URI/EpiVax Westin Immunogenicity Seminar 2013

Westin Tokyo
Thursday, May 9th

Institute for Immunology and Informatics

Supported by:

EpiVax, Inc.

THE UNIVERSITY OF RHODE ISLAND

THINK BIG WE DO

Making a positive impact both locally and globally.

At EpiVax we strive to see beyond the horizon, desiring to be a part of an effort that is larger than ourselves, an effort that contributes to improving human health by moving science forward. In our day-to-day work, we develop vaccines and immunotherapeutics that will afford better protection with fewer side effects through the application of our cutting-edge immunoinformatics tools and immuno-logic techniques. In the long term, we aim to leave behind a legacy for the next generation, and we therefore continually strive to create an environment that enables every member of our team to take part in the building of that legacy.

URI/EpiVax
Westin Immunogenicity Seminar 2013

Thursday, May 9th, 2013
Westin Tokyo
9:30 am – 5:00 pm
1-4-1 Mita Meguro-ku, Tokyo 153-8580 Japan

Program Contents:

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THE UNIVERSITY OF RHODE ISLAND

THINK BIG WE DO

URI/EpiVax

Westin Immunogenicity Seminar 2013

Speakers:

Dr. Keizo Yoshida, PhD.
EpiVax Asia

Dr. Shingo Niimi, PhD
Manager, Division of Medical Devices
National Institute of Health Sciences (NIHS)

Dr. Annie De Groot, M.D.
Professor and Director, Institute of Immunology and Informatics,
University of Rhode Island, CEO/CSO, EpiVax, Inc.

Dr. Naonobu Sugiyama, MD, PhD
JCR-board Certified Rheumatologist
Associate Director, RA & Inflammation
Medical Affairs Pfizer Japan

Dr. Chris Bailey-Kellogg, PhD
Associate Professor of Computer Science,
Dartmouth College

Ms. Frances Terry
Bioinformatics Program Manager
EpiVax, Inc.

Supported by: EpiVax, Inc. Science without Fear

Conference Agenda

Institute for Immunology and Informatics

THE UNIVERSITY OF RHODE ISLAND — THINK BIG WE DO

URI/EpiVax Westin Immunogenicity Seminar 2013 May 9th, 2013		
Time	**Presenter**	**Topic**
9:30	**Dr. Keizo Yoshida, PhD.** EpiVax Asia	Introduction & Welcome
9:40	**Dr. Shingo Niimi, PhD** Manager, Division of Medical Devices, National Institute of Health Sciences (NIHS)	Immunogenicity Evaluation of Biotechnology-derived Drugs Including Biosimilar Therapeutic Monoclonal Antibodies
10:10	**Prof. Annie De Groot, M.D.** Professor, URI and CEO, EpiVax	Immunology Perspective: What Drives Immunogenicity
10:50	***Break***	
11:15	**Dr. Naonobu Sugiyama, MD, PhD**, Associate Director, RA & Inflammation, Medical Affairs Pfizer Japan	Case Study: Immunogenicity and Clinical Outcomes in RA Treatment
12:00	**Dr. Chris Bailey-Kellogg, PhD** Associate Professor of Computer Science, Dartmouth College	New Technologies: Immunogenicity, Deimmunization & 3D Modeling
12:45	***Lunch (Provided)***	
1:30	**Ms. Frances Terry**, Bioinformatics Program Manager EpiVax, Inc.	Live Demonstration: In-Silico Immunogenicity Screening Platform (ISPRI)
2:30	**Prof. Annie De Groot, M.D.** Professor, URI and CEO, EpiVax	Into the Clinic: Immunogenicity Solutions
3:10	***Break***	
3:30	**Prof. Annie De Groot, M.D.** Professor, URI and CEO, EpiVax	Quick Update: Rapid Vaccine Design for (H7N9) Pandemic Readiness
4:00	**All Speakers**	Panel Discussion and Questions from Participants
5:00	***Close***	

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EpiVax and iCubed Introduction:

The team at **EpiVax**, Inc., led by Dr. Annie De Groot and Bill Martin, has pioneered the development of a set of immunoinformatics tools which allows researchers to predict the immunogenicity of peptides and proteins. The potential applications of this technology are vast: for instance, one could be to predict which vaccines will be most effective or which protein therapeutic drugs will have the possibility of eliciting an adverse immune response. It is a powerful research and development tool for designing effective and safe protein/peptide based therapeutics. The leaders of EpiVax, Inc. have been resolute in availing these tools to the research community. To that end, Dr. De Groot and her team, with funding from an NIH U19 grant, have developed the iVAX website where investigators can access their own set of genome sequences, proteins of interest, and tools for the analysis of vaccines and diagnostics. Using the iVAX toolkit, researchers can quickly and efficiently identify the most reactive proteins contained within a given pathogen, and optimize the antigenic content of vaccines. Furthermore, by selecting the highest quality epitopes from a protein sequence new antigens that are relevant for vaccine development can be discovered. www.epivax.com

The **Institute for Immunology and Informatics** (iCubed) was established in 2008 under the leadership of Annie De Groot, M.D. and Denice Spero, Ph.D., as part of the University of Rhode Island's emerging Biotechnology Program. iCubed's research focuses on new and safer vaccines, new methods of predicting and treating adverse immune responses, and improving tolerance in the case of transplantation. iCubed supports a wide variety of training efforts that will provide opportunities to teach the next generation the tools for effective vaccine design.

The iCubed excels in immunoinformatics-driven vaccine development, colloquially known as "Gene-to-Vaccine". The approach involves computer-driven analysis of genome sequences, selection of immunogenic segments, and composition of vaccines in silico. The next step in the process is to validate the vaccine candidates in vitro and in vivo, using methods developed in the iCubed laboratories. A wide array of vaccine delivery technologies are under evaluation, including monoclonal antibodies, liposomes, and DNA vaccines (De Groot with the Department of Defense). Using immunoinformatics tools, research also is being conducted on eliminating parts of vaccines that may contribute to deleterious immune responses. Collaborations extend internationally to Thailand (Dengue virus), and Mali (HIV, TB, HPV). Cross-disciplinary collaborations exist between the iCubed, which is actively developing vaccines using immunoinformatics tools, and the laboratory of Geoff Bothun, where the vaccines are being packaged in liposomes for delivery. Research collaborations also have been developed with Steve Williams (filaria, Smith College), another investigator that will be involved in the iCubed program. In addition, iCubed researchers are actively carrying out field research in vaccines that will accelerate the delivery of new vaccines to the developing world; iCubed student researchers are collaborating with clinicians in Mali to evaluate 'knowledge, attitudes and practices' related to vaccines and the efficacy of existing vaccines (such as HPV) in that setting. Each of these cross-cutting areas of research, comprising experience that covers the biotech field 'from gene to vaccine' is currently being integrated into the activities of the iCubed. www.immunome.org

Speaker Biographies

Dr. Keizo Yoshida, PhD.
EpiVax Asia

Dr. Keizo Yoshida has been an industry professional for over 40 years. After obtaining his undergraduate degree in Agricultural Biological Chemistry, Dr. Yoshida began working for Fujisawa Pharmaceutical Company. During this time, he acted as a scientist in the research division for 27 years, then became involved in drug development for 10 additional years, and completed his doctorate degree in Agricultural Biological Chemistry. For the past five and a half years, Dr. Yoshida has worked for GreenPeptide, a venture company originated from Kurume University School of Medicine, developing personalized cancer peptide vaccines. He was also, the Chairman CMIC Bioresearch Center and is now full time at EpiVax Asia.

Dr. Shingo Niimi, Ph.D.
Manager of Division of Medical Devices
National Institute of Health Sciences (NIHS)

Dr. Shingo Niimi graduated from Tokushima College of Pharmacy, and went on to receive his Ph.D. at Tokushima College of Medicine at 1984. After working at Tokushima College of Medicine as a research assistant, he was transferred to Division of Biological Chemistry and Biologicals of National Institute of Health Sciences. He was promoted to the section director at 1997 and then manager of Division of Medical Devices at 2013. He has been engaged in the approvals of many biotechnology-derived drugs. He is a member of committees on Japanese Pharmacopeia of biologicals, measure for safety of drugs and drug efficacy reevaluation, etc.

Anne S. De Groot, M.D.
CEO/CSO: EpiVax
Professor/Director: iCubed, The University of Rhode Island

Dr. Anne S. De Groot earned her B.S. at Smith College B.S. in 1978, her M.D. at the Pritzker School of Medicine / University of Chicago (1983), worked on field campaigns to vaccinate against measles in Zaire, and then trained in Internal Medicine (New England Medical Center 1986); earned a research (NRSA) fellowship in tropical medicine and vaccinology (NIH, 1986-89), and underwent specialty training in infectious disease at Tufts New England Medical Center (1989-92). She earned board certification in Internal Medicine (1986) and Infectious Disease (1992). Having been awarded her first R01 as a research fellow at NEMC, she moved to Brown University, joined the Medical Faculty (1992-2011), establishing a productive vaccine research laboratory and developing new tools for vaccine design (EpiMatrix, Conservatrix, ClustiMer). Having licensed these tools from her laboratory at Brown, she established EpiVax (1998-present) with business partner Bill Martin. In 2008 she was invited to open the Institute for Immunology and Informatics (iCubed) at the University of Rhode Island. She has served as CEO/CSO at EpiVax from 1998 to present and as Director of the Institute and Research Professor at University of Rhode Island since 2008. Although De Groot is already well established in the fields of immunoinformatics and vaccinology, the co-discovery of Tregitopes resulted in a major shift in her research efforts, to which she has added new explorations in autoimmunity and tolerance.

Dr. Naonobu Sugiyama, MD, PhD.
JCR- Cert. Rheumatologist SCBU Medical Affairs,
Enbrel Medical Lead of Japan,
Pfizer, Inc.

Dr. Naonobu Sugiyama has both basic research and clinical experience in the field of Immunology and Rheumatology. He gained his Medical degree ***(MD)*** and Doctorate degree ***(PhD)*** in the field of Rheumatology, with a focus on IL-27/WSX signaling associated with regulatory T cell, from Kyushu University Japan. He also has a wealth of clinical experience as a Board Certified Member of Internal Medicine and JCR-board certified rheumatologist. He joined Pfizer Japan as a Medical Lead of RA/Inflammation. Soon after joining Pfizer

Speaker Biographies

(Japan), he initiated an immunogenicity expert forum in 2013, to differentiate Enbrel (Etanercept) in Japan, and introduce the importance of Immunogenicity to Japanese physicians and rheumatologist. He has several key publications in the field of Rheumatology.

Dr. Chris Bailey-Kellogg, PhD
Associate Professor of Computer Science,
Dartmouth College

Dr. Chris Bailey-Kellogg is an associate professor of computer science at Dartmouth College. He earned a BS/MS with Sandy Pentland at MIT and a PhD with Feng Zhao at Ohio State and Xerox PARC, and conducted postdoctoral research with Bruce Donald at Dartmouth. He was an assistant professor at Purdue before being recruited back to Dartmouth. He has received an NSF Career award and an Alfred P. Sloan Foundation fellowship, along with regular grants from the NIH, NSF, and other organizations. Research in his lab focuses on embedding computation as a core component in studies of protein structure and function, and in engineering protein variants. He conducted his 2011-2012 academic year sabbatical at the Institute for Immunology and Informatics with Dr. De Groot, where they initiated several such projects at the intersection of computation and immunology.

Ms. Frances Terry, BA
Bioinformatics Program Manager
EpiVax, Inc.

Frances Terry, BA, is Bioinformatics Program Manager at EpiVax, where she oversees informatics-based analysis of commercial therapeutics and development of genome-derived vaccines. Prior to joining the EpiVax team, Ms. Terry amassed expertise in many laboratory techniques including flow cytometry, molecular and immunological assays, tissue culture and animal handling. She has contributed to research projects at Brown University and Roger Williams Medical Center, most recently developing standard operating procedures and serving as primary quality control operator for a cGMP drug manufacturing facility. Ms. Terry holds a degree in Biological Sciences from Smith College, where she investigated gene flow and diversity in coastal protozoans and became interested in host-pathogen co-evolution.

Institute for Immunology and Informatics

Introduction and Welcome

Presented by:

Dr. Keizo Yoshida, PhD.
EpiVax Asia

EpiVax, Inc.
Informatics and Immunology

Notes:


Institute for
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Informatics and Immunology

Immunogenicity Evaluation of Biotechnology-derived Drugs Including Biosimilar Therapeutic Monoclonal Antibodies

Presented by:

Dr. Shingo Niimi, PhD
Manager, Division of Medical Devices, National Institute of Health Sciences (NIHS)

Immunogenicity Seminar 2013
May 9, 2013 The Westin Tokyo

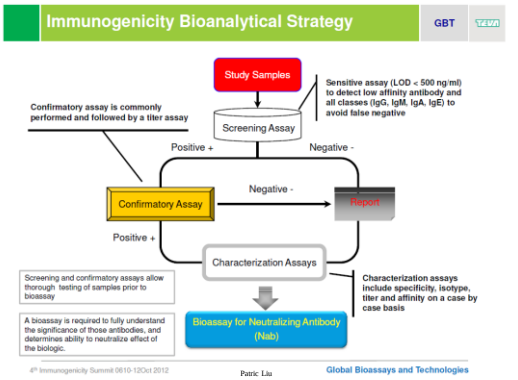
Immunogenicity Evaluation of Biotechnology-derived Drugs Including Biosimilar Therapeutic Monoclonal Antibodies

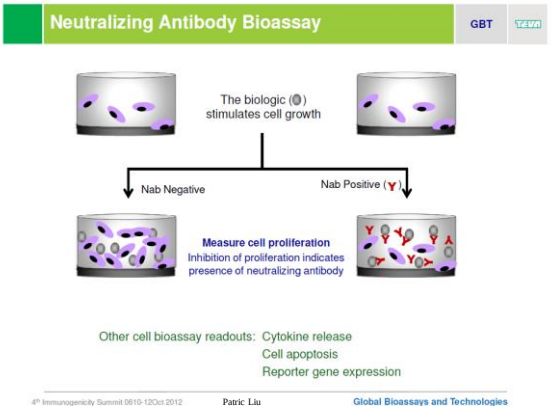
Shingo Niimi, Ph.D.
National institute of Health Sciences, Japan

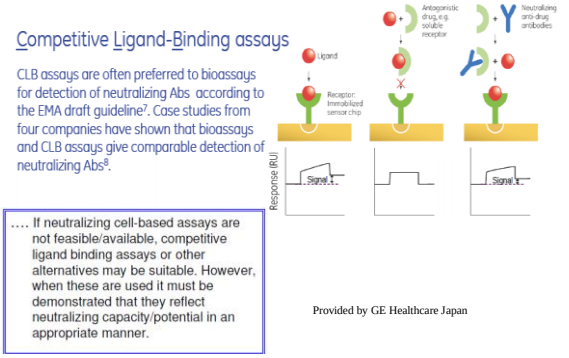
国立医薬品食品衛生研究所
National Institute of Health Sciences

Contents of Presentation

Immunogenicity Assay







Provided by GE Healthcare Japan

Key Points for Immunogenicity Assays

GBT TEVA

- Many assay platforms and technology available, but each has pros and cons. Validated methods for ***an intend use*** must be considered.
- Appropriate setting of the ***cut point*** provides adequacy of the detection of antibody responses
- Ensure the assay measures all ***clinically relevant antibody*** responses:
  - Nab
  - Antibody cross-reacting with endogenous counterpart
- Impact of ADA on PK/PD, safety and efficacy

Challenges of Data Comparison

GBT TEVA

- Statistics aspects
  - Size of the clinical immunogenicity trial
  - Disease populations and heterogeneity of individual response
  - Drug interference
  - Variation from the assays
- Types of the antibody response
  - Pre-existing antibody to the reference product
  - Transient vs persistent response
  - Matrix effect or false antibody response
- How similar is "similar"?
  - The immunogenicity rate is generally low for the reference product (<5%)

Considerations for Immunogenicity Comparison

GBT TEVA

Immunogenicity statement:

- The incidence of antibody development in patients receiving *[reference product]* has not been adequately determined due to:
  - *Assay sensitivity was inadequate to reliably detect lower titers of antibody*
  - *Nature and specificity of these antibodies has not been adequately studied*
- The detection of antibody formation is highly dependent on the sensitivity and specificity of the assay, and the observed incidence of antibody positivity in an assay may be influenced by several factors including sample handling, timing of sample collection, concomitant medications and underlying disease.
- Therefore, comparison of the incidence of antibodies to *[reference product]* with the incidence of antibodies to other products may be misleading

Key factor for successful immunogenicity comparison:

- Well control head-to-head trial design
- Sample collection and handling
- Equivalent validated immunogenicity assays

Clinical Immunogenicity Assessment

GBT TEVA

"The goal of the clinical immunogenicity assessment is to evaluate potential differences between the proposed product and the reference product in the incidence and severity of human immune response." [draft Guidance –Scientific Considerations]

- At least one head to head comparative clinical trial
- One-sided design with non-inferiority margin
- Severity and incidence of immune responses to be considered for the study design
- Tested in the most "sensitive" population
- No clinically meaningful difference in immune response
- Further evaluation on the absence of clinical sequelae if a difference of immune response observed

Points to be Considered

GBT TEVA

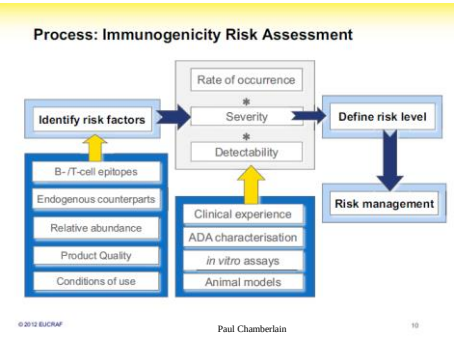
- Immunogenicity is a critical matter for all biologics.
- Immunogenicity of a biosimilar may be less concerned if:
  - *Demonstration of analytical similarity*
  - *Immunogenicity of the reference product has been well studied*
  - *Application to the same indication*
- Limitations of immunogenicity to reveal clinically meaningful differences
  - *Variability of individual immune response*
  - *Assay sensitivity and data variation*
  - *Immunogenicity due to product's impurities and excipients needs to be further investigated.*
- The difference in immune response may not be observed even with significant difference of the products revealed by the current available analytical methods.

