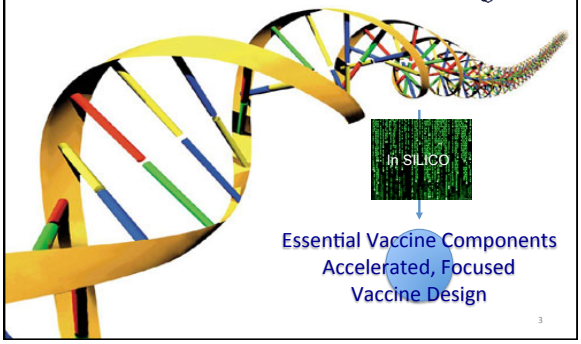
Vaccine Design Using in Silico Tools: How to Improve Vaccine Efficacy by Modifying Treg Epitopes

Westin Immunogencity Seminar
Vaccines and Biologics

Annie De Groot MD
CEO/CSO EpiVax
April 2015

From "Gene to Vaccine"



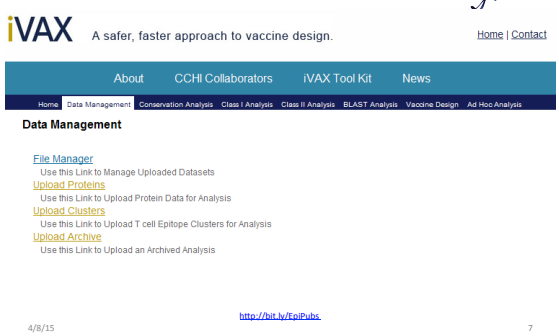
3

Outline

- Vaccine Design Tools
- Predicting Vaccine Efficacy
  - H1N1 and H7N9
- Engineering Better Vaccines
  - H7N9
  - CEA/Mage A3
- Off Target Effects of Vaccines
- Vaccines "on Demand"

4/9/15 5

iVAX Toolkit – Commercial



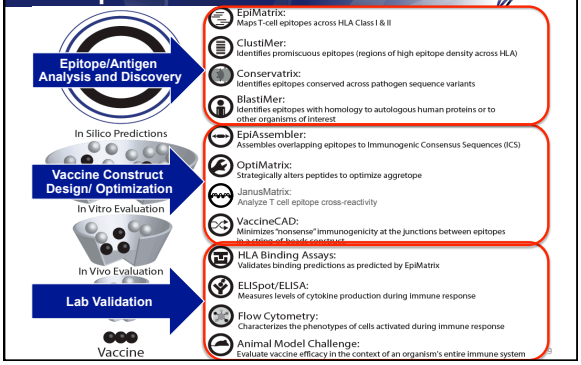
http://bit.ly/EpiPubs

4/8/15 7

iVAX Toolkit – Academic

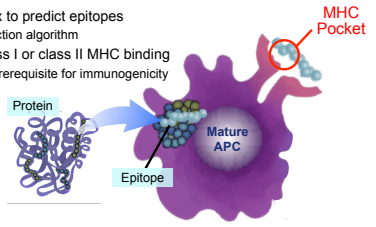
8

Vaccine Design Tools and Techniques



Epitope Predictions
EpiVax

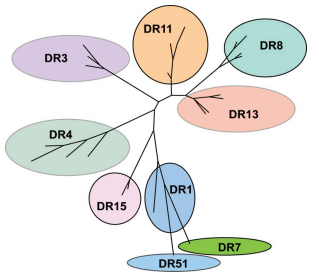
- EpiVax uses EpiMatrix to predict epitopes
  - –matrix based prediction algorithm
- Can predict either class I or class II MHC binding
  - –MHC binding is a prerequisite for immunogenicity



  - –Dedicated website available for vaccine design: iVAX
  - –http://www.epivax.com/vaccine-design-redesign/ivax-web-based-vaccine-design/

