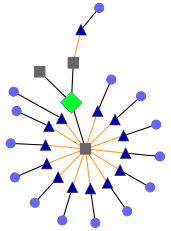

8_H7N9_HA

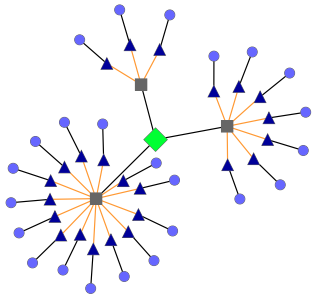

13_H7N9_M1

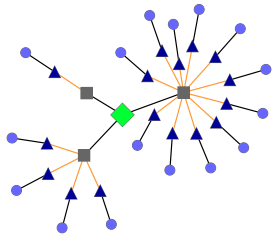

73_H7N9_PB1

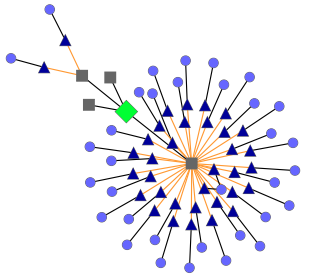

88_H7N9_PB2

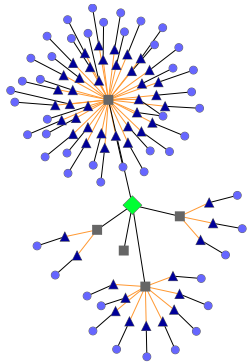

48_H7N9_NS1

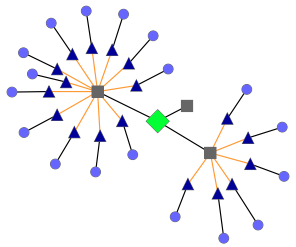

53_H7N9_PA

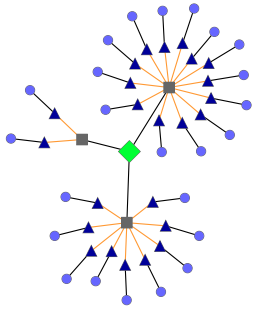

7_H7N9_HA

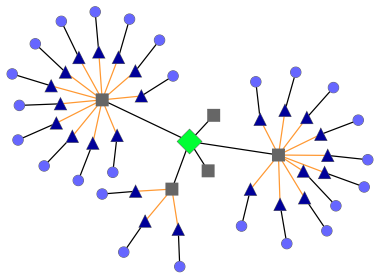

21_H7N9_M2

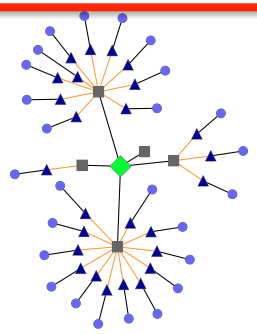

14_H7N9_M1


59_H7N9_PA


29_H7N9_NEP

