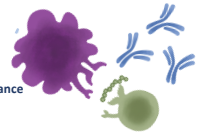
Welcome to Immunogenicity and Tolerance

Anne S. De Groot MD
Katie Porter
Paul von Hoegen
Immunogenicity and Tolerance
Amsterdam, 15 Nov 2019



EpiVax

EpiVax Immune Engineers

Immunogenicity and Tolerance: Setting the stage. . .

Anne S. De Groot MD
CEO/CSO, EpiVax, Inc.
Director, Institute for Immunology and Informatics
Professor (Research) Univ. Rhode Island

EpiVax

Immunogenicity and Tolerance – Relevant to vaccines, allergy therapy, and biologics design

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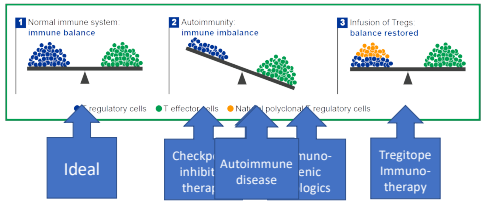


Immunogenicity Assessment by EpiVax Twenty Years of Progress

EpiVax

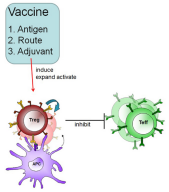
EpiVax designs and develops safer, more effective vaccines and biologics

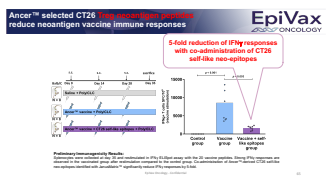
Trusted	Cutting Edge	Engaged
12 of the largest pharma companies and hundreds of small companies and institutions	Continuously responding to our client's needs and developing new, innovative tools	Strong corporate values: *Improving Human Health Everywhere*

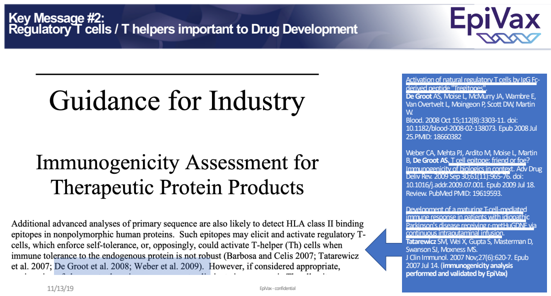
Science without fear.

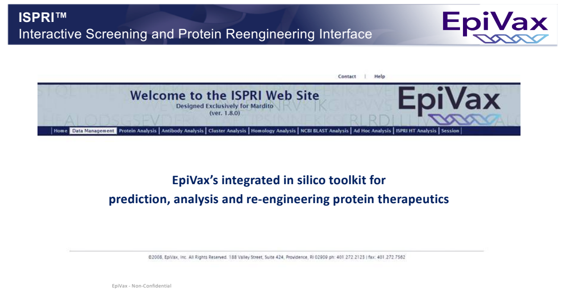
Key Messages #1: Treg and Teff are important in Vaccine Design Role of regulatory T cells in Vaccines including Cancer Vaccines