11

EpiVax HLA "Supertype" Coverage

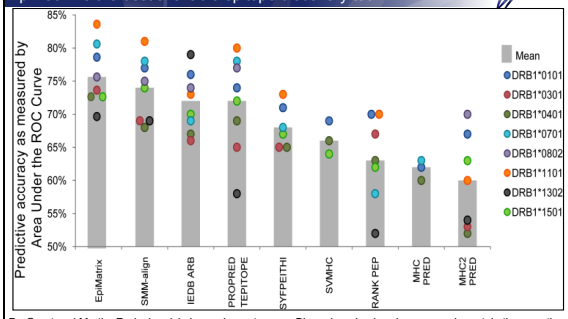


- **EpiVax tests for binding potential to the most common HLA molecules within each of the "supertypes" shown to the left.**
- **This allows us to provide results that are representative of >90% of human populations worldwide* without the necessity of testing each haplotype individually.**

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

EpiVax - Non-Confidential 12

Epitope Identification
EpiMatrix is the best available epitope discovery tool



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

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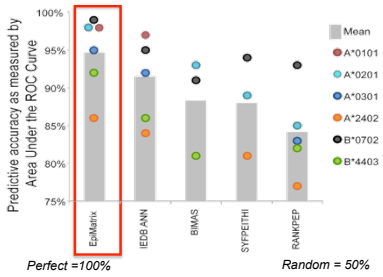
Epitope Identification
EpiMatrix is the best available epitope discovery tool

The accuracy of Class I prediction is even greater



Perfect =100% *Random = 50%*

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.

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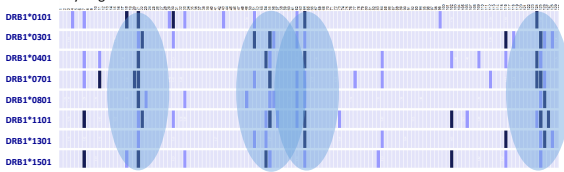
ClustiMer

- 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.
- ClustiMer recognizes T-cell epitope clusters as polypeptides predicted to bind to an unusually large number of HLA alleles.



- T-cell epitope clusters make excellent vaccine candidates:
  - compact; relatively easy to deliver as peptides; highly reactive in-vivo

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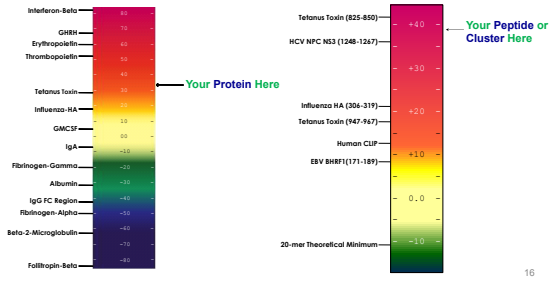
Immunogenicity Scales
EpiVax

EpiVax has developed an immunogenicity scale to compare predicted immunogenicity across **whole proteins**, **clusters** and **antibodies**. This allows for *easy comparison* between different sequences of different lengths and to known compounds.



16

iVAX Fully integrated From genome to vaccine

- EpiMatrix – maps T cell epitopes
- ClustiMer - Promisc ous / Superty
- BlastiMer - Avoiding "self" - toim
- Conservatrix – Identif e
- EpiAssembler - Imm noge Con
- Aggregatrix – Optim zing the cove
- VaxCAD - Processin g and Assemb

Seamless Vaccine Design

Integrated toolkit is *unique* to iVax

The main point . . .

- Significant improvements in Vaccine Production
- Significant improvements in Vaccine Delivery
- *. . . Problems with Vaccine Efficacy*

The main point . . .

- Significant improvements in Vaccine Production
- Significant improvements in Vaccine Delivery
- *. . . Problems with Vaccine Efficacy*
  - We Proposes Three Potential Solutions:
    1. *Better Vaccine Design*
    2. *Faster Vaccine Development*
    3. *More Vaccine Researchers using iVAX toolkit*

Computational Vaccinology *The current status*

- Computational vaccinology tools *are already in use*.
- Using these tools, we predicted the outcome of the H1N1 pandemic in 2009.
- In 2013, 10 months in advance of the clinical trials, we predicted the (poor) efficacy of H7N9 influenza vaccines.
- Using these tools, we can also design more effective vaccines.
- They may help reduce off-target effects for cancer immunotherapies, too.