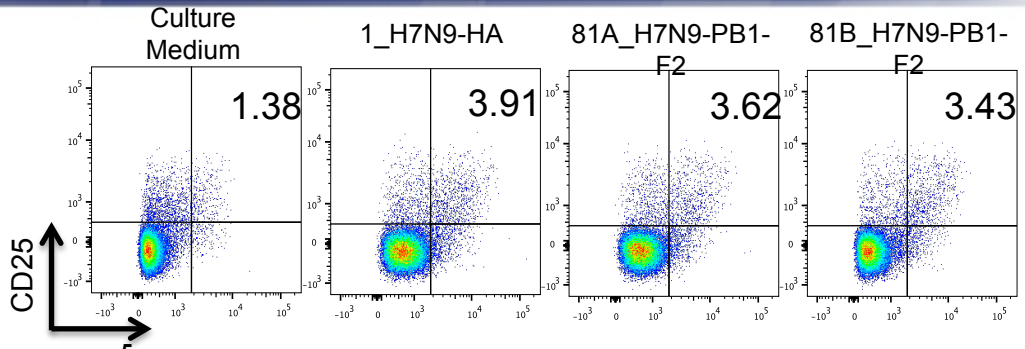

1_H7N9_HA


81A_H7N9_PB1-F2

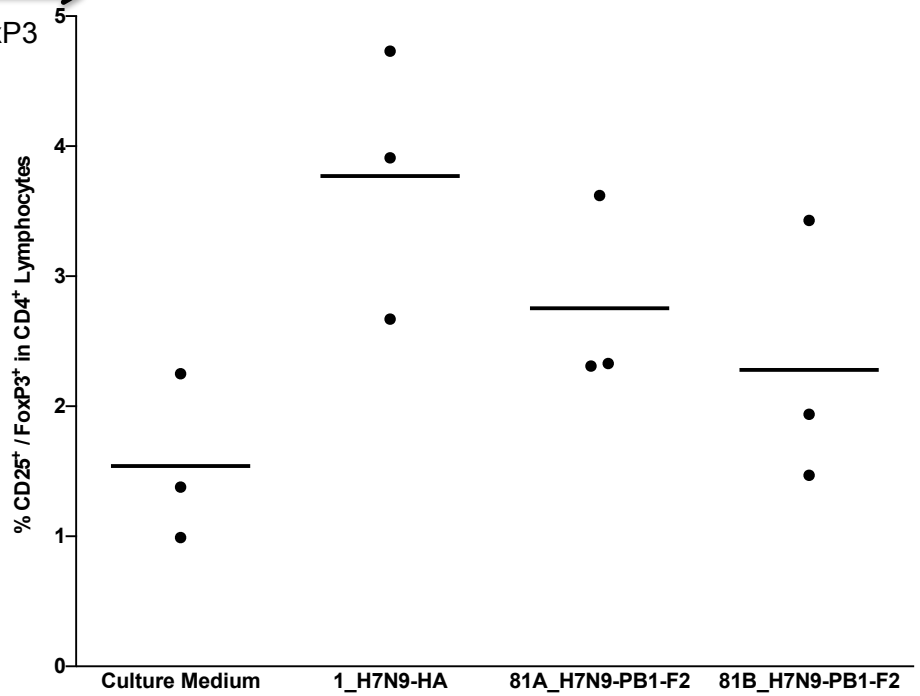

81B_H7N9_PB1-F2

Rui Liu et al., manuscript under revision

Immunosuppressive phenotype of human-like H7N9 peptides



Treg Frequency

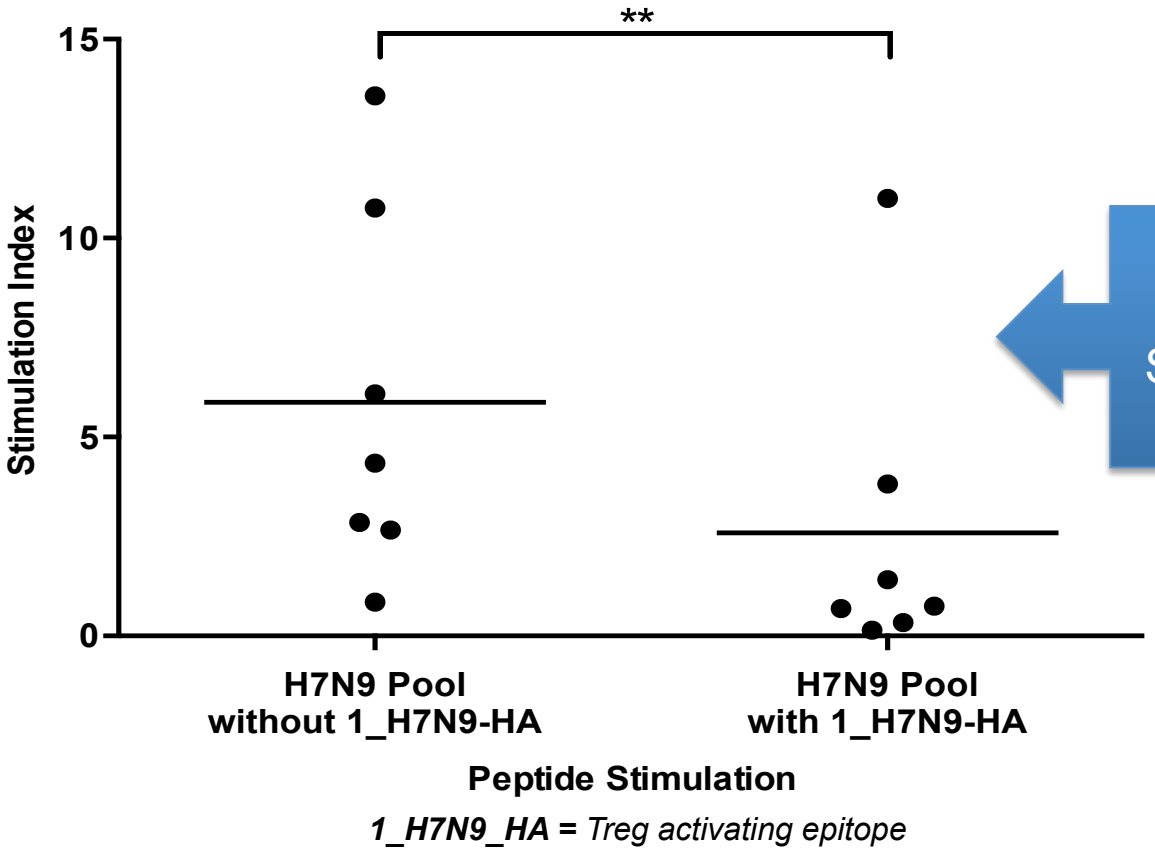


H7N9 human-like peptides

- **H7N9 HA epitopes with high potential for self cross-reactivity elevate Treg frequency in vitro**

Manuscript Accepted

H7N9 Treg activating epitope suppresses IFNγ secretion in co-culture



"Bystander Suppression"