Points to be Considered

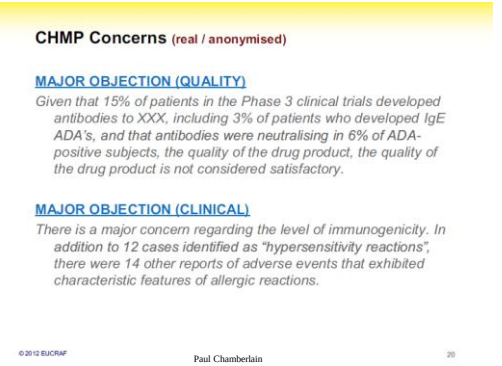
GBT TEVA

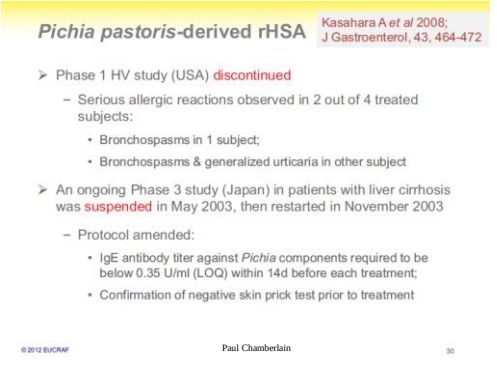
- Risk-based immunogenicity assessment strategy
  - *Indication considerations: chronic treatment, replacement therapy, autoimmune diseases*
  - *Product considerations: endogenous counterpart*
- Interpretation of immunogenicity *data*
  - *The overall clinical observation should be taken into consideration, including PK, PD, safety and efficacy*
  - *Differences in immunogenicity of two products do not necessarily correlate to observed clinical difference*
- Comparative head to head immunogenicity clinical trials should be carefully considered to ensure data can be meaningful.

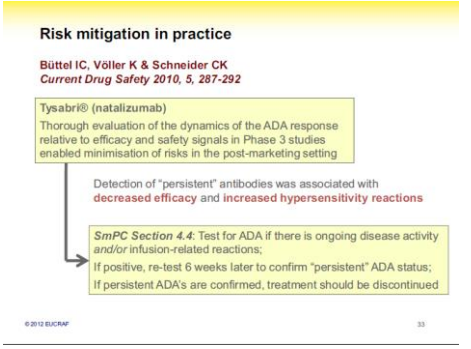
Immunogenicity Risk Assessment



Case Studies (Concerns and Mitigation of Immunogenicity in the Clinical Trials)







Definition of an Acceptable Comparability Threshold for the Immunogenicity Assessment of Biosimilar mAbs Using the Development of Biosimilar Version of adalimumab as an example

Problem Statement

Direct comparative clinical evaluation is required as part of the demonstration of **"biosimilarity"** of **therapeutic mAbs**.

Design of a reliable comparison is limited by a number of factors, most importantly:

- ↘ Incomplete knowledge of impact of ADA's on longer-term treatment outcome with Reference Product
- ↘ Bias caused by choice of bioanalytical method on sensitivity to detect differences

Role of immunogenicity evaluation

Goal is to demonstrate absence of a **clinically-meaningful *difference*** # in immune response to biosimilar product relative to Reference Medicinal Product

Emphasis on confirming ***no increase***, rather than decrease

What is known for Reference Product?

- Anti-adalimumab antibodies likely to be neutralizing & form small immune complexes
  - van Schouwenburg PA *et al*; Ann Rheum Dis 2012
- Negative impact of anti-adalimumab antibodies on drug trough concentration
  - Bartelds GM *et al*; JAMA 2011, 305 (14), 1460-1468
- Allotype mismatch does not increase incidence of anti-adalimumab antibodies in RA patients
  - Bartelds GM *et al*; Arthritis Res & Therapy 2010, 12:R221

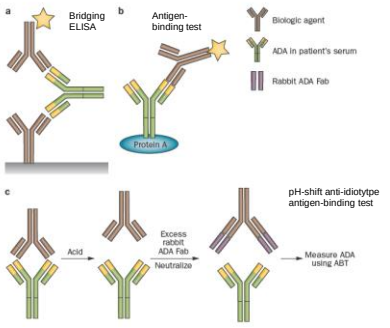
Uncertainties

- Relative role of neutralization via binding of anti-idiotype antibodies *vs.* enhanced clearance of drug?
- Impact of anti-adalimumab antibodies on longer-term clinical outcome in different therapeutic indications:
  - Treatment failure?
  - Type III hypersensitivity?
  - Autoimmune status
    - Diagnostic value of isotyping anti-adalimumab antibodies & monitoring anti-dsDNA?

Humira®: EPAR's for line extensions

Therapeutic indication	Comment
Psoriasis	*"In the ongoing study, a tendency towards tapering of effect was observed in the 48w evaluation"* *"CHMP noted that antibody data was lacking"*
Ankylosing Spondylitis	Immunogenicity evaluated in 208 subjects *"CHMP concluded that the data are too limited to draw definitive conclusions regarding the impact of AAA on both efficacy and safety"*
Crohn's Disease	52w double-blind, maintenance study, (n=854) *"Anti-adalimumab antibody (AAA) levels were not monitored in the pivotal maintenance study M02-404"* Although AAA was evaluated for the induction studies, there were an insufficient number of events to assess impact of AAA on efficacy. *"One of the main issues with anti-TNF therapy is to satisfactorily describe the population intended for treatment because of uncertainties about the long-term safety but also long-term efficacy."*

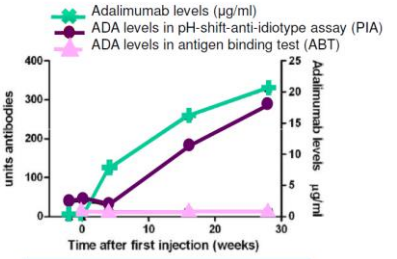
Assays for Detection of ADA



Van Schouwenburg PA et al. Nat Rev Rheumatol 2013 9.164-72

Van Schouwenburg *et al*; JIM 2010, 362, 82-88

Influence of assay format



PIA detects ADA in presence of adalimumab

Relevant parameters ?

INFERENCES

1.Measures of ADA incidence or magnitude are highly subjective, and may have limited relevance *on their own* for the assessment of biosimilarity of therapeutic mAbs;

2.Applicants need to establish the sensitivity of their bioanalytical methods by reference to **clinically-meaningful endpoints**;

3.PK, PD, efficacy & safety parameters should be compared for ADA positive *vs.* ADA negative subjects

Choice of ADA assay

Does drug interference preclude use of bridging format?

- No, but Agency might request:
  - Larger sample size to compensate for lower assay sensitivity
  - Application of nAb assay to detect ADA levels that might have a negative functional influence

Note discussion in van Schouwenburg PA *et al*; Ann Rheum Dis 2012

In the case of adalimumab:
ADA response is highly restricted to anti-idiotype, neutralizing antibodies;
Ligand binding assay adequate to monitor ADA, i.e. without nAb assay

Acceptable margin of difference ?

Guideline on **similar biological medicinal products containing monoclonal antibodies** - Non-clinical & clinical issues
EMEA/CHMP/BMWP/403543/2010

"A higher immunogenicity as compared to the reference mAb may become an issue for the benefit/risk analysis and would question biosimilarity"

No comment regarding what is meant be "higher immunogenicity";

Lower immunogenicity would not preclude biosimilarity

→ Pre-specify additional exploratory subgroup analysis of efficacy and safety in ADA negative subjects

Acceptable margin of difference ?

Draft Guidance for Industry:
Scientific considerations in demonstrating biosimilarity to a Reference Product
FDA/CDER/CBER/February 2012

"Acceptable differences in incidence and other immune response parameters should be discussed with the FDA in advance of the study"

Questions:

1.What is a clinically-relevant difference in ADA response?

2.Is it feasible to power adequately pre-registration studies to detect a pre-defined one-sided statistical difference in ADA response?

→ Uncertainty regarding (i) different sensitivity of methods to detect clinically-meaningful ADA's; *and* (ii) impact of ADA on longer-term clinical outcomes for Reference Product

Acceptable margin of difference?

INFERENCES

1.Because there are insufficient data to understand the clinical impact of a measured difference in the incidence or magnitude of ADA's, **there can be no pre-defined acceptance range for the designation of "biosimilarity" in terms of ADA *only***

- even when measured in a directly comparative study using the most sensitive bioanalytical method

2.At time of registration, there should be sufficient data to indicate impact of increased ADA incidence or magnitude on drug trough levels and other relevant clinical parameters

- **"Totality of evidence"** approach

CHI Immunogenicity Summit 2012 Paul Chamberlain

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How much evidence is required ?

Different therapeutic indications

Could different incidence / magnitude of ADA response in different therapeutic settings alter the "biosimilar" status of a mAb product?

Insufficient historical data on relationship of ADA to clinical efficacy and safety in different indications for reference products

Even if ADA data were collected in the population most responsive in terms of ADA, e.g. monotherapy, any detected difference in ADA should still be interpreted only in relation to impact on clinical parameters

Comparison of ADA in population chosen for Phase 3 study should be sufficient to support registration, on the basis that this were the most sensitive population for detection of **clinically-meaningful** differences

Additional long-term data may be required post-authorisation

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How much evidence is required ?

Post-registration monitoring

Objective = To evaluate impact of differences in ADA response on clinical parameters where insufficient data are available at the time of marketing authorisation of product with demonstrated therapeutic equivalence

Monitoring of **ADA** *and/or* **drug trough concentration** *vs.* **disease activity** during chronic treatment for at least 2 years

Collection of data in **other therapeutic indications** in which **risk factors** have been identified for the Reference Product

Size of population to be monitored post-registration would depend on **severity of consequences** identified for Reference Product *and* **strength of pre-registration database** to detect a **clinically-meaningful difference** in immunogenicity of the biosimilar product

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CHI Immunogenicity Summit 2012 Paul Chamberlain

Acknowledgement

I thank Dr. Paul Chamberlain（NDA Advisory Board）for providing presentation slides and helpful discussion.

Thank you for your attention

If you have any questions or comments about my presentation, please contact me (niimi@nihs.go.jp).

Immunology Perspective: What Drives Immunogenicity

Presented by:

Dr. Annie De Groot M.D.
Professor and Director, Institute of Immunology and Informatics,
University of Rhode Island, CEO/CSO, EpiVax

Immunogenicity of Biologics
The Immunology Point of View

Anne S. De Groot M.D.
EpiVax, Inc.
and the Institute for Immunology and Informatics

http://www.EpiVax.com http://www.immunome.org

Westin Immunogenicity Seminar 2013

Outline

- Why are autologous proteins immunogenic?
- How do we find T cell epitopes in silico?
- What's the difference between good T cell epitopes and bad ones?
- Tregitopes – a paradigm shift
- T cell epitope networks
- Host Cell proteins

Outline

- Why are autologous proteins immunogenic?
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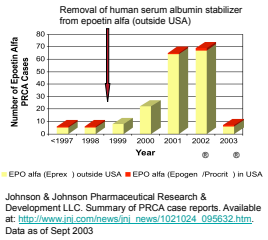
- T cell epitope networks
- Host Cell proteins

Why do Autologous Proteins Cause immunogenicity?



Johnson & Johnson Pharmaceutical Research & Development LLC. Summary of PRCA case reports. Available at: http://www.jnj.com/news/jnj_news/1021024_095632.htm. Data as of Sept 2003

AMGEN DISCONTINUES DEVELOPMENT OF MGDF

FOR IMMEDIATE RELEASE

THOUSAND OAKS, Calif., September 11, 1998 -- Amgen (NASDAQ:AMGN) today reported that it has discontinued development of its megakaryocyte growth and development factor (PEG-rHuMGDF) due to evidence of neutralizing antibodies in a few patients participating in cancer clinical trials and in additional people in platelet donor clinical trials.

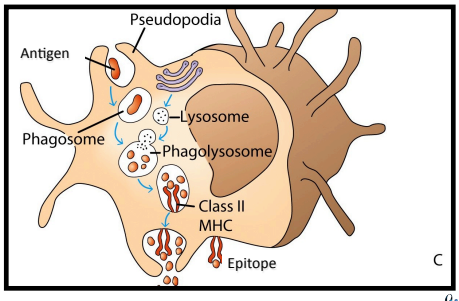
Amgen is a global biotechnology company that discovers, develops, manufactures and markets cost-effective human therapeutics based on advances in cellular and molecular biology.

CONTACT: Amgen, Thousand Oaks
David Kaye, 805/447-6892 (media)
Denise Powell, 805/447-4346 (investors)

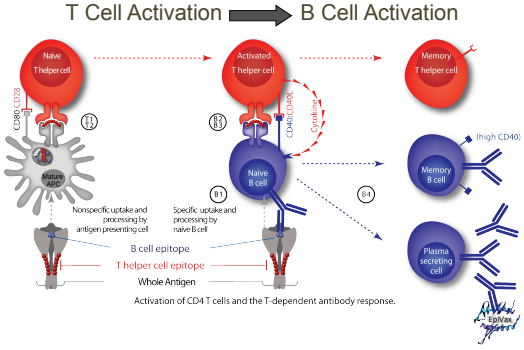
EDITOR'S NOTE: An electronic version of this news release may be accessed via our web site at www.Amgen.com. Visit the Corporate Center and click on Amgen News. Journalists and media representatives may sign up to receive all news releases electronically at time of announcement by filling out a short form in the Amgen News section of the web site.

Severe Adverse Event – Anti-self Antibodies

"Biologics" Drugs are Processed by APC JUST Like Vaccines



With T Cell Help – Drive Ab Response



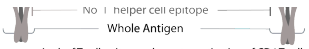
Without T Cell Help – Lose Ab Response

NO T Cell Activation → NO B Cell Activation

No recognition — No recognition — No expansion and

PROVE ME WRONG:
Aggregates without T cell epitopes do not drive ADA

Can removal of T cell epitopes lead to reduction of ADA?



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

Category of Immunogenicity by "Biologic"

(1) Gene Deletion (FVIII)

(2) Danger Signal present

(3) Foreign epitopes (Chimeric, Host Cell proteins)

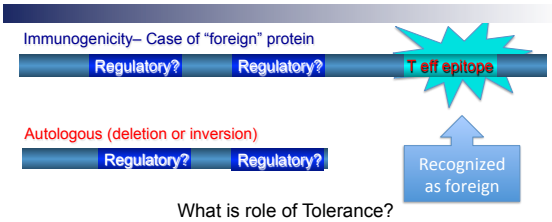
(4) Immunogenic but autologous (human) protein

Some examples - FVIII

Absent or partially deleted protein drives immune response

Response may depend on HLA of patient and # epitopes presented

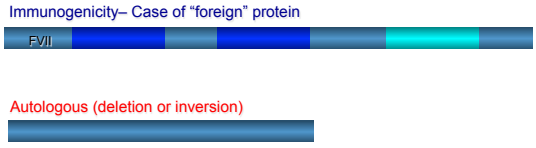
FVIII example: 25% get ADA



What is role of Tolerance?

Do the epitopes that are conserved contribute to tolerance?
What is the balance between neo-epitopes and ?Treg epitopes?
If the patient cannot present the 'foreign' epitope what is the effect?

Experiment of Nature: FVIII + HIV

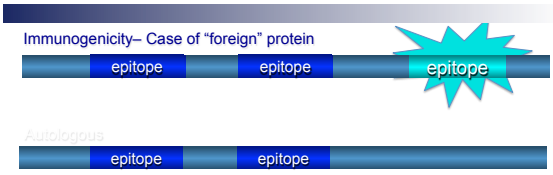


When the T cells are Absent in the Host (HIV)
- the anti-FVIII antibodies are also absent

Evans GD, Mendelson GM, Lever AM, Baglin TP. Development of autoantibodies and factor VIII inhibitor in an HIV-infected haemophiliac following treatment with combination anti-retroviral therapy. Br J Haematol. 1998 Sep;102(5):1382-3.

Werwitzke S, Tiede A, Stoll M, von Depka M. Immune reconstitution inflammatory syndrome (IRIS) as a cause for inhibitor development in hemophilia. J Thromb Haemost. 2004 Jan;2(1):193-4.

Some examples – Foreign epitopes



Epitopes that differ from self contribute to immune response
-this relates to both B and T epitopes

Example: Bovine insulin, animal-sourced replacement proteins
Additional (recent) concern: Host Cell Proteins derived from CHO
Response may depend on HLA of patient and # epitopes presented

Recombinant Human Proteins

Immunogenicity– Case of autologous protein

epitope epitope epitope

Autologous protein

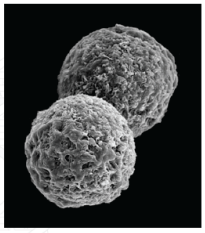
epitope epitope epitope

If there is circulating autologous protein the patient may be "tolerant".