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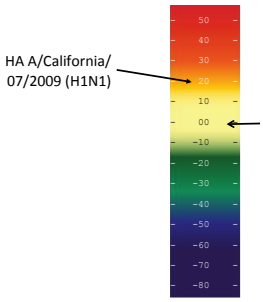
Example: H1N1 Pandemic Flu *Was a new vaccine really needed?*

- 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.

http://bit.ly/Pandemic_H1N1

New H1N1 Pandemic is Predicted to be Highly IMMUNOGENIC



- Immunogenicity Scale
- 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

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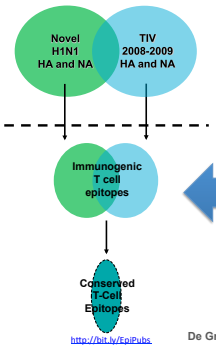
2009 H1N1 contains conserved epitope Sequences – Predicted Cross Protection



http://bit.ly/EpiPubs

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

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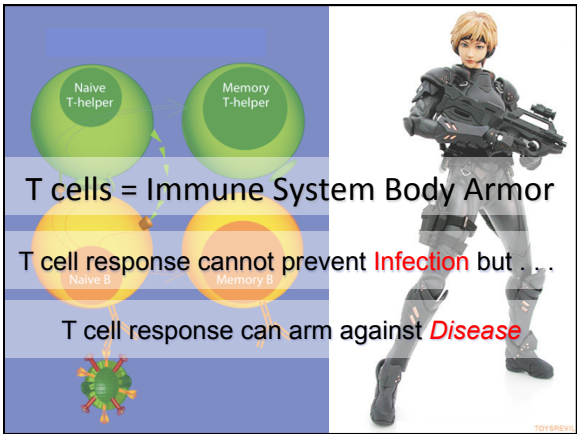

H1N1 Pandemic Flu
Was a new vaccine really needed?

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  - Despite lack of Ab cross-reactivity, we predicted protection against severe disease
  - H1N1 HA predicted to be highly immunogenic.
- Were we right?
  - Decline in cases before the vaccine arrived
  - Morbidity and Mortality substantially lower than expected

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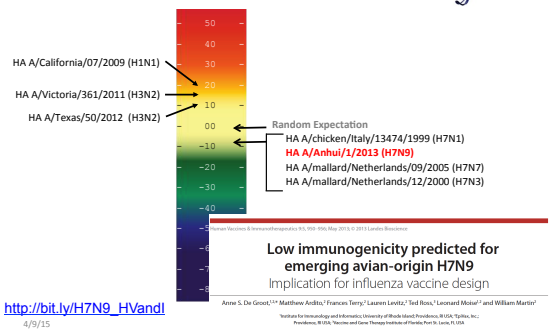
26

H7N9 Flu Predicted to be POORLY IMMUNOGENIC in April 2013



http://bit.ly/H7N9_HVandI

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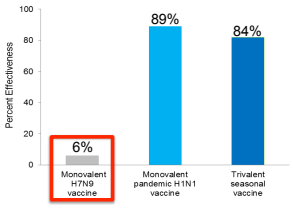
October 2013: New H7N9 Vaccines are CONFIRMED to be POORLY IMMUNOGENIC



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

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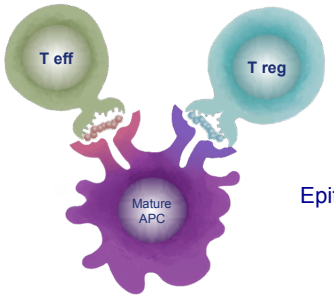
A new tool for finding Treg epitopes

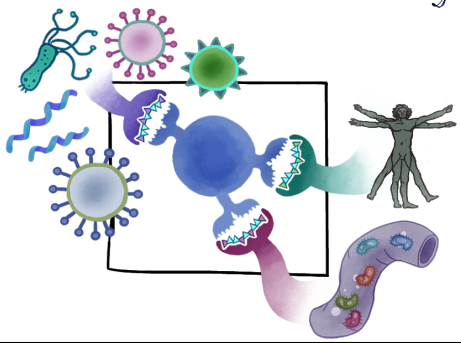
http://bit.ly/EpiPubs

Are Treg epitopes in vaccines?