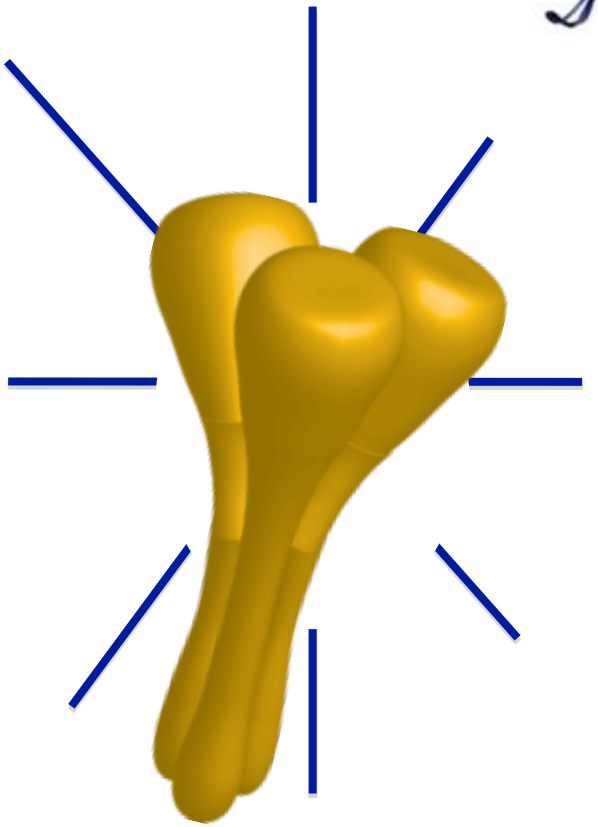
Manuscript accepted

Optimized H7 HA for improved Vaccine Efficacy: Epitope Enhanced H7 HA

Identify potential regions where epitopes can be improved (TREG DELETED)

Result: Epitope–enhanced HA

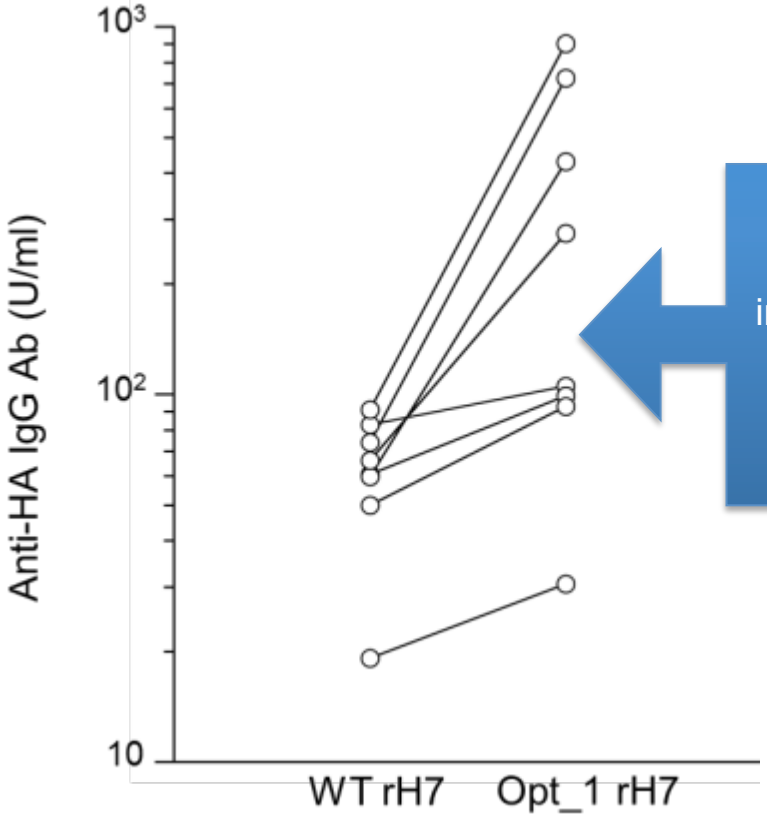
"Opt 1"



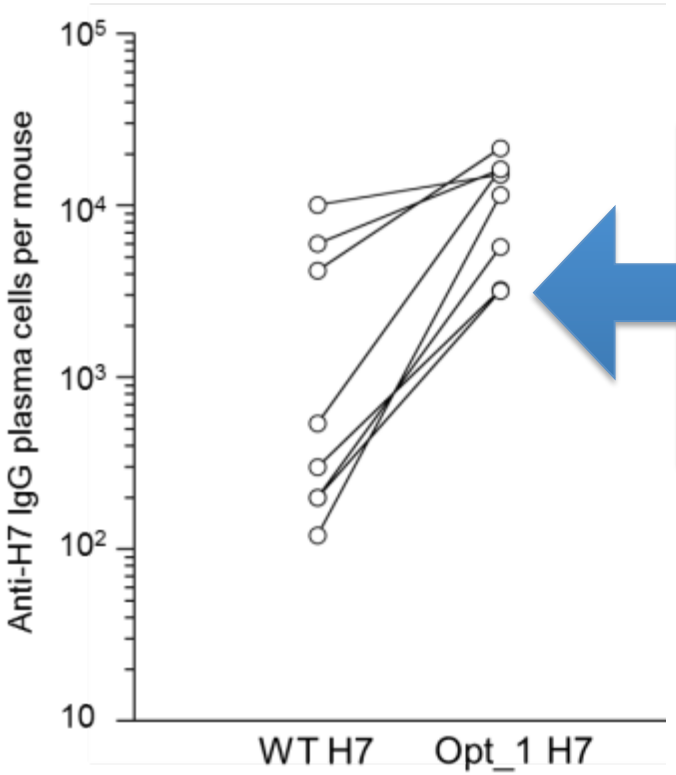
NIID: Opt_1 rH7-HA produced in insect cells