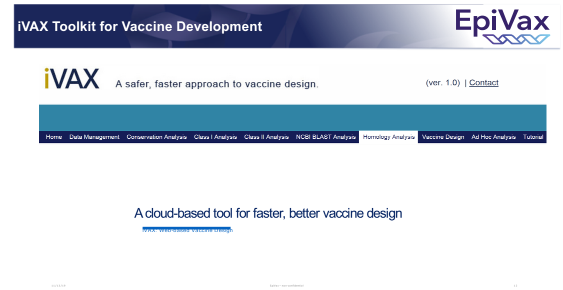
EpiVax

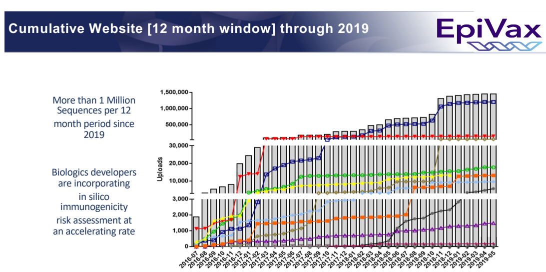


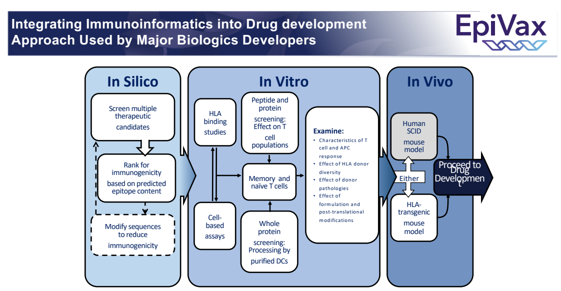


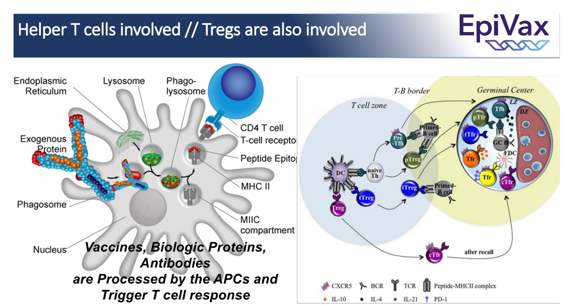

Key Message #2:
Regulatory T cells / T helpers important to Drug Development
EpiVax
Guidance for Industry
Immunogenicity Assessment for Therapeutic Protein Products
Additional advanced analyses of primary sequence are also likely to detect HLA class II binding epitopes in nonpolymorphic human proteins. Such epitopes may elicit and activate regulatory T-cells, which enforce self-tolerance, or, opposingly, could activate T-helper (Th) cells when immune tolerance to the endogenous protein is not robust (Barbosa and Celis 2007; Tatarewicz et al. 2007; De Groot et al. 2008; Weber et al. 2009). However, if considered appropriate,
11/13/19


ISPRI™
Interactive Screening and Protein Reengineering Interface
EpiVax
Welcome to the ISPRI Web Site
EpiVax's integrated in silico toolkit for
prediction, analysis and re-engineering protein therapeutics

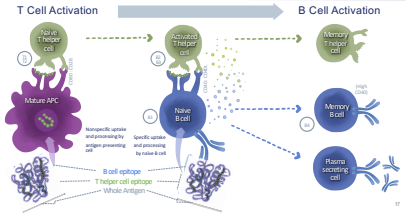

iVAX Toolkit for Vaccine Development
EpiVax
iVAX
A safer, faster approach to vaccine design.
(ver. 1.0) | Contact
A cloud-based tool for faster, better vaccine design


Cumulative Website [12 month window] through 2019
EpiVax
More than 1 Million Sequences per 12 month period since 2019
Biologics developers are incorporating in silico immunogenicity risk assessment at an accelerating rate
Uploads


Integrating Immunoinformatics into Drug development
Approach Used by Major Biologics Developers
EpiVax
In Silico
In Vitro
In Vivo
Screen multiple therapeutic candidates
Rank for immunogenicity based on predicted epitope content
Modify sequences to reduce immunogenicity
HLA binding studies
Cell-based assays
Peptide and protein screening: Effect on T cell populations
Memory and naïve T cells
Whole protein screening: Processing by purified DCs
Examine:
Human SCID mouse model
Either
HLA-transgenic mouse model
Proceed to Drug Development

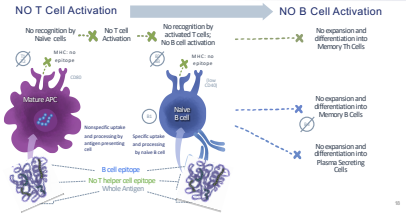

Helper T cells involved // Tregs are also involved
EpiVax
Endoplasmic Reticulum
Lysosome
Phago-lysosome
Exogenous Protein
CD4 T cell T-cell receptor
Peptide Epitope
MHC II
Phagosome
MIIC compartment
Nucleus
Vaccines, Biologic Proteins, Antibodies are Processed by the APCs and Trigger T cell response
T cell zone
T-B border
Germinal Center
after recall

Presence of T cell epitopes drives ADA



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

Absence of T cell epitopes reduces ADA

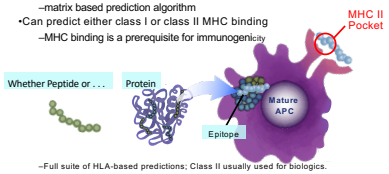


Lack of T cell epitopes abrogates activation of CD4 T cells and T-dependent antibody response

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

Identifying T cell epitopes Is key to assessing Immunogenicity Risk

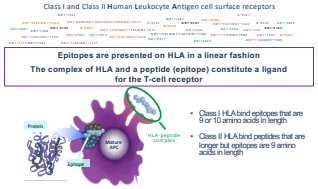
- EpiVax uses EpiMatrix to predict epitopes
  - –matrix based prediction algorithm
- Can predict either class I or class II MHC binding
  - –MHC binding is a prerequisite for immunogenicity



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

6/30/2015 20

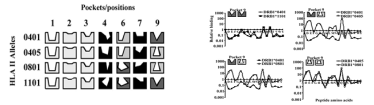
T cell Dependent Immunogenicity HLA binding is a prerequisite for immunogenicity



- Class I HLA bind epitopes that are 9 or 10 amino acids in length
- Class II HLA bind peptides that are longer but epitopes are 9 amino acids in length

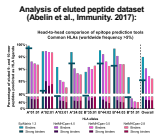
HLA Supertypes Concepts: Pocket Profiles

- Sturniolo and Hammer (below) first described how binding pockets within the HLA DR binding groove are shaped by clusters of polymorphic amino acid residues and therefore different HLA DR alleles have distinct chemical and size characteristics.