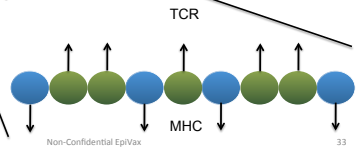
Epitope can be *either* T-eff or Treg

Immune Camouflage

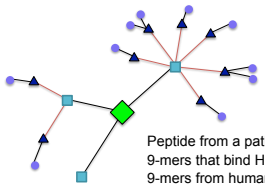


Predicting Treg epitopes: JanusMatrix

JanusMatrix is designed to predict the potential for cross-conservation between epitope clusters and the human genome, based on conservation of TCR-facing residues in their putative HLA ligands. These epitopes are not discovered using BLAST.



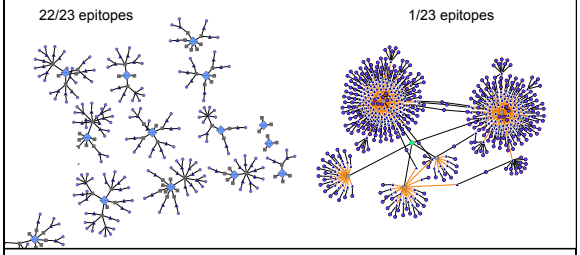
Networks of cross-conservation



Peptide from a pathogen
9-mers that bind HLA
9-mers from human genome that present same TCR face

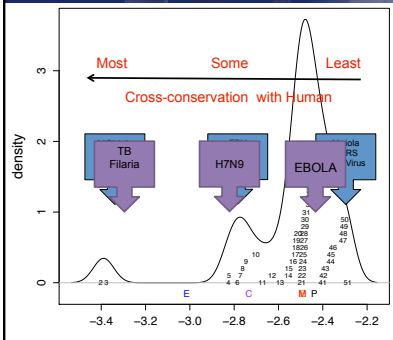
Source proteins of the human 9-mers

Example: HCV Vaccine Project
Cross-reactivity for 23 epitopes



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.

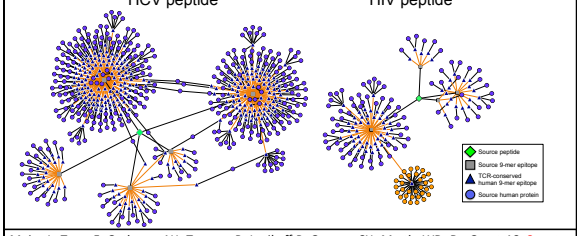
Evaluation of viruses for XR with Human Genome
Human Genome/Human Microbiome/Human Pathogens



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

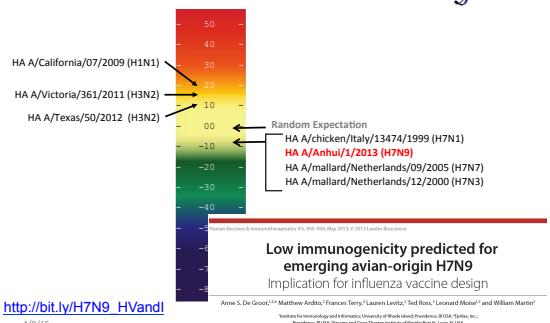
36

A new example:
HIV envelope



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)

Can we fix
POORLY IMMUNOGENIC H7N9 ?