Epitope-Enhanced H7 HA Antigenicity:
"Opt_1 rH7 HA" - H7 HA optimized with 3 AA changes

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



5 fold increase in antibody titer

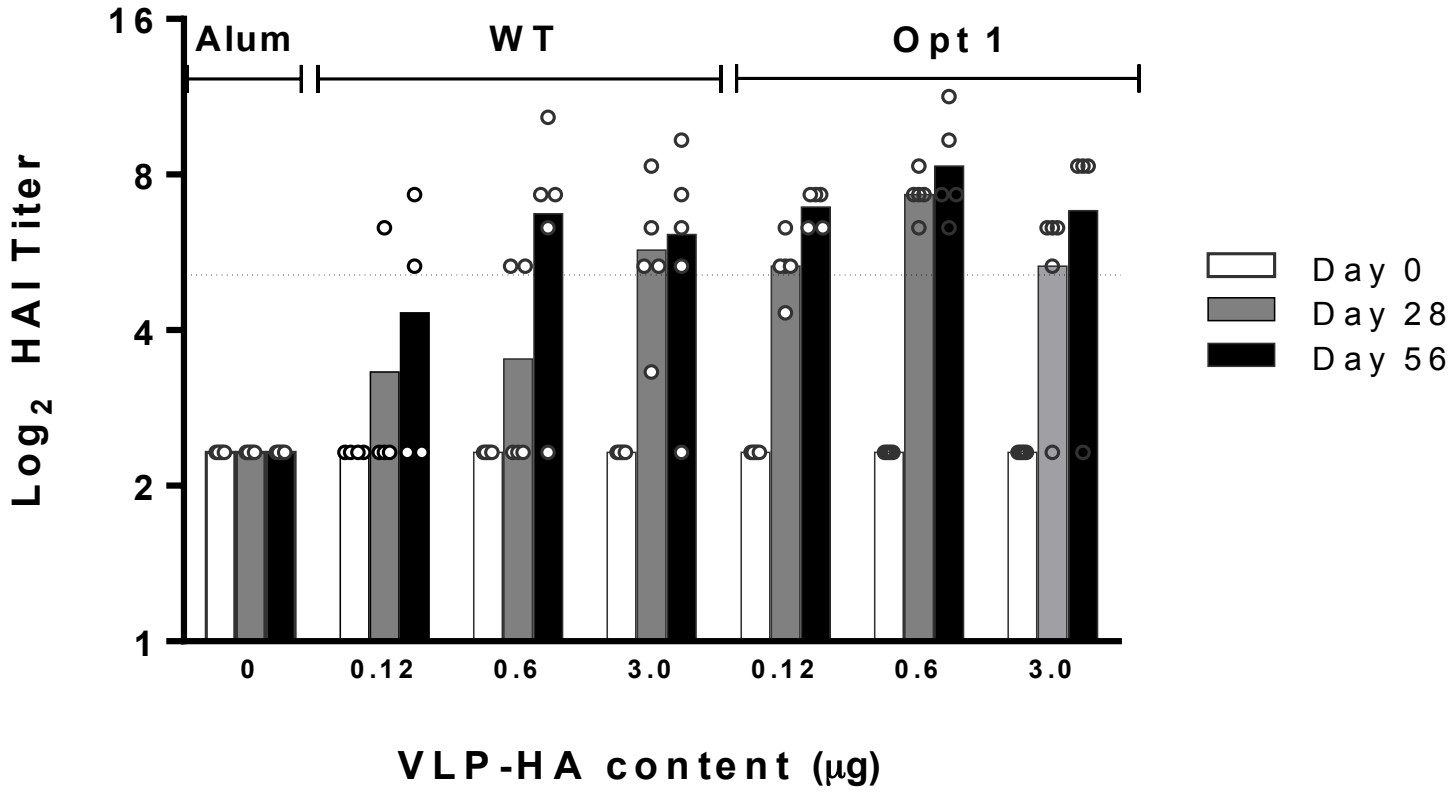


20 fold increase in B cell response

(Study performed in collaboration with NIID Japan)