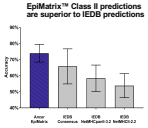


Reference:
Sturniolo T, Bono E, Ding J, Raddrizzani L, Tuereci O, Sahin U, Braxenthaler M, Gallazzi F, Protti MP, Sinigaglia F, Hammer J. Generation of tissue-specific and promiscuous HLA ligand databases using DNA microarrays and virtual HLA class II matrices. Nat Biotechnol. 1999 Jun;17(6):555-61.

Accuracy: EpiMatrix™ is the gold standard for Class I (CD8) and class II (CD4) epitope prediction



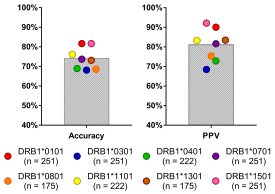
95% of eluted 9- and 10-mers were predicted to bind to HLA according to EpiMatrix®, while only ~68% of ligands were accurately recalled by NetMHCpan.



EpiMatrix™ Class II predictions are **74% accurate** when prospectively tested in *in vitro* HLA binding assays. IEDB predictions are 54-66% accurate when tested against the same set of peptides.

In vitro validation of prospective EpiMatrix™ selection of Class II epitopes — New data

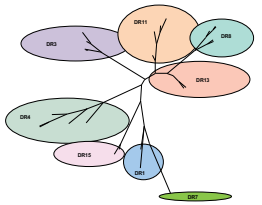
EpiMatrix™ Class II predictions are **74% accurate** when tested in *in vitro* HLA binding assays, average observed **PPV of 81%**.



Metric	Avg [range]
Accuracy	74% [68%-82%]
Sensitivity	88% [83%-90%]
Specificity	25% [13%-34%]
PPV	81% [68%-92%]
NPV	36% [17%-67%]

Source: peptides prospectively selected by EpiMatrix® and tested in *in vitro* HLA binding assays.

EpiMatrix™ HLA "Supertype" Coverage



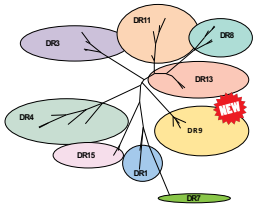
EpiVax tests for binding potential to the most common HLA molecules within each of the "supertypes"* shown to the left.

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

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

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

New: HLA DR9 Supertypes - Updated



Increased Coverage of Asian Populations

MHC restriction data and corresponding population frequencies were uploaded on:

http://tools.immuneepitope.org/tools/population/iedb_input

In order to obtain coverage statistics.

Population frequencies were collected from http://www.ncbi.nlm.nih.gov/projects/gv/mhc/ihwg.cgi?cmd=PRJOV&ID=9

ISPRI Population Coverage by Supertype Allele

(w/ DRB1*08 but w/o DRB1*09)

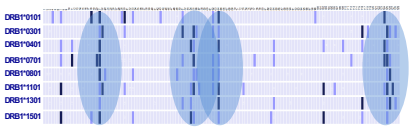
Supertype Allele	Europe + North America	Central + South America	North Africa	Sub-Sahara Africa	Middle East	East Asia
DRB1*01	21.15%	8.58%	10.51%	2.78%	17.80%	2.98%
DRB1*03	26.90%	8.77%	31.11%	26.38%	19.68%	5.91%
DRB1*04	31.61%	27.33%	21.85%	4.55%	15.54%	16.64%
DRB1*07	27.41%	8.01%	28.26%	10.32%	15.01%	5.72%
DRB1*08	5.33%	27.67%	4.94%	17.55%	6.00%	19.36%
DRB1*11	10.70%	9.91%	18.82%	32.10%	26.20%	17.19%
DRB1*13	16.09%	8.58%	20.61%	30.28%	20.03%	6.88%
DRB1*15	31.61%	7.63%	19.54%	23.79%	22.06%	21.50%
Total	**99.31%**	**80.67%**	**97.08%**	**95.67%**	**93.77%**	**75.20%**