Low immunogenicity predicted for emerging avian-origin H7N9
Implication for influenza vaccine design

http://bit.ly/H7N9_HVandI

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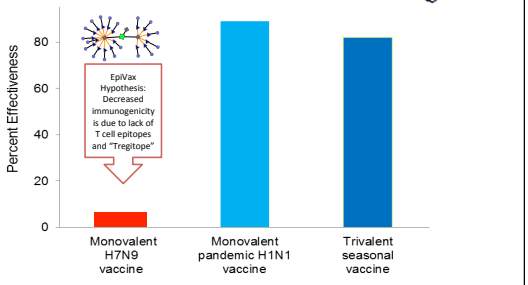
H7N9 contains
a regulatory T cell epitope



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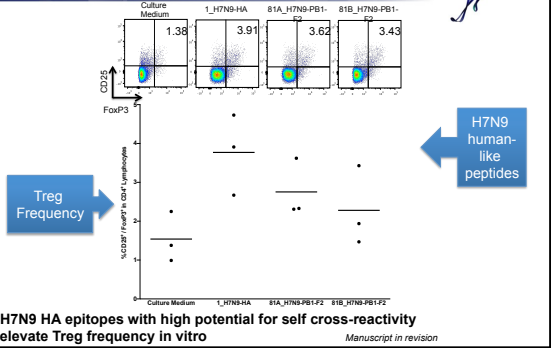
40

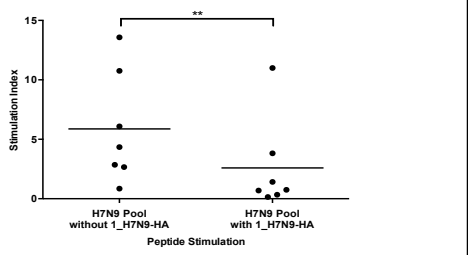
Immunosuppressive phenotype of human-like H7N9 peptides



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

Manuscript in revision

H7N9 Treg activating epitope suppresses IFNγ secretion



1_H7N9_HA = Treg activating epitope

Manuscript in revision

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

Identify potential regions where epitopes can be improved

Result: Epitope–enhanced HA

"Opt 1"

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

NIID: Opt_1 rH7-HA produced in insect cells

Epitope-Enhanced H7 HA Antigenicity: Opt_1 rH7-HA Immunization in Humanized Mice

Does immunization of mice with un-adjuvanted Opt_1 rH7-HA induce higher titers of antibody compared to wild type rH7-HA?

Human PBMC (not H7-immune) Japanese donors

NOD/SCID/Jak3$^{-/-}$

WT rH7-HA ⇨ Weak Ab response

Opt_1 rH7-HA ⇨ Better Ab response?

Endpoints:
1) IgG titers by ELISA
2) Plasma cell numbers by ELISpot

Each pair of mice received one subject PBMC
Eight pairs of mice were tested.

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

Ted Ross - VGTI Epitope Enhanced H7 HA

"Opt 1" in HLA DR tg mice

VGTI FLORIDA

VGTI: Treg epitope-deleted Opt_1 rH7-HA Trimers produced

H7N9 summary

- Sequence analysis of the two faces of T cell epitopes enables prediction of epitope phenotype (Treg vs Teff).
- Epitopes that share a TCR-face with numerous human sequences may activate Tregs.
- Modifying the Treg epitope is possible, and it results in higher titers of antibody, at an earlier timepoint than wild type.
- Two independent laboratories confirmed this result. Unpublished data (not shown in the PDF version of this slideset) from NIID and VGTI confirms this hypothesis.

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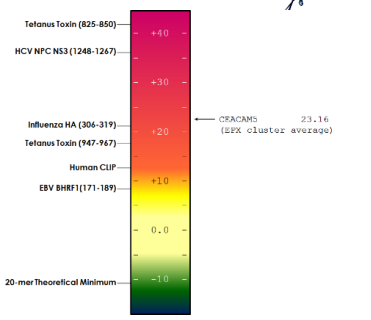
Case Study CEA and Mage A3

- Analysis of Cancer Antigens:
  - Carcinoembryonic antigen **(CEA)**
  - Melanoma-associated antigen 3 **(MAGE-A3)**
    GlaxoSmithKline cancer vaccine
    Failed in Phase III trials

CEA

- JanusMatrix analysis (Class II)



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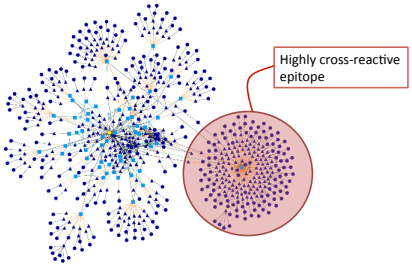