Several situations may overcome tolerance:

(1)Inflammation - adjuvants -may overcome tolerance (e.g. Eprex Story)
(2) Administration of aggregated protein (e.g.. Beta interferon)
(3) Protein is usually "sequestered" (testes origin)

Example: "Particle" of hGH



- PLGA Microspheres (5-10 µm diameter)
- Micronized GH particles embedded in PLG matrix
- GH released as PLG polymer is hydrolyzed to lactic and glycolic acids

Nutropin Depot Sustained Release

PLG hGH more immunogenic

Immunogenicity of Nutropin®, Nutropin AQ® & Nutropin Depot®

ATA metric	Nutropin®	Nutropin AQ®	Nutropin Depot®
ATA rate @ 6 mos	13%	6%	39% (1.5 Q4) 61% (0.75 Q2)
ATA titer @ 6 mos	≤ 3	≤ 2	≤ 2.3 (1.5 Q4) ≤ 3.2 (0.75 Q2)
ABC @ 6 mos	≤ 2 mg/L	≤ 2 mg/L	≤ 2 mg/L
Growth rate @ 6 mos	No impact of ATA	No impact of ATA	No impact of ATA
Subjects	GH naïve pediatric GHD	GH naïve pediatric GHD	GH naïve pediatric GHD

Depot More Immunogenic

Sources: 2001 Product PIs,
Quarmby (2000) Biologics 2000 Talk

Outline

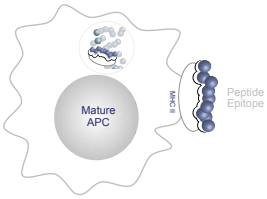
- Why are autologous proteins immunogenic?
- How do we find T cell epitopes in silico?
- What's the difference between good T cell epitopes and bad ones?
- Tregitopes – a paradigm shift

EpiVax Tools and Techniques

We use a comprehensive suite of tools and techniques for screening and deimmunizing therapeutics:

- ✓ EpiMatrix (CTL / T helper)
- ✓ ClustiMer (Promiscuous Epitopes)
- ✓ Immunogenicity Scale (Ranking Proteins)
- ✓ OptiMatrix (Pinpoint Deimmunization)
- ✓ HLA binding assays (Class I / Class II)
- ✓ Ex-vivo immunoassays (ELISpot, ELISA, Searchlight)
- ✓ HLA Transgenic mice (Class II)

How does in silico mapping work?

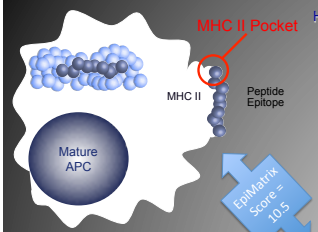


Th cell epitopes are linear and restricted by MHC (HLA).

Because the pockets of the HLA are well known, interactions with peptides can be modeled.

Sturniolo et al, "Pocket Profiles" Nature Biotechnology (Hammer)

EpiMatrix: Pocket Profile Method



HLA (Human MHC), are comprised of a limited number of pockets. EpiMatrix predicts how well a side chain will bind to a specific pocket.

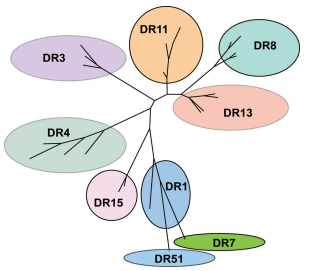
8 class II Archytype matrices which taken together incorporate 95% of human populations (and pockets) worldwide.

Each 9-mer/10-mer is analyzed for binding potential to each of those 8 allele matrices.

The **EpiMatrix Score** describes how well the peptide "fits" into the pockets.

Southwood et al. J. Immunology 1998
Sturniolo et al. Nature Biotechnology, 1999

EpiVax HLA "Supertype" Coverage



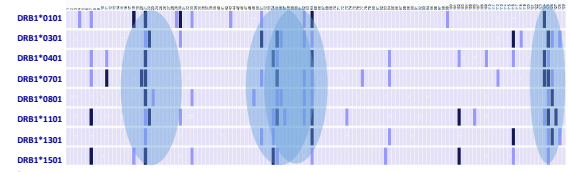
- **EpiVax tests for binding potential to the most common HLA molecules within each of the "supertypes" shown to the left.**
- **This allows us to provide results that are representative of >90% of human populations worldwide* without the necessity of testing each haplotype individually.**

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

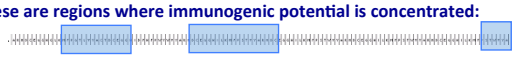
ClustiMer

Method for Finding Regions of High Immunogenicity

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

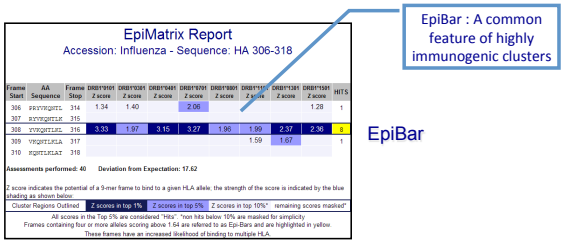


2) These are regions where immunogenic potential is concentrated:



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

What Makes Epitopes Really immunogenic? Clusters that Contain 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

In the same way that a cluster is more immunogenic, for a protein:

Immune Response = Sum of Epitopes

Protein Therapeutic

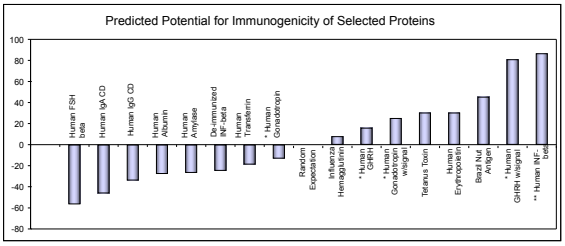
1 + 1 + 1 = Response

T cell response depends on:

T cell epitope content + HLA of subject

Protein Immunogenicity can be Ranked

Immunogenicity scale as published

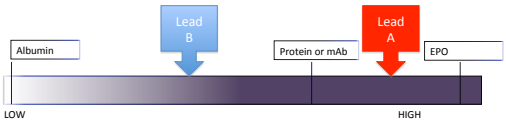


Note- Most common serum proteins have fewer T cell epitopes

Scaling Immunogenicity

How Could We Apply to Triage Biologic Leads?
Select for T cell Epitope Content per AA

EpiVax –ISPRI - Immunogenicity Scale



Proteins ranked by T- Epitope content per Amino Acid

• De Groot A.S., Drug Discovery Today - 2006;
• De Groot A.S., Mire-Sluis, A. Ed.. Dev. Biol. Basel, Karger, 2005. vol 122. pp 137-160.

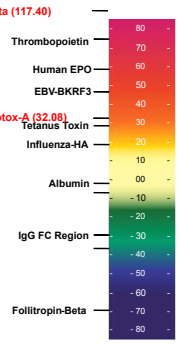
Immunogenicity Scale Whole Proteins

EpiMatrix predicted excess/shortfall in aggregate immunogenicity relative to a random peptide standard.

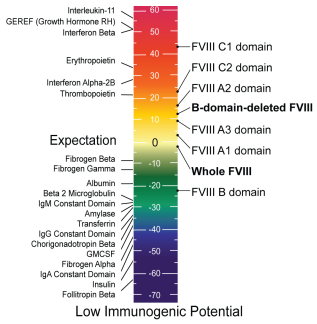
A protein score > 20 indicates a significant immunogenic potential.

Proteins that have previously been demonstrated to be immunogenic have higher potential immunogenicity on the scale.

Those that have rarely been demonstrated to be immunogenicity have lower T cell epitope content.



FVIII immunogenicity



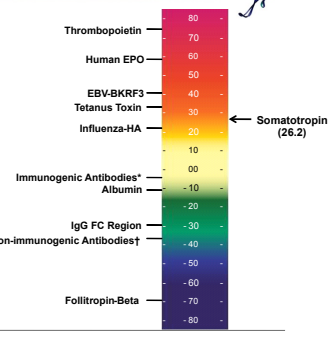
hGH Protein Immunogenicity

Somatotropin contains more T cell epitopes than we would expect to find in a random protein of the same length.

Its EpiMatrix Protein Score of 26.2 indicates a significant potential for immunogenicity.

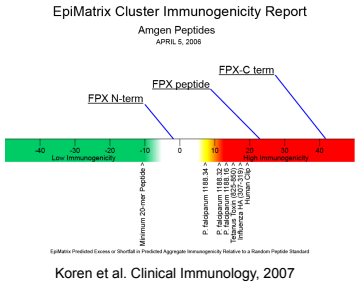
*Average of Antibodies Known to Induce Anti-Therapeutic Responses in More Than 5% of Patients

†Average of Antibodies Known to Induce Anti-Therapeutic Responses in Less Than 5% of Patients



Confidential

FPX peptide – Preclinical Analysis: Immunogenicity at C terminus



Koren et al. Clinical Immunology, 2007

Prospective Study: EpiMatrix Scores and Immunogenicity in Human studies

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

Negative score indicates presence of Treg epitope

Clinical Validation of Predicted Immunogenicity

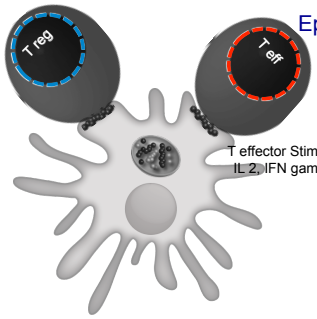
- Koren et al. 2007 FPX
- Moxness et al. 2008 GDNF
- Jawa (Amgen) Comparison of five FPX proteins
- Furfine (Adnexus) not published

ISPRI System

Outline

- Why are autologous proteins immunogenic?
- How do we find T cell epitopes in silico?
- What's the difference between good T cell epitopes and bad ones?
- Tregitopes – a paradigm shift

Presence of Epitope indicates Immune Potential



Epitope can be EITHER T-eff or Treg

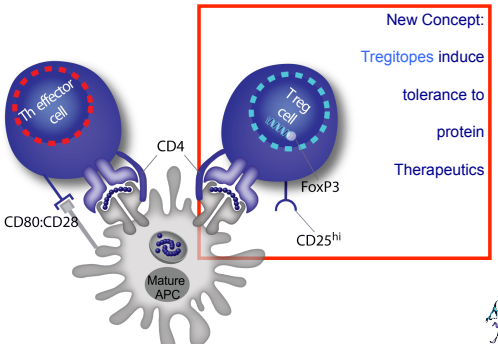
Examples:

Insulin (mice/human)

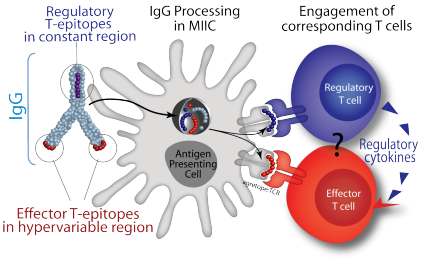
FVIII (hemophiliacs/ not)

Epo?

Not all Epitopes are "Bad" Some Epitopes are your FRIENDS



Tregitopes



Hypothesized tolerizing mechanism of IgG *We have discovered conserved T-cell epitopes in IgG that engage natural regulatory T cells. We hypothesize that antibody-derived Treg epitopes (dark blue epitope) activate regulatory T cells that lead to suppression of effector T cells that recognize effector epitopes (red epitope), like those of IgG hypervariable regions to which central tolerance does not exist.*

Outline

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- Tregitopes – a paradigm shift
- T cell epitope networks
- Host Cell proteins

New approach to analyzing mAbs . . . Immune Response = Sum of Epitopes

Sum includes + (T effectors) and – (Tregs) scores

Protein Therapeutic

epitope | epitope | Treg epitope

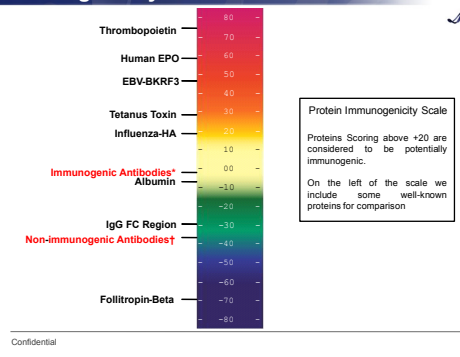
1 + 1 - Treg = Response

T cell response depends on:

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

Protein Immunogenicity can be Ranked

Tregitope-adjusted Immunogenicity Scale



Protein Immunogenicity Scale

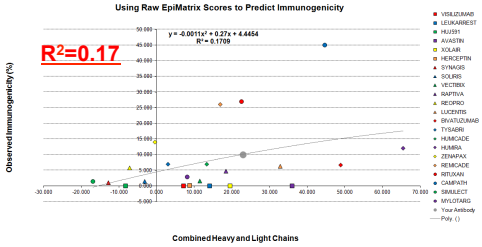
Proteins Scoring above +20 are considered to be potentially immunogenic.

On the left of the scale we include some well-known proteins for comparison

Confidential

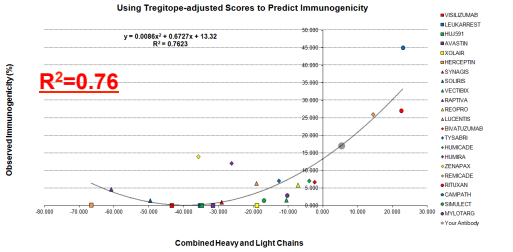
Correlation of antibody immunogenicity without Tregitope adjusted EPX Scores

Correlation to observed Immunogenicity before accounting for Tregitopes



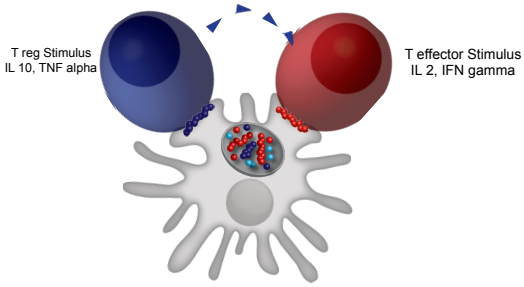
Correlation of antibody immunogenicity with Tregitope adjusted EPX Scores

Correlation to observed immunogenicity after accounting

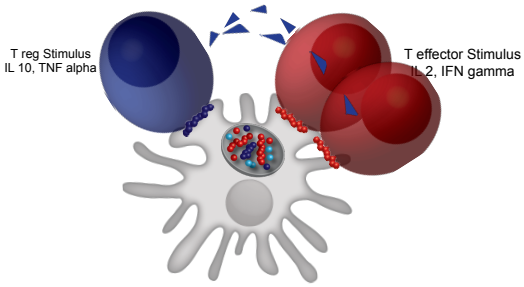


Accounting for Tregitopes results in more accurate predictions.

Thus, in some mAbs – NEW epitopes are Balanced by Treg Epitopes



In some mAbs –TOO MANY NEW epitopes not balanced by Treg Epitopes



mAbs can be "binned" In Two by Two Table:

	High Tregitope	Low Tregitope
Low Neo Epitope	+	++
High Neo Epitope	++	++++

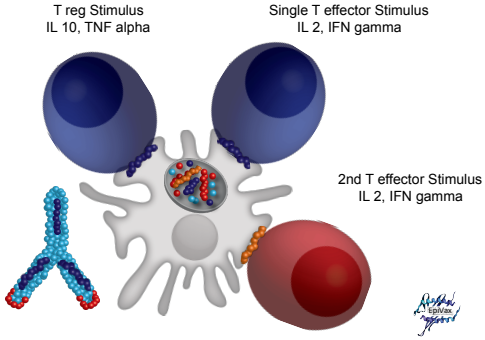
Tregitopes, Neo Epitopes and Immunogenicity

	High Tregitope Content	Low Tregitope Content
Low Neo Epitope Content	Nuvion (0%)	Synagis (1%)
High Neo Epitope Content	Humira (12%)	Rituxan (27%)

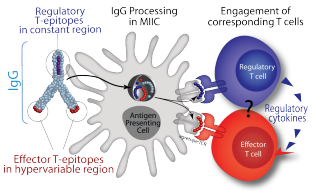
It is our belief that Tregitope content is an important determinant of anti-therapeutic antibody responses. As shown above, antibodies lacking significant T-helper epitopes such as Nuvion and Synagis rarely engender significant anti-therapeutic immune responses. On the other hand antibodies containing significant numbers of T helper epitopes are much more likely to spawn anti-therapeutic responses unless they also contain significant numbers of Tregitopes.