ISPRI Population Coverage (Including DRB1*09) — Updated

Supertype Allele	Europe + North America	Central + South America	North Africa	Sub-Sahara Africa	Middle East	East Asia
DRB1*01	21.15%	8.58%	10.51%	2.78%	17.80%	2.98%
DRB1*03	26.90%	8.77%	31.11%	26.38%	19.68%	5.91%
DRB1*04	31.61%	27.33%	21.85%	4.55%	15.54%	16.64%
DRB1*07	27.41%	8.01%	28.26%	10.32%	15.01%	5.72%
DRB1*08	5.33%	27.67%	4.94%	17.55%	6.00%	19.36%
DRB1*09	1.20%	7.82%	1.99%	2.19%	2.46%	15.73%
DRB1*11	10.70%	9.91%	18.82%	32.10%	26.20%	17.19%
DRB1*13	16.09%	8.58%	20.61%	30.28%	20.03%	6.88%
DRB1*15	31.61%	7.63%	19.54%	23.79%	22.06%	21.50%
Total	99.41%	84.02%	97.41%	96.12%	94.38%	82.69%

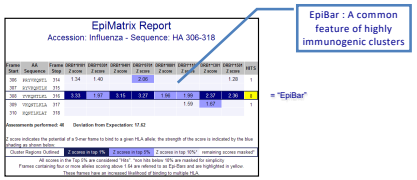
The inclusion of DR9 increases our population coverage for East Asia

ClustiMer/Class II – Regions of Epitope Density



- T cell epitopes are not randomly distributed throughout protein sequences, but instead tend to **cluster** in specific regions.
- These clusters can be very powerful. One or more dominant T cell epitope clusters can **enable significant immune responses to even otherwise low-scoring proteins.**
- ClustiMer is used to identify T cell epitope clusters. It identifies short polypeptides predicted to bind to an unusually large number of HLA alleles.
- T cell epitope clusters make excellent vaccine candidates: they are compact, relatively easy to deliver as peptides, and highly reactive in vivo.

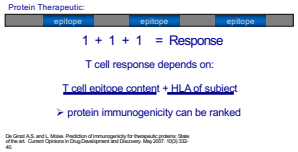
"Promiscuous" epitopes – regions of concentrated immunogenicity – "**EpiBars**"



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

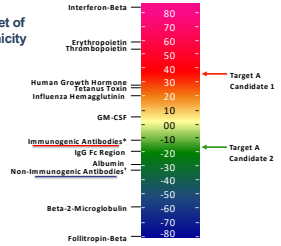
Risk Assessment Approach: Whole Proteins



De Groot A.S. and L. Moise. Prediction of immunogenicity for therapeutic proteins: State of the art. Current Opinions in Drug Development and Discovery. May 2007. 10(3):332-40.

Risk Assessment – Comparing Candidates
Monoclonal antibodies - Adjust for Treg Epitopes (Tregitopes)

Rank your protein relative to a set of proteins with known immunogenicity

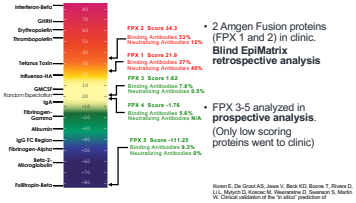


*Average of antibodies known to induce anti-therapeutic responses in **more** than 5% of patients

†Average of antibodies known to induce anti-therapeutic responses in **less** than 5% of patients

All scores are adjusted for the presence of Tregitopes.

Validation: Correlation of EpiMatrix scores and immunogenicity in human studies (Published)



- 2 Amgen Fusion proteins (FPX 1 and 2) in clinic.

Blind EpiMatrix retrospective analysis

- FPX 3-5 analyzed in **prospective analysis**. (Only low scoring proteins went to clinic)

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

Calibration of Immunogenicity Scale New

Question: What is the "True Zero" for the human genome?
Answer: It is lower than the "True Zero" for random proteins.

Method: Analyze the EpiMatrix scores of human proteins from different subcellular locations to include them as references in our immunogenicity scale. (Analysis performed by Andres Gutierrez, not published)

- Gather subcellular location and signal peptide information for the human proteome and generate subsets based on these data.
- Calculate immunogenicity scores for human proteins using 9 supertype Class II alleles, excluding signal peptides.
- Compare predicted immunogenicity of different subsets.
- Determine which subsets will be shown in the immunogenicity scale.

Evaluate hypothesis - *T cell epitopes, which could support deleterious autoimmune activity, will tend to be deleted from the human proteome.*

Results: Human Protein EpiMatrix Scores
Summary of EpiMatrix Scores by subset: Secreted are Lowest

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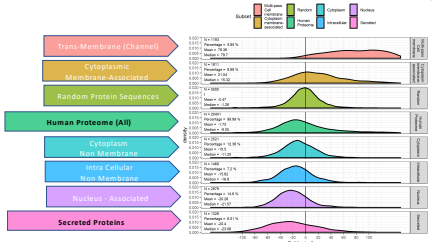
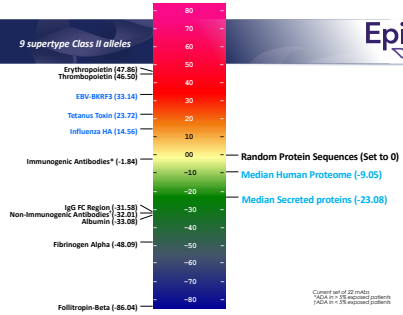