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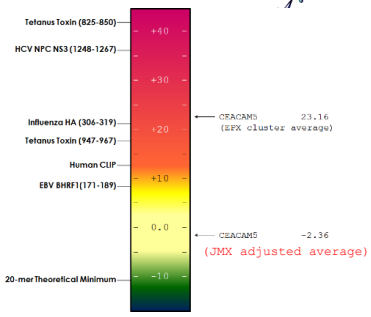


Case Study

- Analysis of Cancer Antigens:
  - Carcinoembryonic antigen (**CEA**)
  - Melanoma-associated antigen 3 **(MAGE-A3)**
    T cell Therapy (CAR)
  - Failed in Phase III trials (GSK)

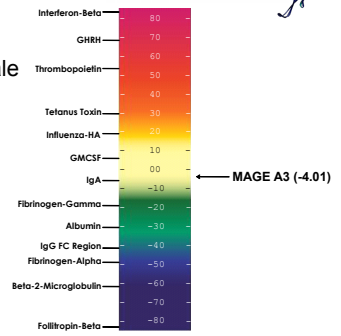
Melanoma-associated antigen 3 MAGE-A3

Clinical Case: Mage-A3

- MAGE-A3 is expressed at the surface of many cancer cells
- MAGE-A3 was the target of GlaxoSmithKline cancer vaccine
- Vaccine failed in Phase III trials due to low immune responses in patients

MAGE-A3 Class II EpiMatrix Results

Class II analysis: immunogenicity scale



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- Vaccines "on Demand" – Lassa Fever, Ebola

Off Target Effects of Vaccines MAGE-A3

Clinical Case: Titin

- MAGE-A3 CAR therapy
- 2 patients died in a clinical trial
- Can JanusMatrix be used to predict off-target effects

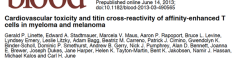
Titin Example: JanusMatrix Cross-Reactivity Case

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.
- These cases highlight the need for improved methods to define the specificity of engineered TCRs
- Affinity-enhanced TCR and importance of specificity to antigens (Adaptimuune)

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



Peptide from Titin Identified as Cross-Conserved with MAGE A3 target

Science Translational Medicine AAAS

Identification of a Titin-Derived HLA-A1–Presented Peptide as a Cross-Reactive Target for Engineered MAGE A3–Directed T Cells
Brian J. Cameron *et al.*
Sci Transl Med **5**, 197ra103 (2013);
DOI: 10.1126/scitranslmed.3006034

Editor's Summary

Cross-Reactive Adoptive Therapy

Engineering T cells with enhanced affinity to cancer targets is a promising therapy. However, one key bottleneck in this strategy is the identification of targets that are expressed on cancer cells but not on normal healthy tissue. One way to identify these antigens is by looking at the family of cancer-testis antigens, which have restricted expression in normal tissue but are frequently up-regulated in tumors. Cameron *et al.* now report that a T cell engineered to target one such antigen —MAGE A3—cross-reacts with a peptide from a muscle protein, Titin.

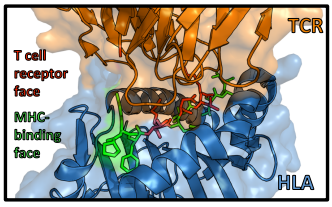
The authors developed a T cell that targeted a MAGE A3 antigen for use in adoptive immunotherapy. Although extensive preclinical investigations demonstrated no off-target antigen recognition, patients who received these T cells had serious adverse events, including fatal cardiac toxicity. The authors then used amino acid scanning to search for potential cross-reactivity of these T cells with an off-target peptide and identified a peptide derived from the muscle protein Titin. Because affinity-enhanced T cells are highly potent, this cross-reactivity was likely the cause of the off-target toxicity. This study highlights methods that may be used to prevent cross-reactivity in future trials of adoptive immunotherapy.