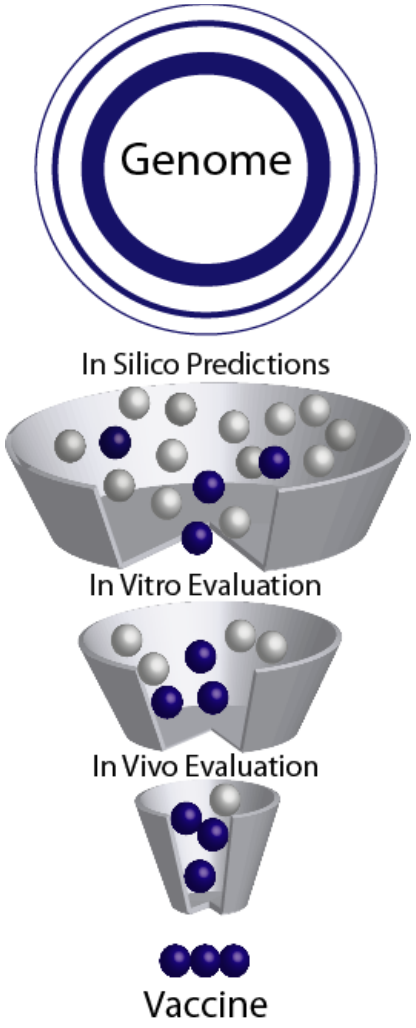
NIID 国立感染症研究所 NATIONAL INSTITUTE OF INFECTIOUS DISEASES

HAI Titers against WT H7N9 (Balb C)



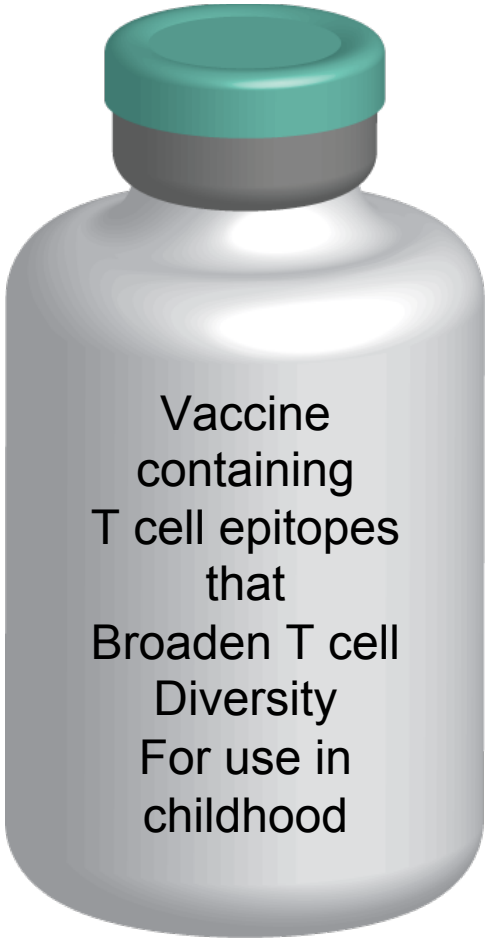
- Modifications of H7-HA in Opt1 preserve neutralizing epitopes
- Opt1 H7N9 VLP vaccine protects earlier and at lower doses than WT vaccine.

Outline



- Epitope mapping basics
- Conserved T cell epitopes may be protective
- Delving deeper – host-pathogen homology
- Engineered antigens for improved efficacy
- Relevance to this topic?

“Good” Cross Reactive epitopes



Given the importance of cross-reactive T cell response, how can we enlist it in developing better vaccines?

“Bad” Cross Reactive epitopes

Do some epitopes induce

adverse off target effects?

Relevance to our discussions

- Sequence analysis of the two faces of T cell epitopes enables prediction of epitope phenotype.
- Epitopes that share a TCR-face with numerous human sequences may activate Tregs.
- Among human-host viruses, chronic viruses appear more like human than do viruses that cause acute infection.
- Vaccines for chronic infections and stealth acute infections must circumvent potential Treg activation that can handicap efficacy.
- Treg induction may not always be a ‘bad outcome’
- We may be able to capitalize on these concepts to advance the development of better vaccines.

Science without fear.