9 supertype Class II alleles

EpiVax



Characterizing Putative T cell Phenotype

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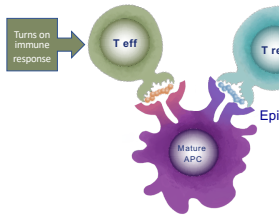


Epitopes can be *either* effector or regulatory

Most in silico algorithms cannot differentiate between these two

Tregitopes™
Regulatory T cell Epitopes

2008 EpiVax

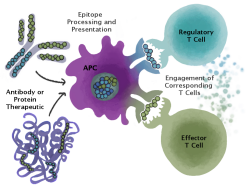
Tregitopes™:
- Discovered in 2008
- In Fc and Fab (framework)
- Bind multiple HLA alleles
- Are highly conserved in IgG
- Are correlated with low immunogenicity
- May be present in other self proteins
- ARE DISCOVERABLE WITH IMMUNOINFORMATICS

We hypothesize that antibody-derived Treg epitopes activate regulatory T cells that lead to suppression of effector T cells that recognize effector epitopes, like those of IgG hypervariable regions to which central tolerance does not exist.

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

Tregitopes Actively Suppress Immune Response and Induce Antigen-Specific Tolerance

EpiVax



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

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

Prediction Approach – Antibodies
In silico tools need to account for Treg epitopes!

EpiVax

Monoclonal Antibodies:

1 + 1 - 1 = Response

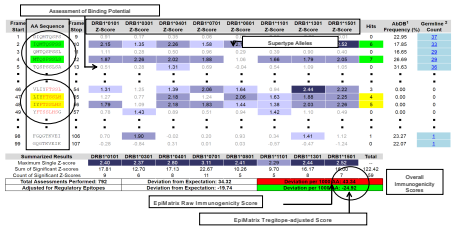
T cell response depends on:

T cell epitope content – Tregitope content and also (HLA of subject)

➢ **mAb immunogenicity can be ranked too**

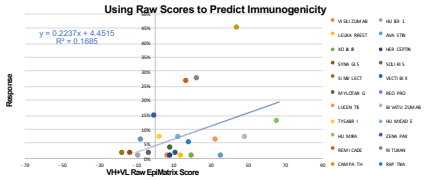
EpiMatrix™ - Tregitope is factored in
Screen Protein Sequence for T Cell Epitopes

EpiVax



Can we predict antibody immunogenicity?
Without Tregitope Adjustment

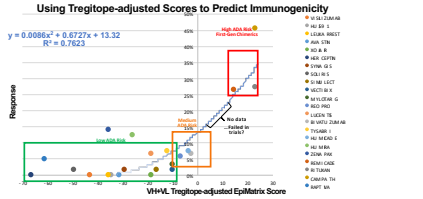
EpiVax



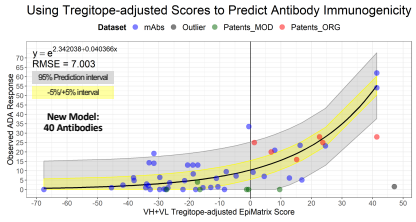
Can we predict antibody immunogenicity?
With Tregitope Adjustment Yes!

2009

EpiVax



Tregitope-Adjusted Antibody Immunogenicity Predictions



Model comparison
Add HLA DR-9, ~20 new mAbs

Know / Risk Yet Integrated Into Score

2019

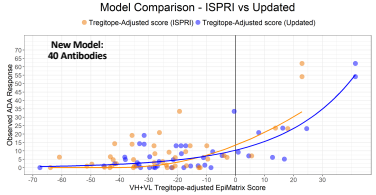
EpiVax

Updated EpiVax Antibody Immunogenicity Risk Assessment tool to be released in 2019.

Model adds 20 new mAb examples with clinical immunogenicity data and predictions for new allele HLA DRB1*0901, increasing coverage in Asian populations.

Original model is also validated, as new model follows highly similar pattern.