. De Groot and Martin Clinical Immunology May 2009

Current Hypothesis: Add more Tolerizing Signals – Suppress Immunogenicity



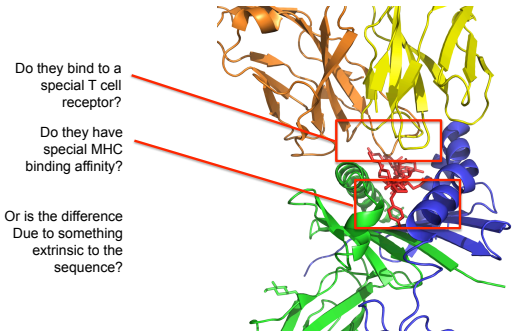
Tregitopes – See 2nd PPTer



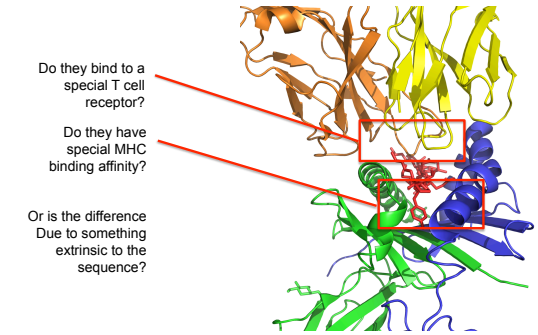
Outline

- Why are autologous proteins immunogenic?
- How do we find T cell epitopes in silico?
- What's the difference between good T cell epitopes and bad ones? ☺
- Tregitopes – a paradigm shift
- T cell epitope networks
- Host Cell proteins

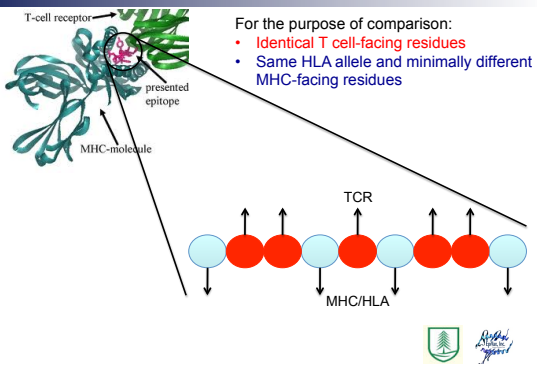
New Concept: Why do Tregitopes Exist?



New Concept: Why do Tregitopes Exist?

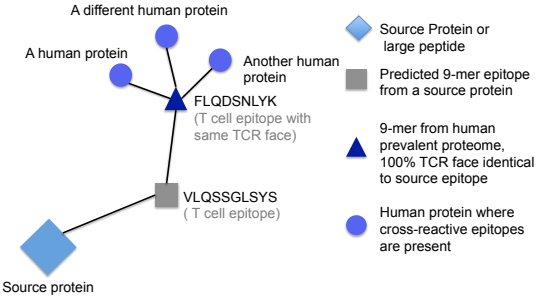


TCR face vs. MHC binding face

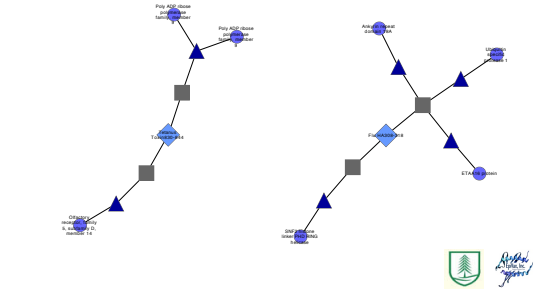


JanusMatrix Publication

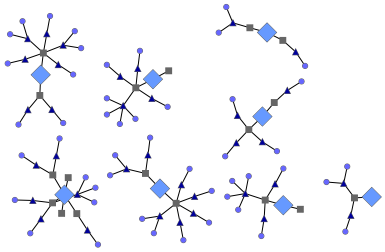
Epitope Networks: A New Concept



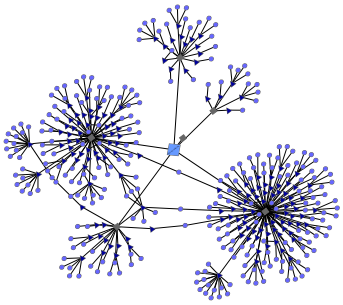
Flu and Tet tox epitopes



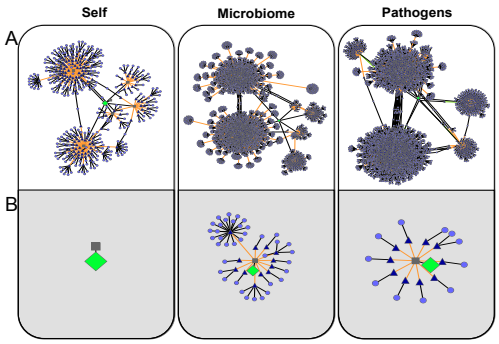
CEFT Peptides (immunogenic) vs. Hu Proteome



Treg-like-Epitope discovered in HCV



Different Types of Epitope Networks



Emerging Concept: Ratio of Cross-reactive epitopes per genome

Table 1. JanusMatrix TCR-cross reactivity frequencies for three types of epitopes.

Database	Median cross-reactive hits (Ratio, 1x10⁰)[a]					Number of		
	T eff epitopes		Treg epitopes		Random[c]	Genomes	Proteins	Amino acids per database
	CEFT	Influenza A	Tregs	HCV				
Self (HG)	2 (0.18)	0 (0.00)	8.5 (0.75)	23.5 (2.08)	1 (0.09)	1	20,248	11,301,336
Microbiome (HM)	29 (0.13)	38 (0.17)	31 (0.14)	103 (0.47)	14 (0.06)	204	705,684	218,452,796
Pathogens (HP)	17 (0.12)	11 (0.08)	19 (0.13)	107.5 (0.73)	10 (0.07)	221	455,237	146,398,849

A

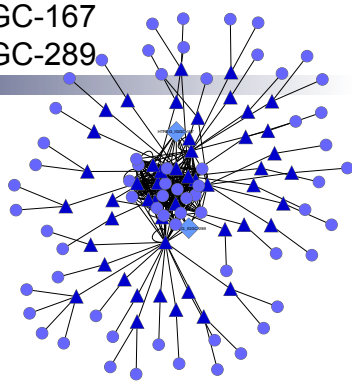
Epitope type	HG	HM	HP
Treg vs. CEFT	**0.00**	0.63	0.62
CEFT vs. Random	**0.00**	**0.05**	0.11
Treg vs. Random	**0.00**	**0.01**	**0.02**

B

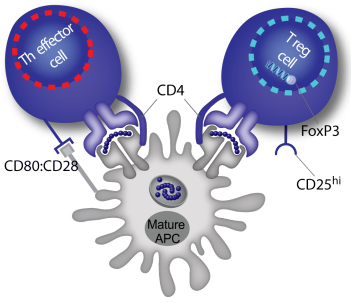
Database	CEFT	Treg	Random
HG vs. HM	0.10	**0.00**	**0.00**
HG vs. HP	0.24	**0.00**	**0.00**
HM vs. HP	0.50	0.15	**0.00**

[a]Ratio of cross-reactive hits per number of amino acids in the comparision database.
[b]Nine-mer predicted to be an epitope.
A) P-values of comparisons between ratios across three types of epitopes by database.
B) P-values of comparisons between ratios across databases by type of epitope.
Median of cross-reactive for T effector epitopes, T regulatory epitopes, and Random 9-mers are shown. CEFT and Tregs are tested as representative sets of T effector and T regulatory epitopes. Examples for both categories are also included; a defined Teff epitope in influenza A and Treg epitope for HCV. TCR cross-reactivity with HG, HM, and and selected human viral and bacterial pathogens (HP) was evaluated. Ratios of cross-reactive hits by number of amino acids in the comparison database are shown in parenthesis. Number of genomes, proteins, and amino acids per database is also shown. P-values of the comparison between the distributions of ratios of cross-reactive hits between databases and types of epitopes are also shown in sub-tables. Sub-table == shows comparisons between type of epitopes by database and sub-table ii comparisons between databases by type of epitope. Analyses were performed with a 95% confidence level.

hTregitope-IGGC-167
hTregitope-IGGC-289



A new definition of Treg epitopes?



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

Higher density of cross-reactive network with self proteins

Immunogenicity analysis of HCP derived from CHO genome

Andres H. Gutiérrez, Chris Bailey-Kellogg, Kristen DaSilva, Frances Terry, Matthew Ardito, Lenny Moise, William Martin, Anne S. De Groot

Why Examine CHO HCP Immunogenicity?

Immune response to HCP (CHO) **led to the recent cancellation of two phase III clinical trials**

The trials were for Inspiration's IB1001, a recombinant factor IX produced in CHO cells.

These trials were cancelled due to the development of **anti-Chinese hamster ovary (CHO) antibodies** at higher levels than expected in patients treated with a protein drug.

We have screened the newly available CHO genome with our immunoinformatics tools (ISPRI) so as to help drug developers reduce HCP immunogenicity risk. A website will soon be available at http://www.immunome.org.

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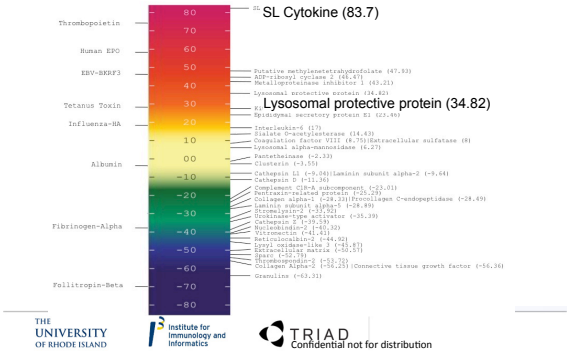
Immunogenicity scale



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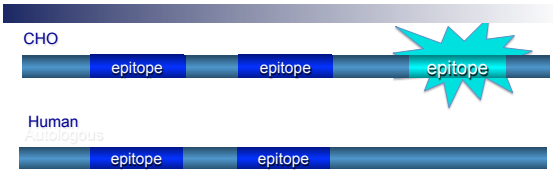
CHO vs. Human – Foreign epitopes



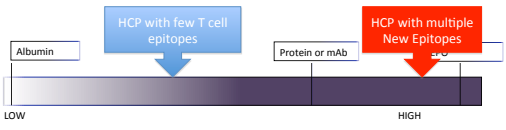
Epitopes that differ from self contribute to immune response -this relates to both B and T epitopes

Example: Bovine insulin

Response may depend on HLA of patient and # epitopes presented

Scaling CHO Immunogenicity

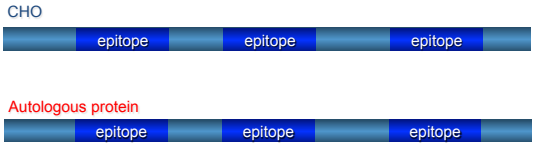
EpiVax –ISPRI - Immunogenicity Scale



Proteins ranked by T- Epitope content per Amino Acid

- De Groot A.S., Drug Discovery Today - 2006;
- De Groot A.S., Mire-Sluis, A. Ed.. Dev. Biol. Basel, Karger, 2005. vol 122. pp 137-160.

CHO vs. Human – Conserved Epitopes



If there is circulating autologous protein that is identical, the patient may be"tolerant" to the CHO protein.

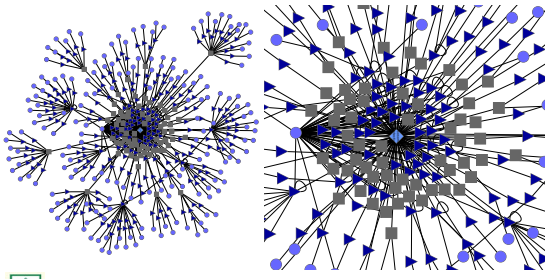
Several situations may overcome tolerance:

(1)Inflammation - adjuvants -may overcome tolerance (e.g. Eprex Story)

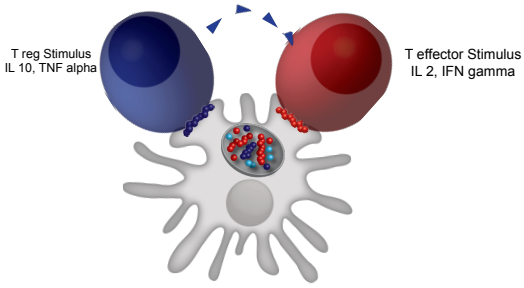
(2) Administration of aggregated protein (e.g.. Beta interferon)

JanusMatrix analysis

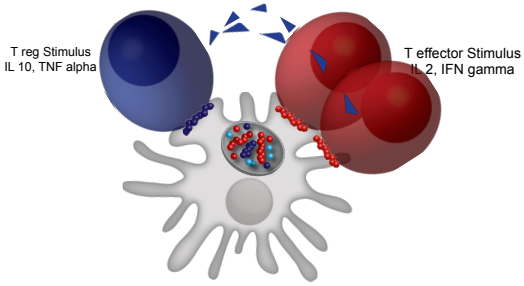
of epitopes contained in ***lysosomal protective protein*** that are similar to (human) self epitopes and probably engage regulatory T cells



Thus, for some CHO proteins – NEW epitopes are Balanced by Self Epitopes



In some HCP –TOO MANY NEW epitopes not balanced by Self Epitopes: Immunogenicity



CHO-Self can be "binned" In Two by Two Table:

	High Self content	Low Self content
Low Neo Epitope	+	++
High Neo Epitope	++	++++

Perhaps we could do it this way. . . Immune Response = Sum of Epitopes

Sum includes + (T effectors) and – (Conserved with human) scores

Protein Therapeutic

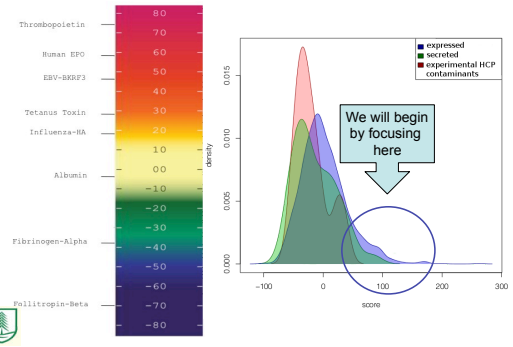
(1 + 1) - epitope conserved with Human = Response

T cell response depends on:

Neo epitope content x HLA – Conserved epitope Epitope content x HLA

Protein Immunogenicity can be Ranked

Epitope-Network-Adjusted Immunogenicity scale



Coming soon: CHOPPI
http://www.Immunome.org

CONCLUSIONS

T cells drive immune response to protein therapeutics

Not all T cell epitopes are the same

Treg epitopes in IgG = Tregitopes, a new concept – correlated with immunogenicity

Tregitope – will be presented later today

CHO (Host Cell Proteins) – drives of immune response when T cell epitopes differ.

Pre-clinical immunogenicity screening can be done in silico, *providing the T-reg predictions have been validated in vitro and in vivo*


Institute for
Immunology and
Informatics

Break


EpiVax, Inc.
Informatics and Immunology

Case Study: Immunogenicity and Clinical Outcome in RA Treatment

Presented by:

Dr. Naonobu Sugiyama, MD, PhD
JCR-board Certified Rheumatologist
Associate Director, RA & Inflammation
Medical Affairs Pfizer Japan

Notes:


Institute for
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Informatics


EpiVax, Inc.
Informatics and Immunology

New Technologies: Immunogenicity, Deimmunization and 3D Modeling

Presented by:

Dr. Chris Bailey-Kellogg, PhD
Associate Professor Computer Science,
Dartmouth College

Notes:


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Informatics


EpiVax, Inc.
Informatics and Immunology


Institute for
Immunology and
Informatics

Lunch


EpiVax, Inc.
Informatics and Immunology

Institute for Immunology and Informatics

Live Demonstration: In-Silico Immunogenicity Screening Platform (ISPRI)

Presented by:

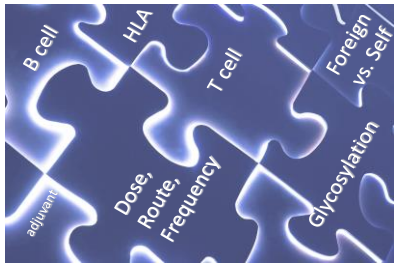
Ms. Frances Terry
Bioinformatics Program Manager,
EpiVax, Inc.

EpiVax, Inc.
Informatics and Immunology

ISPRI: Interactive Screening and Protein Re-engineering Interface

Prepared for:
Westin Immunogenicity Seminar
May 09, 2013

The Immunogenicity Puzzle



T Cell Epitopes

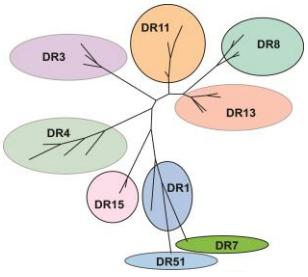
AND

"Foreign-ness"
Aggregation
"Danger signals"
Route, Dose, Frequency
Glycosylation/pegylation
Etc.

Immunogenicity: Perspectives

- **Vaccine Design**
  - Increase immunogenicity, specificity, breadth
- **Allergy and autoimmunity**
  - Identify relevant epitope
  - T cell epitopes to induce tolerance (Tregitopes)
- **Protein Therapeutics**
  - Screening and deimmunizing

EpiVax HLA "Supertype" Coverage



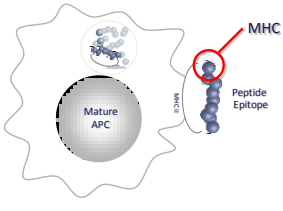
• EpiVax tests for binding potential to the most common HLA molecules within each of the "supertypes" shown to the left.

• 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.**

EpiMatrix

- 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

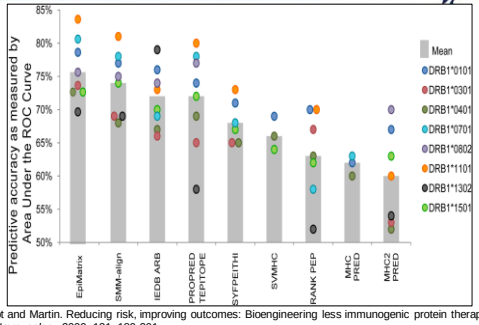


T cell epitopes are linear and directly derived from antigen sequence

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

Epitope Identification

EpiMatrix is a highly accurate epitope discovery tool

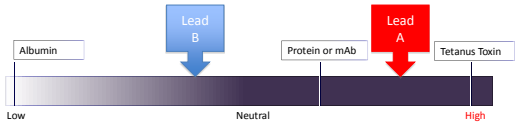


De Groot and Martin. Reducing risk, improving outcomes: Bioengineering less immunogenic protein therapeutics. *Clinical Immunology* 2009, 131, 189-201.