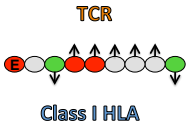
How to Avoid Cross-Reactivity

- In general, for HLA Class I, peptide positions 4-8 can make contact with TCR.
- However, for any given allele-peptide-TCR complex, only some of these positions are important for TCR recognition.
- Further, for some alleles positions 1, 2, and 3 may also be important.
- In this environment bulk screening for potential cross reactivity is impossible.
- **So how can potentially dangerous cross-reactivity** such as observed between A0101, Mage-A3 peptide, and Titin (Linette GP et. al., Blood 2013) **be avoided**?

Class I JanusMatrix

Search for cross-conserved class I T cell epitopes

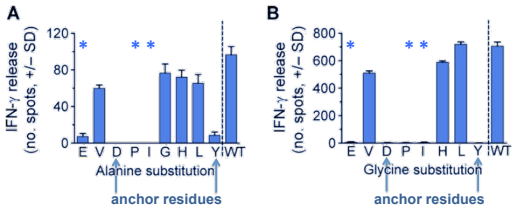




Class I TCR Contacts

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

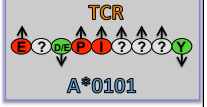
Cameron et al. identified key TCR contact residues (*) in MAGE A3 peptide:



How to Avoid Cross-Reactivity

- Alanine scan of candidate peptide can be used to identify peptide positions relevant for this allele-peptide-TCR complex.
- Based on results of alanine scan, we can screen the human genome for potential cross-reactivity.
- Alanine scan of MAGE-A3 peptide identifies positions 1, 4, and 5 as important to TCR contact and positions 3 and 9 as important for MHC binding.
- Peptide: EV D PIGHLY
- Regular expression: E?[D|E]PI???Y
- EpiVax allowed D or E in position 3, based on knowledge of A*0101 binding preferences.
- This detail, missed by Cameron et al., yields one additional target.

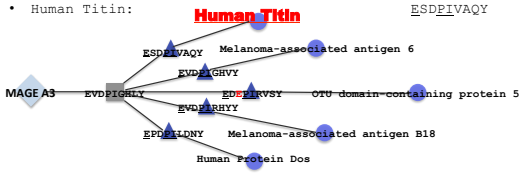
New assumptions:



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: EPEPIRVSY
- 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.

Outline

- Vaccine Design Tools
- Predicting Vaccine Efficacy
  - H1N1 and H7N9
- Engineering Better Vaccines
  - H7N9
  - CEA/Mage A3
- Off Target Effects of Vaccines
- Vaccines “on Demand” – Lassa Fever, Ebola

FastVax™

A Platform for Delivering Faster Vaccines for Bio- and Agro-Defense

Mandate – Faster, Safer Vaccines

The mandate – Develop vaccines for WMD – pathogen unknown, immediately

Does this capacity exist? Yes.

The components:

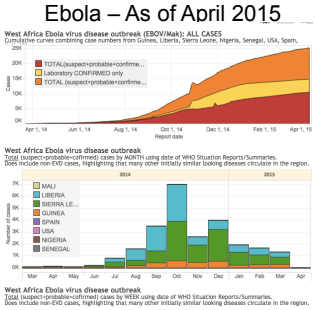
- •High throughput computing
- •Immunoinformatics
- •Vaccine design algorithms
- •DNA synthesis, scale up, preparation of delivery device
- •Delivery via iontophoresis / skin patch
- •Animal safety/tox/immunogenicity/validation
- •Deployment by mail/pharmacy distribution systems

Rapid deployment when genome sequence is in hand

This can be prebuilt!!

Vaccines on Demand

Ebola – As of April 2015



http://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/index.html

Ebola vaccines now in clinical trials

ChAd3-ZEBOV GlaxoSmithKline and PHAC	Phase II	TBD, Cameroon TBD, Ghana TBD, Mali TBD, Nigeria TBD, Senegal	1Q2015
rVSV-ZEBOV NewLink Genetics and Merck Vaccines USA	Phase III	By WHO and MOH Guinea in Conakry, Guinea	March 2015 – Ring vaccination trial design