Tregitope-Adjusted Antibody Immunogenicity Predictions



Case Study – Pfizer Discontinues PCSK9 antibody
Drug Fails (Produced Prior to incorporating ISPRI assessment)

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NEWS / Pfizer Discontinues Global Development of Bococizumab, Its Investigational PCSK9 Inhibitor

PFIZER DISCONTINUES GLOBAL DEVELOPMENT OF BOCOCIZUMAB, ITS INVESTIGATIONAL PCSK9 INHIBITOR

Company will record a charge to GAAP and Adjusted earnings in the fourth quarter of 2016 estimated to be approximately $0.04 per share

Tuesday, November 1, 2016 - 6:30am
EDT

Pfizer Inc. announced today the discontinuation of the global clinical development program for bococizumab, its investigational Proprotein Convertase Subtilisin Kexin type 9 inhibitor (PCSK9). The totality of clinical information now available for bococizumab, taken together with the evolving treatment and market landscape for lipid-lowering agents, indicates that bococizumab is not likely to provide value to patients, physicians, or shareholders. As a result, Pfizer has decided to discontinue the development program, including the two ongoing cardiovascular outcome studies.

With the completion of six bococizumab lipid-lowering studies, Pfizer has observed an emerging clinical profile that includes an unanticipated attenuation of low-density lipoprotein cholesterol (LDL-C) lowering over time, as well as a higher level of immunogenicity and higher rate of injection-site reactions with bococizumab than shown with the other agents in this class. The goal of treating elevated cholesterol is to reduce the occurrence of cardiovascular events such as heart attacks and stroke, which requires long-term

Recent Example - Bococizumab
Anti-PCSK9 antibodies

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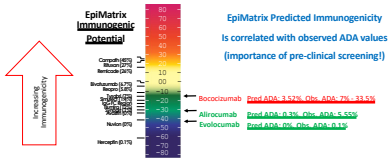
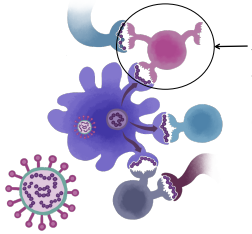


T cells Recognize Epitope Surfaces – TCR facing contour
May be conserved with Self or Other Pathogens



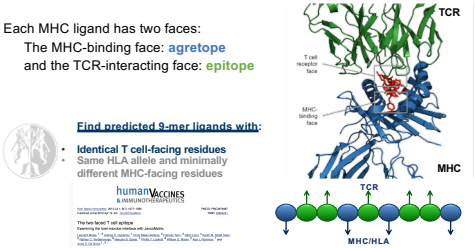
Pathogens may be able to evolve T cell epitopes that trigger Tregs instead of T effectors .

Tool for defining Tregs using "Epitope Networks"
JanusMatrix

2013

EpiVax

Each MHC ligand has two faces:
The MHC-binding face: agretope
and the TCR-interacting face: epitope



Find predicted 9-mer ligands with:

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

humanVACCINES & IMMUNOTHERAPEUTICS

Networks used to provide visual map of epitope cross-conservation

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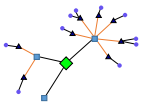

- Peptide from a drug, antigen, etc.
- 9-mers that bind HLA
- 9-mers from human genome that present same TCR
- Source proteins of the human 9-mers

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

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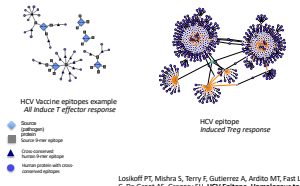

Loskioff PT, Mishra S, Terry F, Gutierrez A, Ardito MT, Fast L, Nevola M, Martin WD, Bailey-Kellogg C, De Groot AS, Gregory SH. **HCV Epitope, Homologous to Multiple Human Protein Sequences, Induces a Regulatory T Cell Response in Infected Patients.** J Hepatol. 2014 Aug 22. pii: S0168-8278(14)00613-8.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

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

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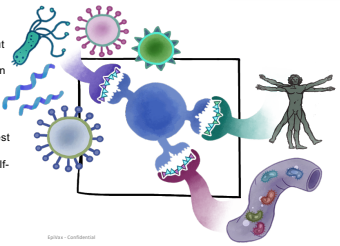
Immune Camouflage
Originated with discovery about pathogens "copy/pasting" epitopes that looked like human Treg epitopes in their own genomes

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



humanVACCINES & IMMUNOTHERAPEUTICS

JanusMatrix reveals differences between hit-and-run and hit-and-stay viruses

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

BMC Bioinformatics

RESEARCH Open Access

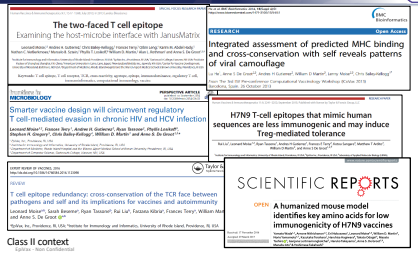
Integrated assessment of predicted MHC binding and cross-conservation with self reveals patterns of viral camouflage