Scaling Immunogenicity

How Could We Apply to Triage Biologic Leads?
Select for T cell Epitope Content per AA

EpiVax –ISPRI - Immunogenicity Scale



Proteins ranked by T- Epitope content per Amino Acid

• De Groot A.S., Drug Discovery Today - 2006;
• De Groot A.S., Mire-Sluis, A. Ed.. Dev. Biol. Basel, Karger, 2005. vol 122. pp 137-160.

ISPRI

Interactive Protein Screening and Reengineering Interface

- EpiVax has developed a **secure**, interactive work environment that is seamlessly linked to EpiVax's proprietary in silico immunogenicity screening toolkit.
- This **interactive** biologics screening and optimizing work environment gives access to the same in silico tools used by the EpiVax bioinformatics team.
- ISPRI can be used for **high throughput** unlimited screening of partial and complete sequences of biological (protein therapeutic) candidates.
- The toolkit can be used to identify within each protein sequence potentially immunogenic regions (known as **epitope clusters**) and to fine map those individual amino acids which contribute most to the immunogenic potential of the cluster.
- The output is **customized** to best fit the needs and preferences of the client.

ISPRI

Available Tools

- EpiMatrix
  - Screen the protein sequences of product candidates for the presence of putative T cell epitopes.
- Immunogenicity Protein Scale
  - Rate the immunogenic potential of each submitted sequence on a normalized scale and compare each protein to other immunogenic proteins and antibodies
- Tregitope Analysis
  - For antibodies, identify within each submitted sequence putative regulatory T-cell epitopes (i.e. sub-regions contained within the submitted sequences which may relate to natural regulatory T cells and which may help to dampen the immune potential of the submitted antibody sequence)
- ClustiMer
  - Identify T-cell epitope clusters contained within product candidates
- Immunogenic Cluster Scale
  - Rate the immunogenic potential of each T-cell epitope cluster on a normalized scale and compare each T-cell epitope cluster to other well-known immunogenic epitope clusters
- BlastiMer
  - Blast T-cell epitope clusters against the non-redundant protein or patent database at GenBank
- OptiMatrix
  - The protein re-design algorithm that provides a list of critical amino acid residues and potential amino acid substitutions that are conserved in existing databases (based on published sequences) and that do not introduce new epitopes.

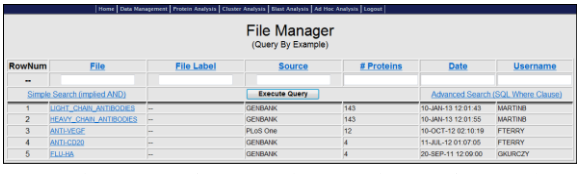
Data Management

ISPRI Data Management

Upload Proteins

File Manager

Description



List of uploaded files

List of optional file labels

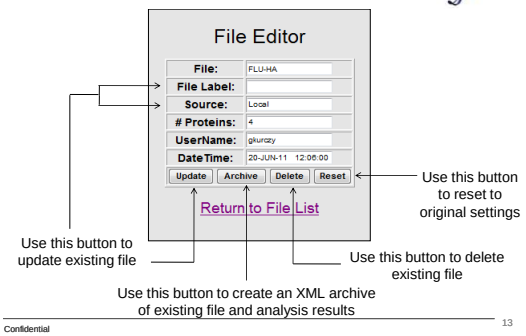
List of file sources

# of proteins in associated file

Date of upload

User labels

File Editor
Description



Protein Analysis

Protein Analysis
Protein Summary Report

Protein Analysis

ISPRI Protein Summary Report
Use this Link to Display the Protein Summary Report
ISPRI Protein Immunogenicity Scale
Use this Link to Display the Protein Immunogenicity Scale
ISPRI Protein Immunogenicity Report
Use this Link to Display the Results of EpiMatrix Analysis
ISPRI Antibody Immunogenicity Report
Use this Link to Analyze an Antibody for Immunogenicity

Approach – Whole Antigens

EpiVax Immunogenicity Hypothesis As Applied:
Immune Response = Sum of Epitopes

Protein Therapeutic

1 + 1 + 1 = Response

T cell response depends on:

T cell epitope content + HLA of subject

Protein Immunogenicity can be Ranked

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

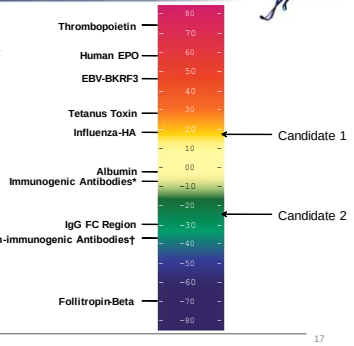
ISPRI Protein Immunogenicity Scale
Rates immunogenic potential relative to standardized controls

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

All scores are adjusted for the presence of Tregitopes.

*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



ISPRI Protein Detail Report
Provides detailed map of epitope content

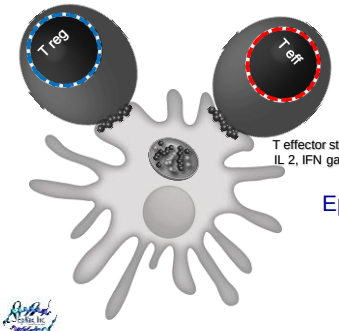
EpiMatrix Report
Accession: YOUR_PROTEIN - Sequence: YOUR_PROTEIN

Frame Start	AA Sequence	Frame Stop	DRB1*0101 Z-Score	DRB1*0301 Z-Score	DRB1*0401 Z-Score	DRB1*0701 Z-Score	DRB1*0801 Z-Score	DRB1*1101 Z-Score	DRB1*1301 Z-Score	DRB1*1501 Z-Score	Hits
1	DIQMTQSPS	9	0.91	0.17	0.35	0.06	0.09	0.86	-0.08	-0.01	0
2	IQMTQSPSS	10	2.15	1.35	2.26	1.58	2.17	1.88	2.24	2.52	6
3	QMTQSPSSL	11	1.11	0.28	0.5	0.96	0.29	0.39	0.9	0.4	0
4	MTQSPSSLS	12	1.87	2.26	2.02	1.88	1.06	1.66	1.79	2.05	7
5	TQSPSSLSA	13	0.51	0.28	1.31	0.69	-0.04	0.54	1.09	1.05	0
.	.	.	.	.	.	.	.	.	.	.	.
46	LLIYAASTL	54	1.41	1.08	0.99	2.16	1.54	0.85	2.28	2.3	3
47	LIYFASTLQ	55	1.91	1.85	2.46	2.07	1.79	1.08	1.79	2.1	7
48	IYFASTLQS	56	1.73	2.01	2.68	1.26	1.58	1.82	2.11	2.07	6
49	YFASTLQSG	57	0.64	1.6	0.59	0.32	1.42	1.28	0.04	1.09	0
.	.	.	.	.	.	.	.	.	.	.	.
98	FQQKTVEI	106	0.7	1.9	-0.02	0.2	0.93	0.34	1.41	1.12	1
99	QQKTVEIK	107	-0.28	-0.84	0.31	0.01	0.03	-0.57	-0.47	-1.24	0
Summarized Results			DRB1*0101	DRB1*0301	DRB1*0401	DRB1*0701	DRB1*0801	DRB1*1101	DRB1*1301	DRB1*1501	Total
Maximum Single Z score			2.4	2.26	2.8	3.11	2.41	2.29	2.28	2.52	--
Sum of Significant Z scores			23.41	12.17	15.96	18.16	10.2	13.2	16.47	16.99	126.56
Count of Significant Z Scores			12	6	7	9	5	7	8	8	62

Total Assessments Performed: 792	Deviation from Expectation: 38.46	Deviation per 1000 AA: 48.56
Adjusted for Regulatory Epitopes	Deviation from Expectation: -21.24	Deviation per 1000 AA: -26.82

Assessment
EpiBar
Hit
EpiMatrix Immunogenicity Score
EpiMatrix Tregitope-adjusted Score

Presence of epitope indicates immune potential



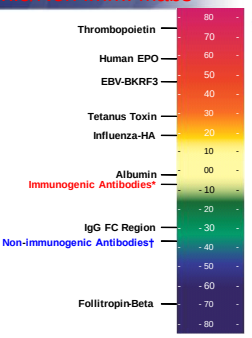
Epitope can be *either* T-eff or Treg

Validation in Clinical Practice
Compare Immunogenic/Non Imm. Mabs

EpiMatrix predicted excess/shortfall in aggregate immunogenicity relative to a random peptide standard.

All scores are adjusted for the presence of Tregitopes.

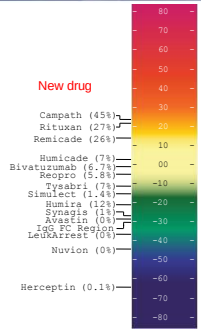
A protein score > 20 indicates a significant immunogenic potential.



*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

Antibodies: A Special Case



Due to the presence of Tregitopes, antibodies tend to fall lower on the immunogenicity scale.

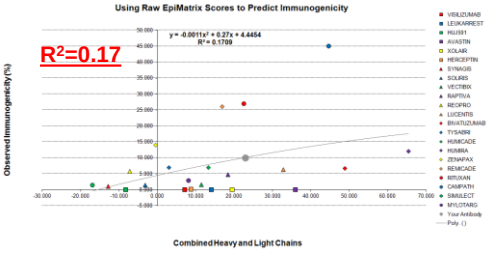
We have developed a refined method using regression analysis to predict the immunogenicity of antibody sequences based on observed clinical responses.

We have found that a balance in favor of Tregitope (regulatory) content over neo-epitope (effector) content is correlated with reduced clinical immunogenicity.

Neo Epitope Content	Tregitope Content: High	Tregitope Content: Low
Low	Avastin (0%) Herceptin (0%)	Mylotarg (3%) Simulect (1%) Synagis (1%)
High	Campath (45%)	Remicade (26%) Rituxan (27%)

Correlation of antibody immunogenicity without Tregitope adjusted EPX Scores

Correlation to observed Immunogenicity before accounting for Tregitopes



Immunogenicity Scale for Monoclonals

Factoring in Tregitopes. . .

Protein Therapeutic

1 + 1 - Regulatory T cell epitope* = Response

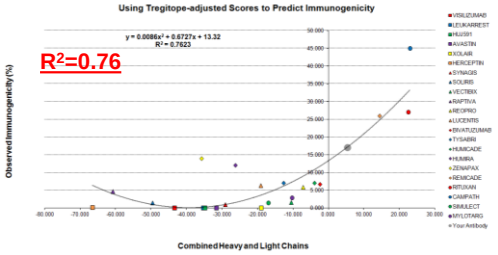
T cell response depends on:

T cell epitope content – Tregitope content + HLA of subject

Protein Immunogenicity can be Ranked

Correlation of antibody immunogenicity with Tregitope adjusted EPX Scores

Correlation to observed immunogenicity after accounting for Tregitopes



Accounting for Tregitopes results in more accurate predictions.

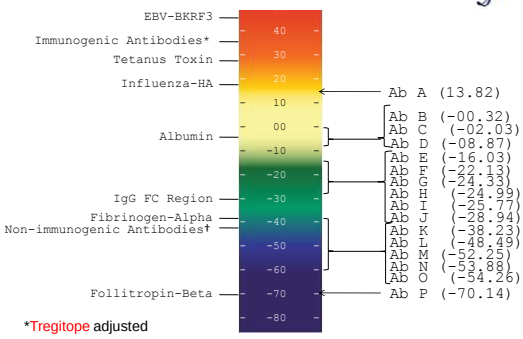
Analyzing Antibodies

Antibody Reports rate immunogenic potential on a standardized scale

Categorized Immunogenic Potentials

Optimal Antibodies (Low Effector Content - High Tregitope Content)				
Antibody	**Tregitope-Adjusted EpiMatrix Protein Score[1]**	**Tregitope Content[2]**	**Predicted Ab Response**	**Observed Ab Response**
Herceptin	-66.56	75.27	0.00	0.10
Raptiva	-60.84	79.23	0.00	4.70
Soliris	-49.67	46.50	0.00	1.50
Visilizumab	-43.41	50.57	0.00	0.00
Zenapax	-35.76	35.32	0.26	14.00
LeukArrest	-35.29	49.39	0.29	0.00
HUJ591	-34.88	26.67	0.32	0.00
Your Antibody	**-31.61**	**67.57**	**0.65**	**n.a.**
Avastin	-31.61	67.57	0.65	0.00
Humira	-26.29	91.75	1.58	12.00
Lucentis	-19.13	51.94	3.60	6.30
Xolair	-19.01	38.54	3.64	0.00
Vectibix	-10.49	21.94	7.21	1.60
Bivatuzumab	-2.48	51.35	11.70	6.70

Application – Germline Abs*



*Tregitope adjusted

Cluster Analysis

Cluster Analysis

ISPRI Find T Cell Epitope Clusters

Cluster Analysis

ISPRI Find T Cell Epitope Clusters
Use this Link to Screen Protein Sequences for T Cell Epitope Clusters

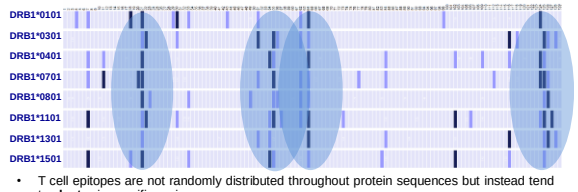
Interactive Cluster Immunogenicity Report
Use this Link to Display the Results of an EpiMatrix Cluster Immunogenicity Analysis

Printable Cluster Immunogenicity Report
Use this Link to Print or Download an EpiMatrix Cluster Analysis

Printable Logo Report
Use this Link to Print or Download an EpiMatrix Logo Analysis

ISPRI

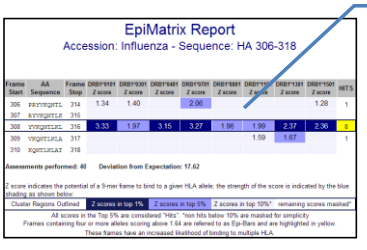
ClustiMer finds promiscuous epitopes



- 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 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

What Makes Proteins Really immunogenic?

Sequences that Contain EpiBars

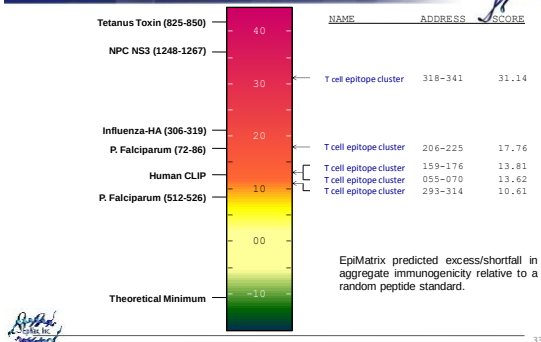


Roberts CGP, Meister GE, Jesdale BM, Lieberman J, Berzofsky JA, A.S. De Groot, Prediction of HIV peptide epitopes by a novel algorithm, AIDS Research and Human Retroviruses, 1996, Vol. 12, No. 7, pp. 593-610.

ClustiMer - Locates highly immunogenic regions

Immunogenicity Scale - Peptides

Cluster Immunogenicity Scale

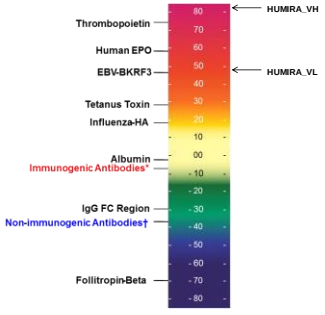


NAME	ADDRESS	SCORE
T cell epitope cluster	318-341	31.14
T cell epitope cluster	206-225	17.76
T cell epitope cluster	159-176	13.81
T cell epitope cluster	055-070	13.62
T cell epitope cluster	293-314	10.61

EpiMatrix predicted excess/shortfall in aggregate immunogenicity relative to a random peptide standard.

Humira – Case Study

EpiMatrix Raw Scores

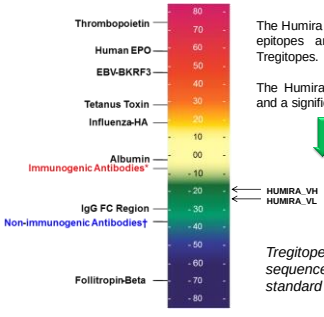


With respect to raw EpiMatrix scores, Humira scores extremely high

Indicates significant potential for immunogenicity

Humira – Adjusted Immunogenicity Scale

Tregitope Adjusted Scores



The Humira heavy chain contains a significant neo-epitopes and a significant number of known Tregitopes.

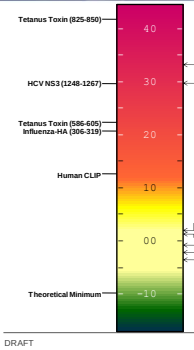
The Humira light chain contains one neo-epitope and a significant number of known Tregitopes.

Indicates a drop in significant potential for immunogenicity

Tregitope adjusted scores of the submitted sequences rate against the same series of standard controls.

Key Humira Epitopes EMX Scale

Tregitope Adjusted Scores



NAME	ADDRESS	SCORE
HUMIRA_VH	091-113	33.92
HUMIRA_VL	043-065	29.62

HUMIRA_VH	01.69
HUMIRA_VL	01.48
HUMIRA_VH	01.32
HUMIRA_VH	-00.45
HUMIRA_VH	-01.65
HUMIRA_VL	-02.47

Two epitopes with high EMX scores are expected to contribute to immunogenicity . . .