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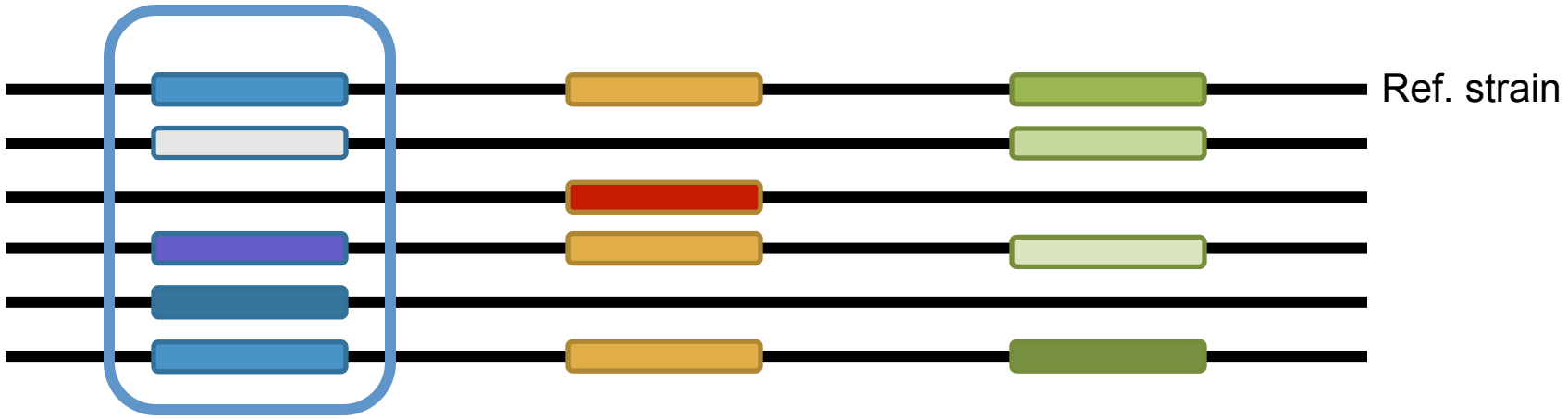
Sequence Homology within the same species - Conservatrix

Use Conservatrix to find most conserved epitope



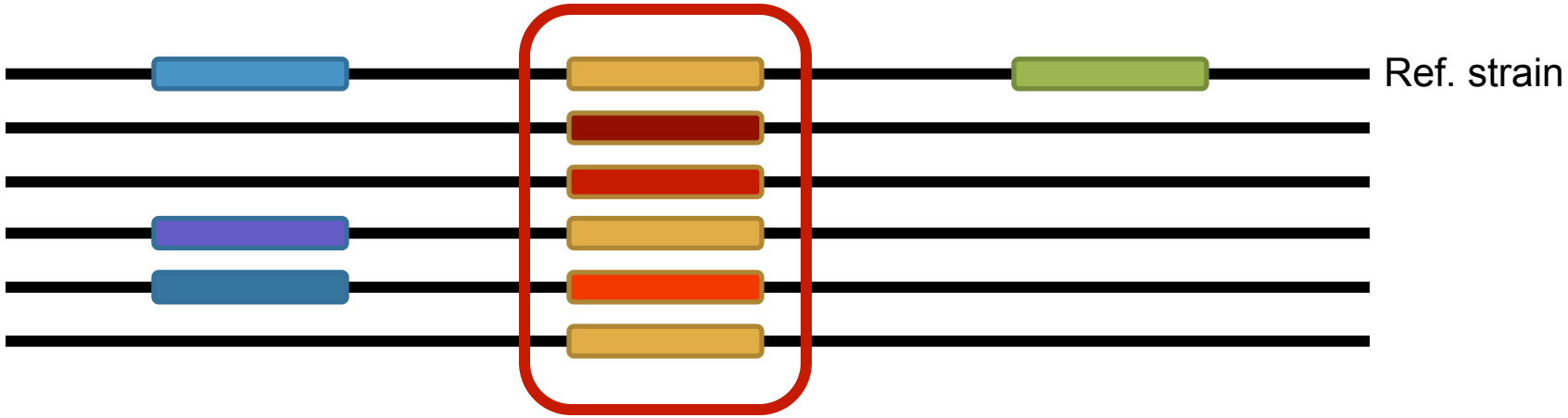
Identify epitope that is conserved across multiple strains, but… (see JMX)

TCR Homology within the same species

JanusMatrix

… due to minor AA differences, homologous epitopes may not be cross-conserved at TCR face

JanusMatrix specifically tracks conservation of the TCR-facing residues; improved conservation analysis now possible



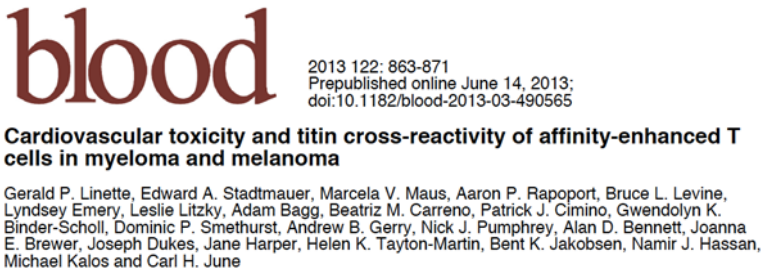
Can we avoid Off-Target effects? JanusMatrix Cross-Reactivity Case

Titin cross-reactivity:

Cardiovascular toxicity and titin cross-reactivity of affinity enhanced T cells in myeloma and melanoma.
Linette GP, et al. *Blood 2013;122(6):863-71.*