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

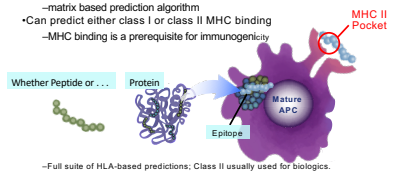
JanusMatrix Publications



Class II context

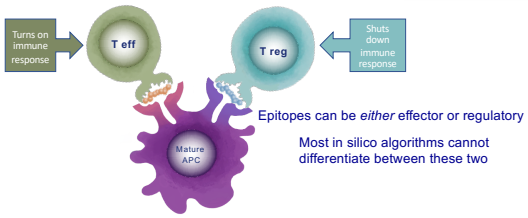
Identifying T cell epitopes Is key to assessing Immunogenicity Risk

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



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

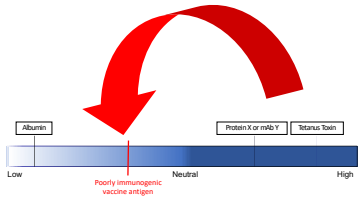
Characterizing Putative T cell Phenotype



Epitopes can be *either* effector or regulatory

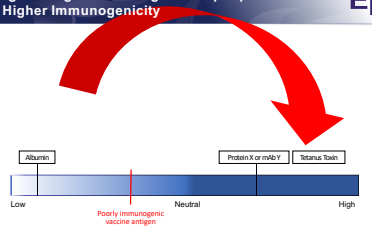
Most in silico algorithms cannot differentiate between these two

Immune-engineering - Lower Effector T Cell Epitope Content = Lower Immunogenicity



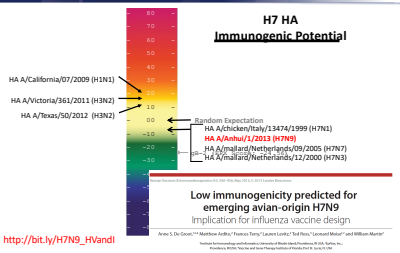
In fact, 'deimmunization' already happens "naturally!" in the context of infectious disease (HIV, HCV etc.)

Immune-engineering – Increasing T cell Epitope Content = Higher Immunogenicity



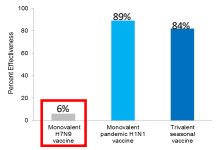
New H7N9 Flu *Emerges in China*
We predict that it is *poorly immunogenic*

EpiVax



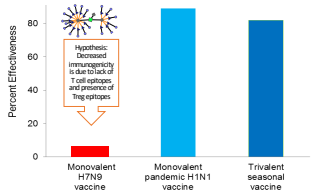
http://bit.ly/H7N9_HVandI

First H7N9 Vaccines are POORLY IMMUNOGENIC

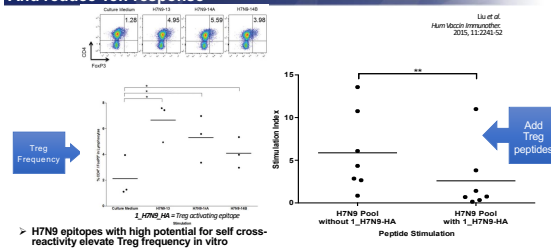


Griffin MR et al. PLoS One 2011 Aug 6(8);
Goodwin et al. Vaccine 2006(24):1159-1169; CDC MMWR September 20, 2013/ 62(RR07): 1-43

Does H7N9 contain a regulatory T cell epitope?



Treg-like H7N9 peptides Induce Tregs And reduce Teff response



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

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

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	297	309
H7-HA	PRYVKQRSILLAT	Treg Epitope
	306	318
H3-HA	PRYVKQNTLKLAT	T Eff Epitope
	297	309
H7-HA-Opt1	PRYVKQNTLKLAT	T Eff Epitope

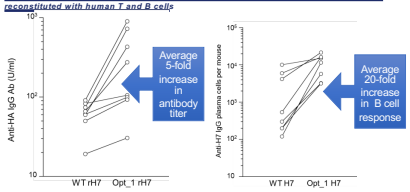
simultaneous Treg epitope knock-out and Teff epitope knock-in

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

Epitope-Enhanced H7 HA Antigenicity
"Opt_1 rH7 HA" Optimized with 3 AA changes – Tested in mice by NIID

EpiVax

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



(Study performed in collaboration with NIID Japan)

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

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

EpiVax

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

Identify potential regions where epitopes can be improved
Remove Treg Epitopes

Result: **20-Fold More Immunogenic**

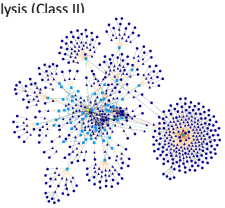
SCIENTIFIC REPORTS
OPEN
A humanized mouse model identifies key amino acids for low immunogenicity of H7N9 vaccines