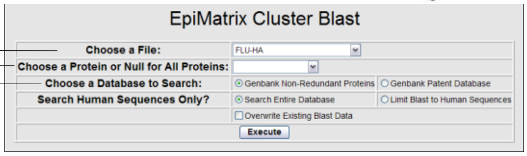
Other clusters, when Tregitope adjusted – may cause proliferation but phenotype of proliferating cells should be evaluated . . . Could be Tregitopes

Homology Analysis

BLAST Analysis

Submit Epitope Clusters to BLAST

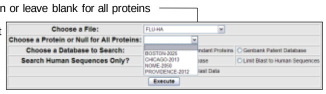
Submit Epitope Clusters to BLAST
Description



Click arrow to select desired File

Click arrow to select desired protein or leave blank for all proteins

Chose which database to BLAST against



BlastiMer
BLAST helps to find viable substitutions

- BLAST functions:
  - Submit T cell epitope clusters to NCBI GenBank BLAST
    - Compare to Non-redundant Database
      Patent Database
      Human Sequence Database
    - View Summary or detailed Alignment Reports

Cluster Blast Summary Report for SEASONAL_H3N2_HA_CLASS2_ICS-3-1
Analysis of Homologous Sequences
November 18, 2011
Click to Print Click to Download Back to Cluster Summary

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
I	N	Q	I	N	G	K	L	N	R	L	I	G	K	T
I 9/15	N 7/15	E 7/15	I 13/15	N 13/15	T 7/15	E 7/15	L 8/15	N 15/15	K 8/15	L 15/15	I 13/15	NONE 9/15	NONE 9/15	NONE 15/15
NONE 6/15	D 6/15	H 6/15	V 2/15	L 2/15	M 6/15	N 6/15	I 7/15		Q 7/15		NONE 2/15	E 6/15	K 6/15	
	S 2/15	Q 2/15			G 2/15	K 2/15								

BlastiMer
BLAST helps to find human-like sequences

Blast Alignment Report for ENV_SEPT01-OCT01_CVX-DATA-1
Analysis of Homologous Sequences
September 23, 2011
Click to Print Click to Download Back to Cluster Summary

	# BLAST HITS	SEQUENCE	AA IDENTITIES	FILE	DESCRIPTION	ORGANISM
	--	[illegible]	--	ENV_SEPT01-OCT01	--	n/a
TOP 11 BLAST HITS	8	[illegible]	8/16	AAH04950	PHRF1 protein	[Homo sapiens]
	3	[illegible]	8/16	AAI44296	PHRF1 protein	[Homo sapiens]
Total:	11					

Blast Alignment Report for ENV_SEPT01-OCT01_CVX-DATA-2
Analysis of Homologous Sequences
September 23, 2011
Click to Print Click to Download Back to Cluster Summary

	# BLAST HITS	SEQUENCE	AA IDENTITIES	FILE	DESCRIPTION	ORGANISM
	--	[illegible]	--	ENV_SEPT01-OCT01	--	n/a
TOP 0 BLAST HITS	No significant matches found. See footnotes for a complete explanation.					
Total:	0					

Deimmunization

OptiMatrix – Interactive Engineering
Overview

- Identifies those individual amino acids that contribute the most to binding affinity across peptide frames and HLA alleles.
- Displays that changes in these "sensitive" amino acids can have a disproportional impact on the immunogenicity of the underlying sequence.
- Shows, in real-time, the impact each amino acid mutation has on the overall immunogenicity of the peptide

OptiMatrix
Use OptiMatrix to redesign potentially immunogenic clusters

OptiMatrix: Interactive Peptide Deimmunization
Accession: FLU-HA - Sequence: BOSTON-2025 - Cluster: 254
September 25, 2009 (Epx Ver. 1.2)
Click to Print Save Deimmunized Sequence Back to Summary Report

ORIGINAL SEQUENCE

254	255	256	258	259	260	261	262	263	264	265	266	267	268	269
P	R	G	F	K	I	R	T	G	K	T	T	I	M	R
0	4.72	0	15.79	15.94	12.99	16.69	3.71	2.98	12.23	3.32	3.32	4.63	1.78	1.72

MODIFIED SEQUENCE

254	255	256	258	259	260	261	262	263	264	265	266	267	268	269
P	R	G	F	K	I	R	T	G	K	T	T	I	M	R
0	4.72	0	15.79	15.94	12.99	16.69	3.71	2.98	12.23	3.32	3.32	4.63	1.78	1.72

A C D E F G H I K L M N P Q R S T V W Y

The number below each amino acid indicates that residue's relative impact on EpiMatrix scores averaged across all alleles and frames. In this Logo Report the size and color of each amino acid is keyed to its EpiMatrix score. Higher scoring amino acids are represented larger, indicating that they are more "sensitive" than lower scoring amino acids.

Show Suggested Substitutions Show ISPRI Cluster Report Show ISPRI Blast Summary Best Single Change

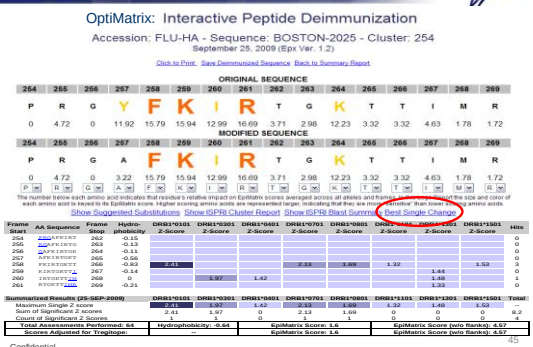
Frame Start	AA Sequence	Frame Stop	Hydro-phobicity	DRB1*0101 Z-Score	DRB1*0301 Z-Score	DRB1*0401 Z-Score	DRB1*0701 Z-Score	DRB1*0801 Z-Score	DRB1*1101 Z-Score	DRB1*1301 Z-Score	DRB1*1501 Z-Score	Hits
254	PRGYFKIRT	262	-0.23									0
255	RGYFKIRTG	263	-0.2									0
256	GYFKIRTGK	264	-0.19									0
257	YFKIRTGKT	265	-0.9	2.38		2.41	2.51	1.4	2.2		1.98	5
258	FKIRTGKTT	266	-0.83	2.41			2.13	1.69	1.32		1.53	3
259	KIRTGKTTI	267	-0.14							1.44		0
260	IRTGKTTIM	268	0		1.97	1.42				1.48		1
261	RTGKTTIMR	269	-0.21							1.33		0

Summarized Results (25-SEP-2009)	DRB1*0101	DRB1*0301	DRB1*0401	DRB1*0701	DRB1*0801	DRB1*1101	DRB1*1301	DRB1*1501	Total
Maximum Single Z score	2.41	1.97	2.41	2.51	1.69	2.2	1.48	1.98	--
Sum of Significant Z scores	4.79	1.97	2.41	4.64	1.69	2.2	0	1.98	19.68
Count of Significant Z Scores	2	1	1	2	1	1	0	1	9

Total Assessments Performed: 64	Hydrophobicity: -0.84	EpiMatrix Score: 13.08	EpiMatrix Score (w/o flanks): 16.05
Scores Adjusted for Tregitope:	--	EpiMatrix Score: 13.08	EpiMatrix Score (w/o flanks): 16.05

OptiMatrix

See the effects of amino acid substitution in real-time



ISPRI v1.5

New Features in 2013

- Protein Analysis (early 2013)
  - CDR Identification and Scoring Tool
    - Using any of the four most popular antibody numbering schemes, identify and score CDRs for immunogenic potential
  - Updated Antibody Immunogenicity Regression
    - Systematic review of newly released immunogenicity data for licensed monoclonals and incorporation into Antibody Immunogenicity Prediction tool
- Homology Analysis (early 2013)
  - Improved BLAST submission interface and reporting
    - Direct identification of successful vs. unsuccessful submissions to NCBI BLAST
  - Construction and maintenance of new, local databases
    - Investigate and score homology with human and human microbiome genomes without releasing proprietary sequences into public search space

ISPRI v1.5

New Features in 2013

- EpiMatrix v1.3 (late 2013)
  - New curation of known epitope sequences
    - Rebuild predictive matrices and add tools for HLA DRB1*0901 and *1201

ISPRI Development Team

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  - Email: martinb@epivax.com
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  - Email: fterry@epivax.com
- Jacob Tivin, Immunoinformatics Programmer
  - Tel: (401) 272-2123 (ext. 128)
  - Email: jtivin@epivax.com

Science without fear.

www.epivax.com • info@epivax.com • 401.272.2123

Applications of the ISPRI System

Clinical Validation

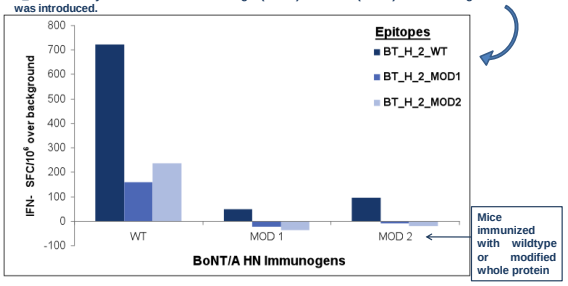
Immunogenicity proof of principle
in the following PROSPECTIVE* clinical studies:

- Koren et al. 2007 FPX
- Moxness et al. 2008 GDNF
- De Groot and Martin Clinical Immunology 2009
- Jawa (Amgen) 3 low immunogenicity compounds in the clinic (in press)

*In each of these studies, EpiVax provided screening prior to learning immunogenicity results

BoNT/A MODs are less antigenic and less immunogenic

H_2 is the anonymized locus at which a single (MOD1) or double (MOD2) deimmunizing mutation was introduced.



Recall response to modified peptides was decreased compared to wildtype in all groups.

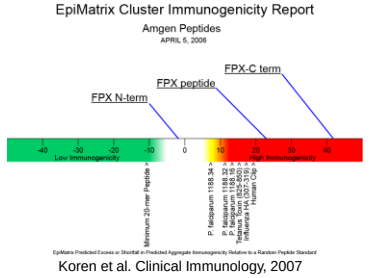
Correlation of EpiMatrix Scores and Immunogenicity in Human studies

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

Negative score indicates presence of Treg epitope

Case study: HLA/immunogenicity: FPX

Preclinical Analysis: Immunogenicity at C terminus



Koren et al. Clinical Immunology, 2007

Why is the response to Biologics Variable by Subject? Immune Response = Sum of Epitopes

Protein Therapeutic

1 + 1 + 1 = iTEM

T cell response depends on:

T cell epitope content + HLA of subject

Protein Immunogenicity can be ranked for a population
However depending on HLA of the individual, results may vary

If the subject only has one allele that can present the epitope, the response to the protein may be diminished*

Protein Therapeutic

T cell response depends on:

T cell epitope content + HLA of subject

iTEM captures this variability:
The individualized T-cell Epitope Measure

EMX score HLA allele #1 + EMX Score HLA allele #2 . . . = iTEM

* Recently confirmed in a large scale studies of HIV/HLA

Individualized T cell Epitope Measure = iTEM

• iTEM = HLA Allele x EMX score + HLA Allele x EMX score + . . . = measures potential strength of response to antigen

Can be used to predict which patients (with which HLA) will develop an antibody response in a clinical trial…

. . . *Immunopharmacogenomics*

Observed Correlation between HLA Haplotype, iTEM , Antibody Concentration and Response

Seq 11-24

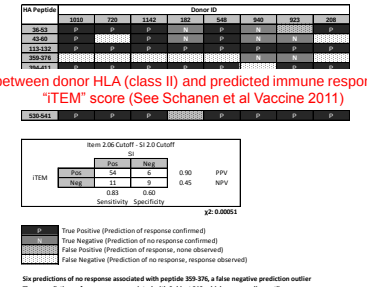
HLA DRB1	iTEM	Ab conc (mg/mL)	IFN-g SFC ratio	IL-4 SFC ratio
*0301/0701	4.75	5.60	1.74	2.60
*0101/0103	2.83	2.80	2.00	3.34
*0701/1501	6.25	20.20	26.0	89.0
*0301	1.67	NA	1.04	1.30

Observed Correlation between HLA Haplotype, iTEM , Antibody Concentration and Response

Seq 11-24

HLA DRB1	iTEM	Ab conc (mg/mL)	IFN-g SFC ratio	IL-4 SFC ratio
*0301/0701	4.75	5.60	1.74	2.60
*0101/0103	2.83	2.80	2.00	3.34
*0701/1501	6.25	20.20	26.0	89.0
*0301	1.67	NA	1.04	1.30

Further study of Cross Conserved epitopes – T cell recognition based on HLA Thus – HLA determines extent to which cross-conserved peptides may protect



Correlation between donor HLA (class II) and predicted immune response to peptide – "iTEM" score (See Schanen et al Vaccine 2011)

Institute for Immunology and Informatics

Into the Clinic: Immunogenicity Solutions

Presented by:

Dr. Annie De Groot M.D.
Professor and Director, Institute of Immunology and Informatics,
University of Rhode Island, CEO/CSO, EpiVax

Notes:


Institute for
Immunology and
Informatics


EpiVax, Inc.
Informatics and Immunology

Institute for Immunology and Informatics

Quick Update: Rapid Vaccine Design for (H7N9) Pandemic Readiness

Presented by:

Dr. Annie De Groot M.D.
Professor and Director, Institute of Immunology and Informatics,
University of Rhode Island, CEO/CSO, EpiVax

EpiVax, Inc.
Informatics and Immunology

Extremely Rapid H7N9 Immunogenicity Analysis and Vaccine Design

Or – 10 steps to Rapid Flu Vaccine Design

April 28, 2013

Annie De Groot M.D.
CEO/CSO
www.epvax.com

Adapted from a Presentation to NIAID

Universal Flu Vaccines: Now More than Ever

EpiVax has a flu SBIR that was scored and we are waiting for a decision about funding

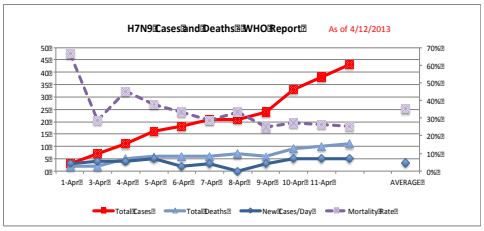
21 March 2013
Presented by Annie De Groot MD
to Rachelle Salomon, NIH, NIAID, DMID
EpiVax: Lenny Moise, Frances Terry, Bill Martin
Mindy Cote, Ryan Tassone, Howie Latimer
Lauren Levitz, Christine Boyle
VGTI: Ted Ross

Before starting a vaccine, Consider the 10 steps:

1. Define Disease (identify individuals who fit disease criteria)
2. Isolate and Define Pathogen (helps develop diagnostic test)
3. Is there Immunity (if not you are in trouble)
4. Correlates of Immunity one or many? (Ab? Innate? CMI?)
5. Critical Antigens - one or many?
6. Animal Model? Does it predict protection?
7. Prototype Vaccine – Obtain Preclinical Proof in Animal Model
8. Safety and Toxicity, GMP production, Stability
9. FDA "IND" (Investigational New Drug)
   - Clinical trials (Phase I, II, III)
10. FDA "NDA" (New Drug Application) Approval
    - Distribution / Access

Emergent H7N9 disease in China

The Problem: Ongoing Transmission

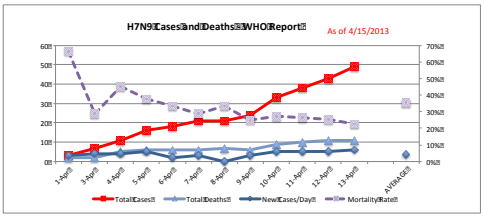


http://www.who.int/csr/don/2013_04_12/en/index.html

http://pandemicinformationnews.blogspot.com

http://crofsblogs.typepad.com

New Case in Beijing



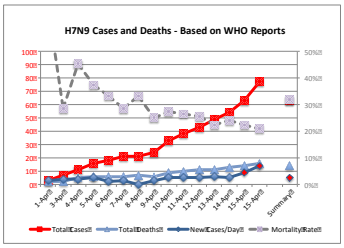
New Case in Beijing

Spread to Beijing on 4/13/13
Spread to Henan on 4/14/ 13

Wide Distribution of Cases

This picture shows the geographically wide distribution of flu cases - suggesting widespread distribution of the virus rather than a point outbreak.