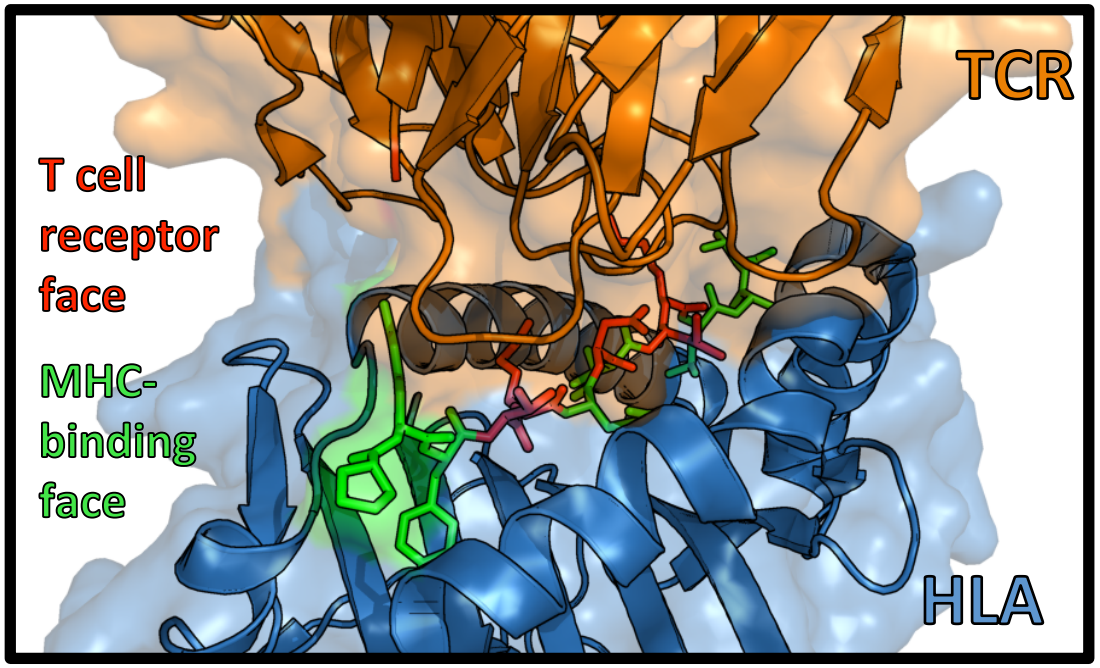
- T cell therapy with affinity-enhanced TCR against HLA-A*01-restricted MAGE-A3.
- **The first two treated patients developed cardiogenic shock and died** within a few days of T cell infusion
- The events were not predicted by pre-clinical studies of the high-affinity TCR. Cross-reactivity to titin was identified later.

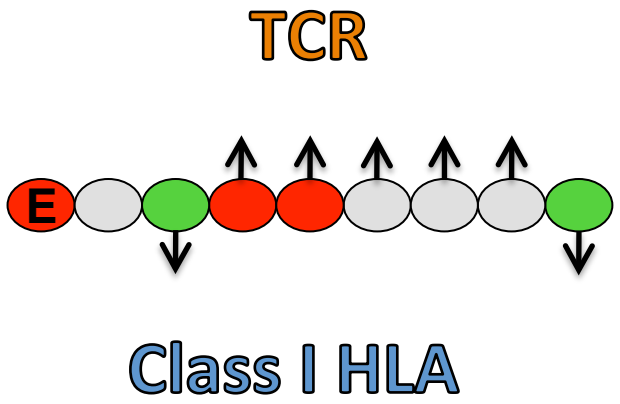
HLA-A*01 restricted MAGE-A3 peptide
[EVDPIGHLY]