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

Cancer Antigens also contain Treg epitopes

EpiVax

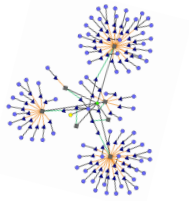
- JanusMatrix analysis (Class II)



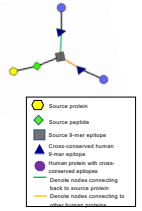
Melan A (MAR1, Uniprot ID: Q16655)

EpiVax

Peptide 1; JanusMatrix Score: 9.37



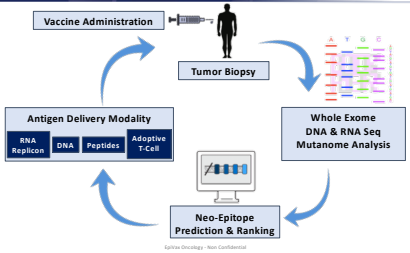
Peptide 2; JanusMatrix Score: 1.14



EpiVax - confidential

EpiVax Oncology NEO-PCV approach based on novel algorithm platform – Ancer™

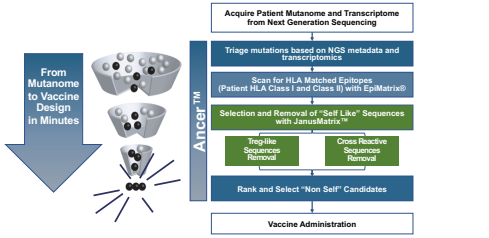
EpiVax ONCOLOGY



EpiVax Oncology - Non Confidential

EpiVax Oncology NEO-PCV approach based on novel algorithm platform – Ancer™

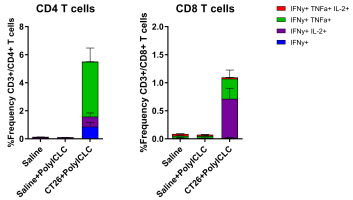
EpiVax ONCOLOGY



EpiVax Oncology - Non Confidential

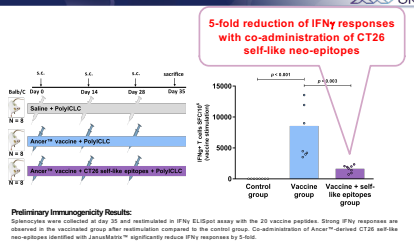
Ancer™ - selected CT26 neoantigen peptides stimulate multifunctional CD4⁺ and CD8⁺ T cells

EpiVax ONCOLOGY



EpiVax Oncology - Non Confidential

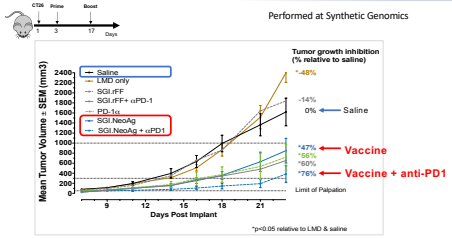
Ancer™ selected CT26 Treg neoantigen peptides reduce neoantigen vaccine immune responses



Preliminary Immunogenicity Results:

Splenocytes were collected at day 35 and restimulated in IFNγ ELISpot assay with the 20 vaccine peptides. Strong IFNγ responses are observed in the vaccinated group after restimulation compared to the control group. Co-administration of Ancer™-derived CT26 self-like neo-epitopes identified with JanusMatrix™ significantly reduce IFNγ responses by 5-fold.

Vaccination of CT26-bearing mice with Ancer™ neoantigen RNA replicons inhibits tumor growth



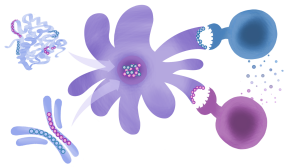
Conclusions

- Defining T cell Epitopes In Silico – *Yes, we can.*
- Comprehensive Immunogenicity Risk Assessment *includes In Vitro*
- Defining Tregs In Silico? – *Yes, we can.*
- Immune Engineering Immunogenicity and Tolerance? – *Yes, we can.*
- Personalizing Immunogenicity Risk ? – *Yes, we can.*
- . . . Can we **immune-engineer**? – *Yes, we can.*
- *Be attentive to potential Treg epitopes!*

Tools for better Biologics and Vaccine Design

- Balance of T-eff and T-reg is relevant to immunogenicity.
- In silico analysis can provide a 'first pass' evaluation of biologics and vaccines.
- Epitopes that share a TCR-face with numerous human sequences may activate Tregs – CD8 T cell response AND antibody responses can be reduced !
- Pathogens use Treg epitopes to avoid immune responses.
- Cancer does too.
- Optimized vaccines reduce Treg response and include better T eff epitopes, driving protection.
- Epitope-engineered proteins are better vaccines and drugs!

Questions?



EpiVax 20 YEARS Fearless Science