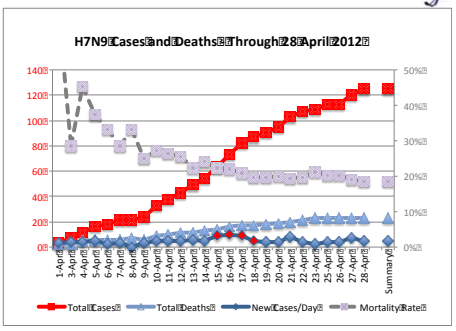
16 April 2013 – Continued Expansion



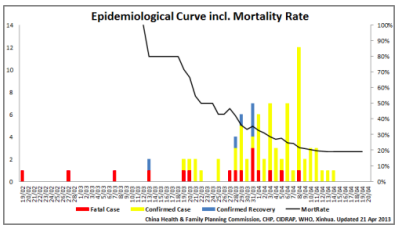
http://www.who.int/csr/don/2013_04_12/en/index.html

http://crofsblogs.typepad.com

28th April 2013 – Continued Expansion

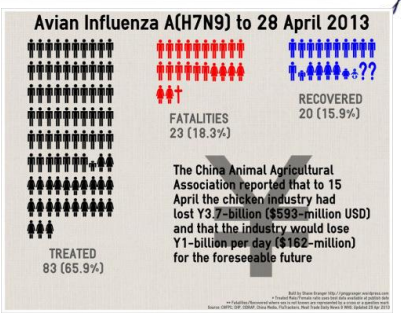


Epidemiology



http://gmggranger.wordpress.com/2013/04/17/random-analytics-influenza-ah7n9-viru

Epidemiology



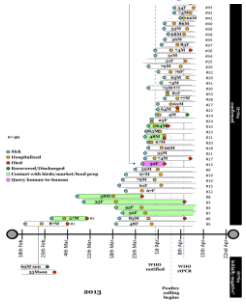
http://gmggranger.wordpress.com/2013/04/17/random-analytics-influenza-ah7n9-virus-mar-apr-2013/

H7N9 Morbidity and Mortality

Quick numbers… 28th April 2013

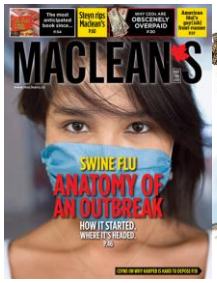
- Total confirmed human cases of influenza A virus H7N9: **125**
- Total deaths attributed to infection with influenza A virus H7N9: **23**
- Case Fatality Rate (CFR): **18%**
- Average time from illness onset to first confirmation of H7N9 (days): **10**
- Average age of the H7N9-confirmed cases (including deaths; years): **60**
- Number of people who have actually recovered (after hospitalization): **9**
- Mode age of the H7N9-confirmed cases (including deaths; years): **74**
- Average age of the deceased (years): **59**
- Males: **70%** of cases, **82%** of deaths
- Younger patients are recovering . . .

http://pandemicinformationnews.blogspot.com



http://www.uq.edu.au/vdu/VDUInfluenza_H7N9.htm

The "New" Flu (H1N1 2009 California)

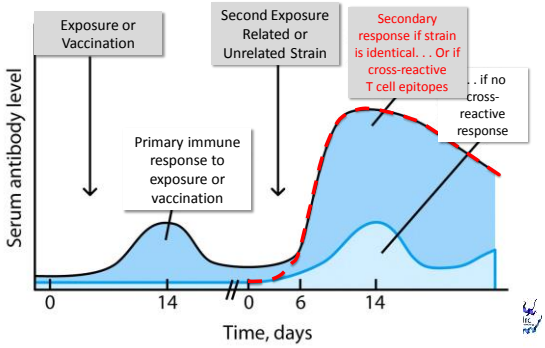


2009 Worry: CDC - No Cross-reactive Ab

- Preliminary studies of individuals showed that antibodies induced by seasonal influenza vaccination were not cross-reactive with novel H1N1.
- What if the T cell epitopes were cross-reactive? Would that help?

Centers for Disease Control and Prevention. Serum antibody response to a novel influenza A (H1N1) virus after vaccination with seasonal influenza vaccine. MMWR Morb Mortal Wkly Rep 2009;58(19):521–4.

But – X-reactive T cell response boosts Ab response



Time to consider T cell epitopes?

Hum Vaccin. Author manuscript; available in PMC 2011 August 1.
Published in final edited form as:
Hum Vaccin. 2010 February 19; 6(2): 11333.
Published online 2010 February 19.
PMCID: PMC2936654
NIHMSID: NIHMS208863

Time for T? Thoughts about the 2009 novel H1N1 influenza outbreak and the role of T cell epitopes in the next generation of influenza vaccines

Anne S. De Groot, M.D.,[1,2,3] Elizabeth McClaine,[1] Lenny Moise,[1,2] and William Martin[1]

Author information ▶ Article notes ▶ Copyright and License information ▶

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2936654/

EpiVax Predicted Cross-Protection

Vaccine
Volume 27, Issue 42, 25 September 2009, Pages 5740-5747

Short communication

Immunoinformatic comparison of T-cell epitopes contained in novel swine-origin influenza A (H1N1) virus with epitopes in 2008–2009 conventional influenza vaccine

Anne S. De Groot[a, b, c], Matt Ardito[a], Elizabeth M. McClaine[a], Leonard Moise[a, b], William D. Martin[a]

[a] EpiVax, 146 Clifford Street, Providence, RI 02903, USA
[b] Institute for Immunology and Informatics, University of Rhode Island, 80 Washington Street, Providence, RI 02903, USA
[c] Alpert Medical School, Brown University, Providence, RI 02903, USA

http://dx.doi.org/10.1016/j.vaccine.2009.07.040, How to Cite or Link Using DOI
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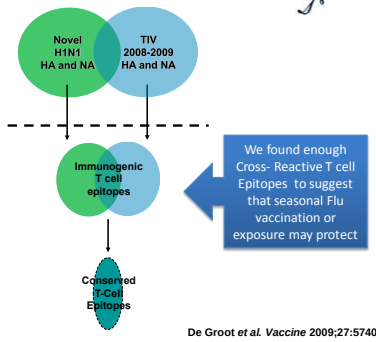
http://www.ncbi.nlm.nih.gov/pubmed/19660593

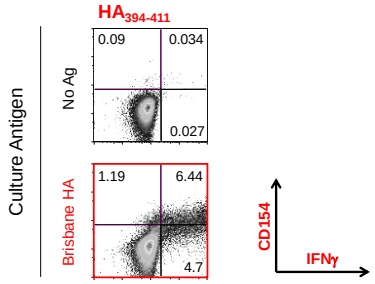
Identified immunogenic and conserved Sequences – Predicted Cross Protection



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

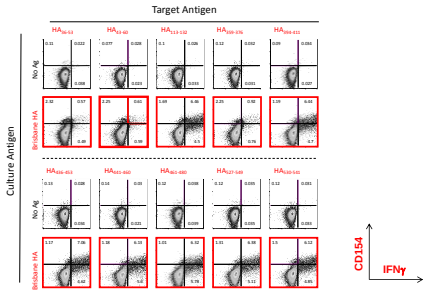
Validation that cross-conserved T cell epitopes are antigenic

"Immunized" with Brisbane HA whole Flu vaccine - Response to X-Conserved T cell epitopes



VAXDESIGN http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3130614/ Schanen *et al. Vaccine* 2011;29:3299-309

'FluVax' cross-conserved T cell epitopes are antigenic



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

Schanen et. al – worth a read. Shows cross-reactive T cell responses

Coupling sensitive *in vitro* and *in silico* techniques to assess cross-reactive CD4+ T cells against the swine-origin H1N1 influenza virus

Brian C. Schanen[a], Anne S. De Groot[b,c,d], L. Moise[b,d], Matt Ardito[b], Elizabeth McClaine[b], William Martin[b], Vaughan Wittman[a], William L. Warren[a], Donald R. Drake III[a,*]

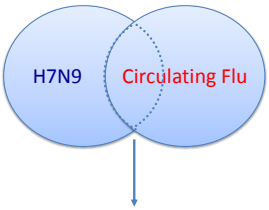
[a] sanofi pasteur, VaxDesign Campus, Orlando, FL, USA
[b] EpiVax, Inc., Providence, RI, USA
[c] The Warren Alpert Medical School of Brown University, Providence, RI, USA
[d] Institute for Immunology and Informatics, University of Rhode Island, Providence, RI, USA

The "Stealth" Flu (H7N9 2013 Shanghai)

FluVax Status

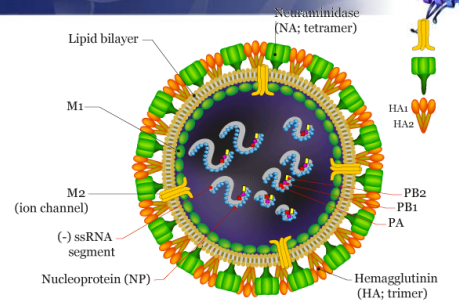
- This work recapitulates other projects already completed: Complete protection using ONLY T cell epitopes (*H. pylori*, Tularemia, VennVax)
- Results of these studies suggest conserved influenza sequences, important to viral fitness, also may be immunologically significant contributors to protection against newly emerging influenza strains.
- The *conserved epitope approach promises to answer the need for prompt preparedness and delivery of a safe, efficacious vaccine without requiring a new vaccine for every emergent influenza strain*.

What Can We Learn About H7N9? (1) Epitopes Novel or Conserved?



Very Poor Cross-Conservation – Only within Internal Proteins

Which H7N9 Proteins



Ian Mackey http://www.uq.edu.au/vduVDUInfluenza_H7N9.htm

Conservation Analysis H7N9: Th

Class II (Thelper) epitope Mapping by EpiMatrix (www.EpiVax.com) Comparison of Emerging H7N9 with Current Circulating Influenza Strains (TIV)

	A/California/7/2009 (H1N1)	A/Victoria/361/2011 (H3N2)	B/Wisconsin/1/2010
	Conserved T helper Epitopes:	Conserved T helper Epitopes:	Conserved T helper Epitopes:
Conservation at 70%	78	3	14
	of 101, or	of 101, or	of 101, or
	77%	3%	14%
	Are over 70% conserved	Are over 70% conserved	Are over 70% conserved
	55	0	0
Conservation at 90%	Of 101, or	101	101
	54%	0%	0%
	Are more than 90% conserved	Are more than 90% conserved	Are more than 90% conserved
	Of a total of 101 epitopes	Of a total of 101 epitopes	Of a total of 101 epitopes
Conservation In HA or NA only	0	3	0
	Or	or	Or
	0%	3%	0%
	Are conserved in HA and NA	Are conserved in HA and NA	Are conserved in HA and NA

Poor conservation in internal proteins for all previously circulating (and seasonal flu vaccine) strains
Except for internal proteins of H1N1 – 55% conservation with H7N9.

Conservation Analysis H7N9: CTL

H7N9 Class I (CTL) epitope Mapping by EpiMatrix (www.EpiVax.com) A Comparison of Emerging H7N9 with Current Circulating Influenza Strains (TIV)

A/California/7/2009 (H1N1)	A/Victoria/361/2011 (H3N2)	B/Wisconsin/1/2010
Total Class I Epitopes Conserved	Total Class I Epitopes Conserved	Total Class I Epitopes Conserved
522	2	12
586	1103	76
89%	0%	16%
(Are 100% conserved)	(Are 100% conserved)	(Are 100% conserved)
of which	of which	of which
8	2	0
epitopes or a total of	epitopes or a total of	epitopes or a total of
1%	0%	0%
. . . are conserved in HA and NA	. . . are conserved in HA and NA	. . . are conserved in HA and NA

Poor conservation in internal proteins for all previously circulating (and seasonal flu vaccine) strains
Except for internal proteins of H1N1 – 89% conservation with H7N9.

This is a unique virus

- Low conservation of HA, NA surface proteins is not surprising (completely new strain).
- Internal proteins are more conserved with H1N1 "pandemic" influenza CA 2009.
- And – HA is has unusually low immunogenicity see next slides.
- Could that explain why infection is widespread?
- Difficult to make antibodies to the HA, and if people are asymptomatic, they spread it easily.

What Can We Learn About H7N9? (2) Immunogenicity of HA

HA (hemagglutinin) is used for Flu vaccines – which are currently in production "subunit vaccines" based only on HA.

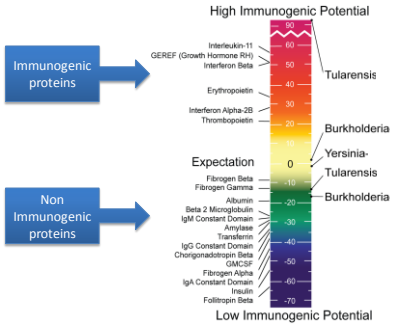
How do we measure Immunogenicity?

Vaccine antigen

1 + 1 + 1 = Response

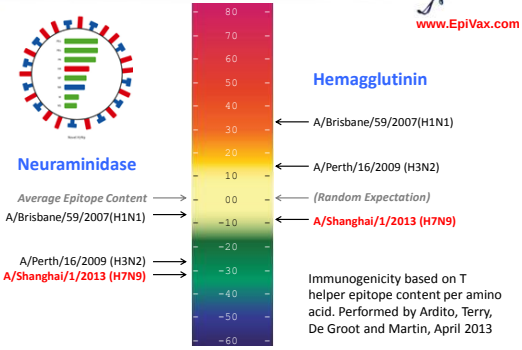
Immune response to a vaccine antigen can be predicted by measuring the number of T cell epitopes contained in the antigen with immunoinformatics tools.

"Immunogenicity Scale"



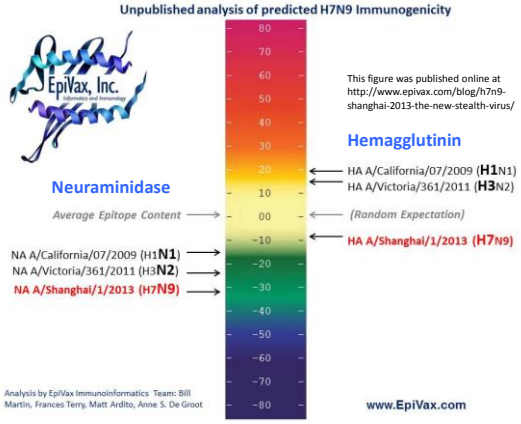
New H7N9 Flu is Predicted to be POORLY IMMUNOGENIC

www.EpiVax.com



Immunogenicity based on T helper epitope content per amino acid. Performed by Ardito, Terry, De Groot and Martin, April 2013

Unpublished analysis of predicted H7N9 Immunogenicity

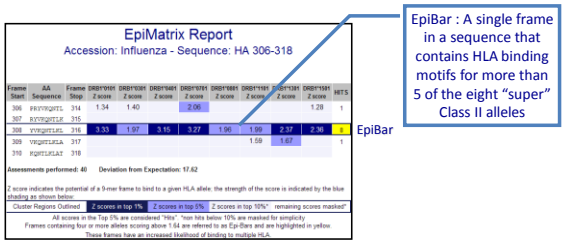


This figure was published online at http://www.epivax.com/blog/h7n9-shanghai-2013-the-new-stealth-virus/

Analysis by EpiVax Immunoinformatics Team: Bill Martin, Frances Terry, Matt Ardito, Anne S. De Groot

www.EpiVax.com

Most Immunogenic Epitopes Contain HLA binding motif Clusters (we call them EpiBars)

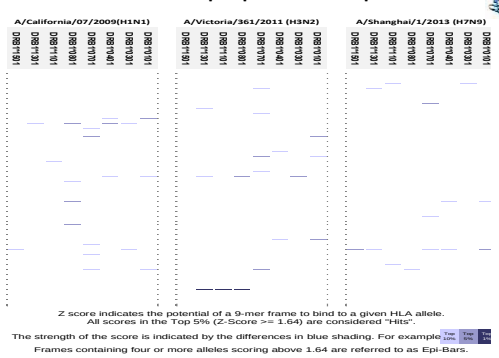


EpiBar : A single frame in a sequence that contains HLA binding motifs for more than 5 of the eight "super" Class II alleles

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

Predicted HA Immunogenicity Based on T cell Epitope Content - EpiMatrix



http://www.EpiVax.com

Z score indicates the potential of a 9-mer frame to bind to a given HLA allele.
All scores in the Top 5% (Z-Score >= 1.64) are considered "Hits".
The strength of the score is indicated by the differences in blue shading. For example
Frames containing four or more alleles scoring above 1.64 are referred to as Epi-Bars.

Potential Solution?

Building Better Biotherapeutics and Vaccines by Design: EpiVax, Inc., an Immunology Company

LEONARD MOISE, PhD; ANTHONY MARCELLO; RYAN TASSONE; LESLIE COUSENS, PhD; WILLIAM MARTIN; ANNE S. DE GROOT, MD

ABSTRACT

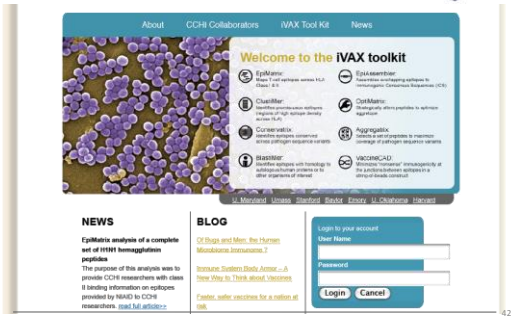
EpiVax, Inc., is an early-stage informatics and immunology biotechnology company in Providence, Rhode Island. It applies computational tools to harness immunity in three major areas: immunomodulation, biotherapeutic immunogenicity risk assessment and de-risking, and vaccine development. Immunotherapy, bio-better and vaccine candidates under development at EpiVax promise to improve the health outcomes of millions of people affected by devastating immune-related diseases.

KEYWORDS: vaccines, immunoinformatics, immunotherapy, immunomodulation, autoimmune diseases

What is the EpiVax Approach?