Class I JanusMatrix

Search for cross-conserved class I T cell epitopes



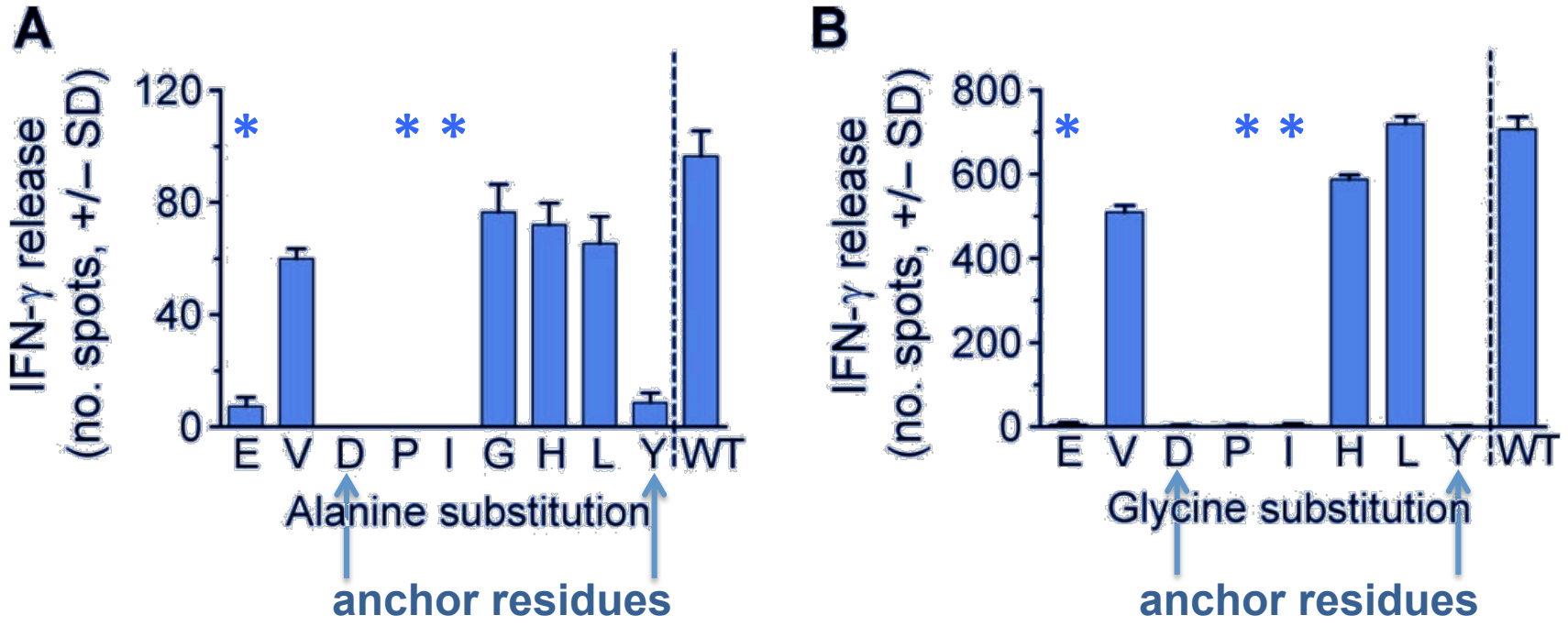


Class I TCR Contacts

blood®

Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma.

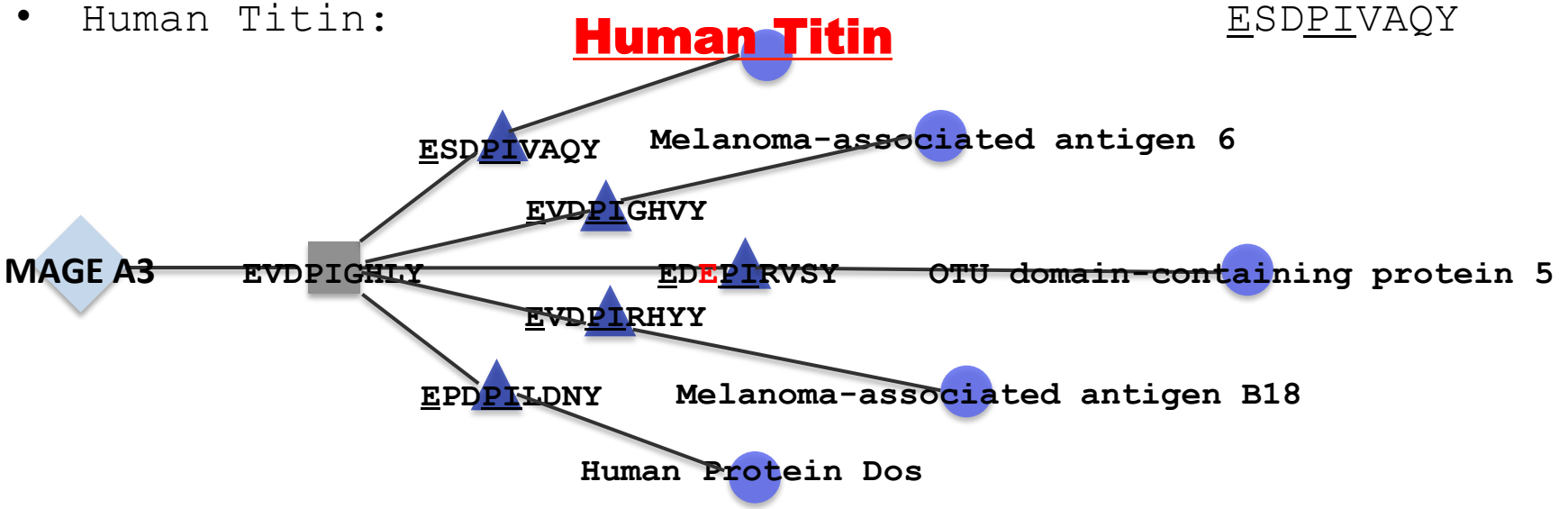
Cameron et al. identified key TCR contact residues (*) in MAGE A3 peptide:



How to Avoid Cross-Reactivity

Results of scan

• Melanoma-associated antigen(MAGE A)3:	**EVDPIGHLY**
• Melanoma-associated antigen 6:	EVDPIGHVY
• Melanoma-associated antigen B18:	EVDPIRHYY
• Human OTU domain-containing protein 5:	EDEPIRVSY
• Human Protein Dos:	EPDPILDNY
• Human Titin:	ESDPIVAQY



- With JanusMatrix, peptides are screened for cross-conservation with human proteins.
- Cross-reactive peptides can then be set aside, avoiding dangerous cross-reactivity.