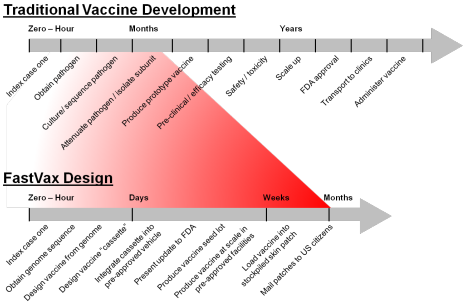
Encodes wild type glycoprotein (GP) from Zaire Ebolavirus

Encodes wild type glycoprotein (GP) from Zaire Ebolavirus

- Dramatic progress within 12 months
- These vaccines were "on the shelf"
- What about the next pandemic?
- Do we need a "vaccine on the shelf" for every EID?

http://www.who.int/medicines/emp_ebola_q_as/en/

FastVax Accelerated Timeline



SAV-L (Lassa): 18 Peptides For Class I/Class II Coverage Across Six HLA Superclasses

Class I

Lassa Protein	Peptide Length	Frame Start	AA Sequence	Frame Stop	A0101 Z-Score	A0201 Z-Score	A0301 Z-Score	A2402 Z-Score	B0702 Z-Score	B4403 Z-Score	Hits	Sum of Scores
GP	9	411		419	4.59	-0.84	1.65	0.01	-0.11	-0.01	2	6.24
GP	9	434		442	1.67	1.05	1.56	1.27	1.26	1.28	3	6.6
GP	9	312		320	-0.24	0.99	3.22	0.4	-0.03	-0.62	1	3.22
GP	9	139		147	0.16	-0.89	0.55	3.38	1.13	1.92	2	5.29
GP	9	237		245	-0.04	1.36	-0.35	1.29	1.16	1.59	1	3.16
GP	10	269		278	1.84	-0.73	0.49	0.04	-0.12	3.51	2	5.35
GP	10	120		129	4.19	-1.31	2.05	-0.19	-0.29	-0.12	2	6.24
GP	10	478		487	0.17	2.23	0.51	-0.19	0.1	0.54	1	2.23
GP	9	344		352	1.58	1.06	2.72	-0.39	-0.56	-0.97	1	2.78
GP	9	392		400	0.28	0.13	-0.07	2.48	-0.54	-0.57	1	2.48
GP	9	452		460	-1.33	-1.28	-0.3	-0.33	2.44	-0.11	1	2.44
GP	9	328		336	0.18	0.53	-0.48	0.92	0.94	2.09	1	2.09

Class II

Lassa Protein	Cluster Address	Cluster Sequence	Hits	Cluster Score	EpiBars? (#)
GP	159 - 177		16	30.57	Yes (3)
GP	438 - 460		18	30.24	Yes (2)
GP	330 - 348		16	30.18	Yes (1)
GP	127 - 151		17	27.81	Yes (2)
GP	238 - 255		12	24.45	Yes (2)
GP	315 - 336		15	24.34	Yes (2)

Can Computational Vaccinology *Design vaccines on demand?*

- Computational vaccinology tools *are already in use*.
- Where sequences are available, vaccine antigens and/ or immunogenic epitopes can be down-selected within 24 hours.
- Were a suitable vector available that had been pre-approved, a vaccine could be designed and produced 'on demand'.
- In the case of Ebola, that was not necessary.
- Will we be ready to make a 'vaccine on demand' in the near future?

Summary

- Computational vaccinology tools like iVAX can:
  - Rapidly analyze pathogens
  - Evaluate immunogenic epitopes for HLA Class I and II MHC
  - Quickly identify regions of interest for vaccine development
  - Screen candidates against human germline sequences
  - Eliminate deleterious epitopes
  - Accelerate development of vaccines for biothreats
- Ebola Virus analysis is posted on EpiVax.com
- We hope our analysis and predictions will help in the fight against Ebola

Summary

- Using computational tools available today, we can analyze a new pathogen's sequence in 24 hours, and tell you whether a vaccine is needed, and which vaccine will be effective, and which will not.
- The 21st century is the *century of vaccines and computational vaccinology*.
- We can see the future.
- Using computational tools, we can make progress towards the future.

Science without fear.

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