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

EpiVax Vaccine Toolkit: iVAX



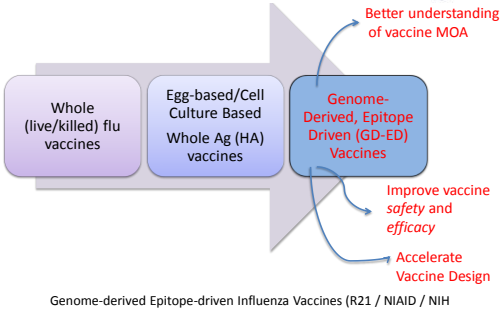
Fully integrated
From genome to vaccine

- EpiMatrix – maps T cell epitopes
- ClustiMer - Promiscuous / Superty
- BlastiMer - Avoiding "self" - Autoim
- Conservatrix – Identifi
- EpiAssembler - Imm Cor
- Aggregatrix – Optim zing the cove
- VaxCAD - Processi g and Assembl

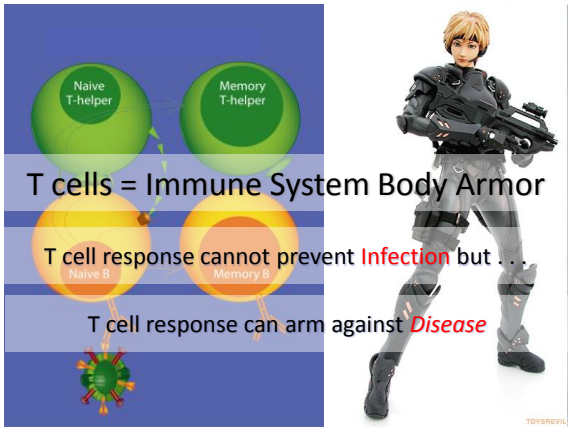
Seamless Vaccine Design

Integrated toolkit is *unique* to iVax

A better/faster way to make flu vaccine?



Genome-derived Epitope-driven Influenza Vaccines (R21 / NIAID / NIH



FastVax: Vaccines on demand

Rapid deployment when genome sequence is in hand

- High throughput computing
- Immunoinformatics
- Vaccine design algorithms

Prebuilt

- Vaccine Production
- Delivery device
- Animal safety/tox/immunogenicity/validation
- Deployment by established distribution systems

Pilot program
Funded by DARPA

20 hours - April 05 – April 06 2013
Extremely Rapid H7N9 Vaccine Design

April 05, 2013: Obtain H7N9 Sequences (4 human-sourced; GISAID)

Obtain all available H7N9 sequences

EpiMatrix Analysis: Identification of H7N9 Class I and Class II Epitopes

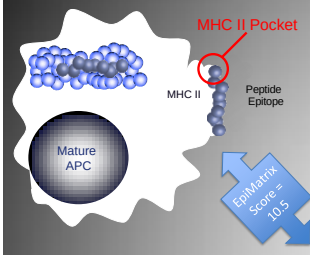
Compare with previous epitopes (IEDB) And other H7N9 strains; create final list 20 hours (Logged).

101 H7N9 ICS* Class II Epitopes + 586 Class I Epitopes

Eliminate Epitopes highly conserved with Human Design vaccine: 12 hours (Logged).

April 06, 2013: H7N9 Vaccine: Two Constructs, Class I and Class II

Predicting Epitopes that Drive Immune Response is our Expertise



HLA (Human MHC), are comprised of a limited number of pockets. EpiMatrix predicts how well a side chain will bind to a specific pocket.

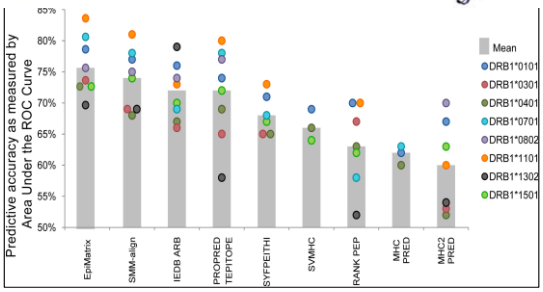
8 class II Archetype matrices which taken together incorporate 95% of human populations (and pockets) worldwide.

Each 9-mer/10-mer is analyzed for binding potential to each of those 8 allele matrices.

The **EpiMatrix Score** describes how well the peptide "fits" into the pockets

Southwood et al. J. Immunology 1998
Sturniolo et al. Nature Biotechnology, 1999

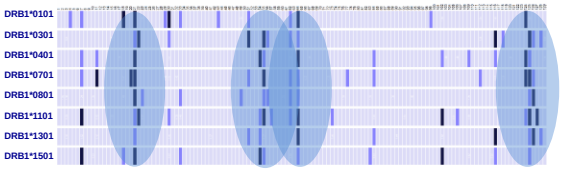
Published Benchmark 2009



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

Epitope Clusters = Immunogenic

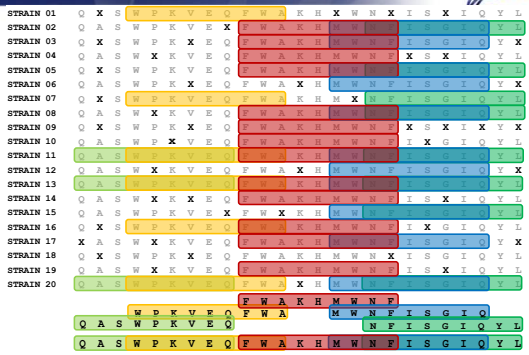
- A Key Discovery – Epitopes are Clustered in Protein Sequences



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

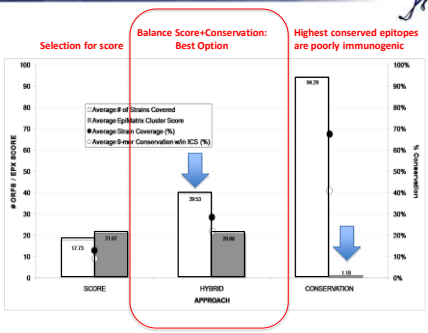
Building ICSs

EpiAssembler – Final Immunogenic Consensus Sequence

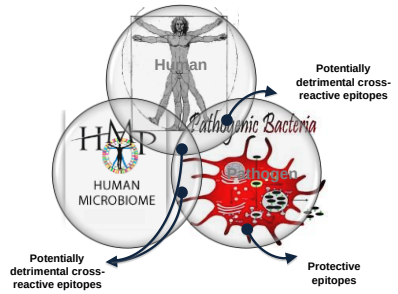


Building ICSs

EpiAssembler – Final Immunogenic Consensus Sequence

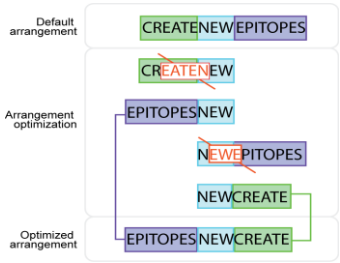


Safer: remove conserved epitopes

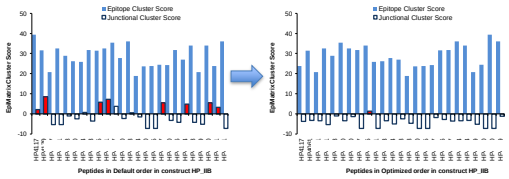


VaxCAD

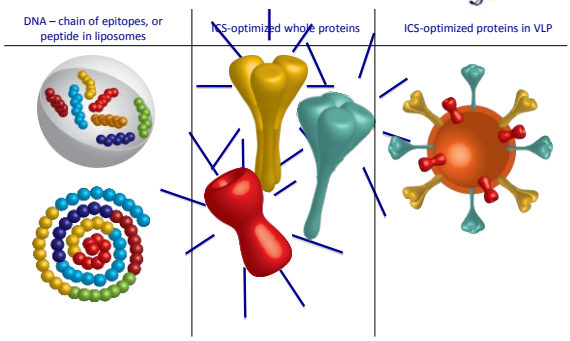
VaxCAD will identify junctional epitopes and rearrange chosen epitopes to reduce junctional epitope formation



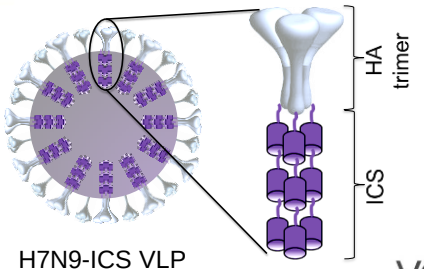
Create String of Beads-Vaccine



GD-IDV Formulations (platform independent)

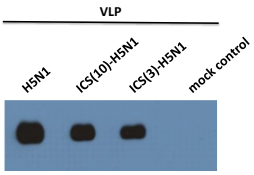


Broadly Reactive Influenza VLP Vaccine



VGTI FLORIDA

Feasibility: Expression of ICS-H5-HA fusions in H5N1 VLPs



Western blot probed for H5-HA

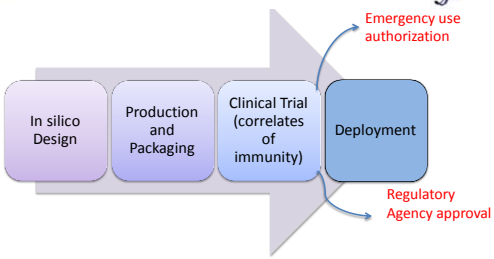
HA-Opitimzed-COBRA-like VLP Vaccine

Proposed: Add EpiVax ICS epitopes for induction of broadly cross- reactive H7N9 immunity to COBRA HA

COBRA: *Computationally Optimized Broadly Reactive Antigen*

- Align amino acid sequences from Clade 2 human isolates
- Assemble 'Layered' Consensus
- Limit sampling bias
- Confirm presence of conserved linear epitopes (Immune epitope database; www.immuneepitope.org)

Getting FastVax into the clinic: 4 Steps



As Currently Proposed with Genome-derived Epitope-driven Influenza Vaccines (R21 / NIAID / NIH)

EpiVax Proposed Vaccines

- String-of-epitopes DNA vaccine (Aldevron)
- String-of-epitopes Phage vaccine (Ft. Detrick)
- Optimized HA (TBN)
- Optimized HA + epitopes (with Ted Ross)

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Anne S. De Groot CEO/CSO annied@epivax.com

Active Collaborators / Vaccines

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Lenny Moise
Frances Terry
Leslie Cousens
Ryan Tassone
Howie Latimer
Mindy Cote
Lauren Levitz
Christine Boyle

DARPA
Mark Poznansky
Tim Brauns
Pierre LeBlanc

Ted Ross

VAXDESIGN
Don Drake, Brian Schanen

University of Massachusetts Medical School
Hardy Kornfeld
Jinhee Lee
Liisa Selin

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Saint Louis University
Sharon Frey
Mark Buller
Jill Schreiwer

Institute for Immunology and Informatics
Alan Rothman
Carey Medin
Andres Guitierrez
Danielle Aguirre
Joe Desrosiers
Thomas Mather
Wendy Coy
Loren Fast

USAMRIID
Connie Schmaljohn
Lesley C. Dupuy

Science without fear.

Media Contact: Anthony Marcello, BDA, amarcello@epivax.com

Panel Discussion and Questions from Participants

Presented by:

All Speakers

Notes:


Institute for
Immunology and
Informatics


EpiVax, Inc.
Informatics and Immunology

Anne Searls De Groot M.D.
Professor, University of Rhode Island, and CEO/CSO EpiVax, Inc.

Current Position

CEO/CSO EpiVax, Inc.	1998-present
Professor, Director Institute for Immunology and Informatics (URI)	2008-present

Education

B.A., Smith College, Northampton, Massachusetts	1974-1978
M.D., Pritzker School of Medicine, University of Chicago	1979-1983

Post-Graduate Training

Residency in Internal Medicine 1983-1986
Department of Internal Medicine
Tufts New England Medical Center, Boston MA

Fellowship in Parasitology and Vaccine Research 1986-1989
National Research Service Award Fellow, National Institutes of Health
Malaria Section, Laboratory of Parasitic Diseases, NIAID (Russell Howard, Michael Good)
Metabolism Branch, NCI, National Institutes of Health (Jay A. Berzofsky)

Fellowship in Infectious Disease 1989-1992
Division of Geographic Medicine and Infectious Disease,
Tufts New England Medical Center, Boston MA

Biography

Educated at **Smith College** (BA, 1978), Pritzker School of Medicine / **University of Chicago** (MD, 1983), Internal Medicine (New England Medical Center, 1986); additional training in immunoinformatics and vaccinology with Russell Howard and Jay Berzofsky at the **National Institutes of Health** (Laboratory for Parasitic Diseases and National Cancer Institute, 1986-89), followed by clinical training in infectious disease at **New England Medical Center** (1989-92). Board certified Internal Medicine (1986) and Infectious Disease (1992). Joined **Brown University Medical School**, and opened the TB/HIV Research Laboratory in 1992. Licensed EpiMatrix vaccine design technology from her laboratory at Brown and established **EpiVax** with Bill Martin, 1998. Invited to direct the activities of the **Institute for Immunology and Informatics** at University of Rhode Island, beginning October, 2008 and awarded $13M to initiate research on epitope-driven vaccines at the Institute in 2009.

Recipient of a NFID-Eli Lilly Award, two RI Foundation awards and a **Commercial Innovation Award** (Slater Biomedical Foundation), given a **Genius Award** in Science and Technology by Esquire Magazine (2003) and honored by the RI Tech Collective (2006) for work on the GAIA HIV vaccine. Awarded RI **Woman Physician of the Year** in 2006, received the Alvan Fisher **Red Ribbon Award** for Medical Advocacy from AIDS project Rhode Island in December 2007 and the **Woman of Achievement** Award, 2008 from the YWCA for her work relating to Access to Care in Providence and West Africa. Has published more than 120 research papers, chapters and reviews in addition to numerous essays and blogs. On May 22, 2009, was given the **Lifetime Achievement Award** at the Business Women Awards by Providence Business News. Featured as a **Top Doctor in Rhode Island** in the RI Monthly, May 2010. Awarded Smith Medal, RI Bioscience Award, 2013.

Professional Licenses and Board Certification

Internal Medicine	1987
Infectious Disease	1992

Academic Appointments (last 10 years)

Associate Professor (Research); Adjunct 08-11 — 07/02-05/11
Division of Biology and Medicine
Brown University School of Medicine

Professor, Biotechnology (Research) — Sept 1, 2008-
College of Environmental and Life Sciences
University of Rhode Island

Director, Institute for Immunology and Informatics — Sept 1, 2008-
University of Rhode Island Biotechnology Program

Other Appointments (Current)

Founder and CEO, **EpiVax Inc.** — 5/98-present

EpiVax is a specialized biotech company that holds the exclusive license to the EpiMatrix technology. The company is located at 146 Clifford Street, in Providence. Clients have included vaccine companies (Wyeth, Chiron), pharmaceutical companies (Genentech, Roche, Bristol Meyers Squibb, Amgen), other biotechnology companies (Sequella), the Department of Defense (BDRD, DARPA) and academic groups (CDC, Forsythe Institute, and the USAMRIID, NMRC, UMD, UCSF, SLU, UPitt, etc. (See http://www.EpiVax.com).

Editor/Editorial Boards (Last 10 years)

Invited Editor for issue of Methods (Academic Press)	2004
Co-editor (with L. Moise) of Special Issue in Medicine and Health Rhode Island	2007
Editorial Board, Emerging Infectious Diseases	2001-present
Editorial Board, Human Vaccines	2008-present
Editorial Board, Immunome Research	2004-present
Editorial Board, Current Opinion in Immunology.	2012-2013

Key Publications (out of more than 150)

1. A.S. De Groot, Johnson AH, Maloy WL, Quakyi IA, Riley EM, Menon A, Banks S M, Berzofsky JA, and Good MF, **Human T cell recognition of polymorphic epitopes from malaria circumsporozoite protein**, *J. Immunol.*, 1989, Vol.142, No.11, pp. 4000-4005.

2. Meister GE, Roberts CGP, Berzofsky JA, A.S. De Groot, **Two novel T cell epitope prediction algorithms based on MHC-binding motifs; comparison of predicted and published epitopes from *Mycobacterium tuberculosis* and HIV protein sequences**, *Vaccine* 1995, Vol. 13, No. 6, pp. 581-591.

3. A.S. De Groot, H. Sbai, C. Saint Aubin, J.A. McMurry, William Martin· **Immuno-informatics: Mining the genome for Vaccine Components.** Immunology and Cell Biology (2002) 80, 255–269.

4. De Groot AS, Goldberg M, Moise L, Martin W. **Evolutionary deimmunization: An ancillary mechanism for self-tolerance.** Cell Immunol. 2007 Apr 17; Epub. Cellular Immunology. Volume 244, Issue 2, December **2006**, Pages 148-153. http://dx.doi.org/10.1016/j.cellimm.2007.02.006

5. Koren E, De Groot AS, Jawa V, Beck KD, Boone T, Rivera D, Li L, Mytych D, Koscec M, Weeraratne D, Swanson S, Martin W. **Clinical validation of the "in silico" prediction of immunogenicity of a human recombinant therapeutic protein**. Clin Immunol. **2007** Jul;124(1):26-32.

6. De Groot A.S., L. Moise, J.A. McMurry, Erik Wambre, Laurence Van Overvelt, Philippe Moingeon, W. Scott, W. Martin, **Activation of Natural Regulatory T cells by IgG Fc-derived Peptide "Tregitopes".** Blood, **2008,**112: 3303. http://tinyurl.com/ASDeGroot-Blood-2008.
7. De Groot A.S., W. Martin,. **Reducing Risk, Improving Outcomes: Bioengineering less immunogenic protein therapeutics**. (Andy Saxon, Ed.). Clin Immunol. 2009 May;131(2):189-201. http://tinyurl.com/ASDeGroot-Clin-Immunol-2009.

(See EpiVax Website for additional publications)

Primary Research Papers Accepted and/or Published 2012-2013

Protein Therapeutics and Tolerance (to Protein Drugs)

1. Moise L, Song C, Martin WD, Tassone R, **De Groot AS**, Scott DW. **HLA DR epitope de-immunization of FVIII in vitro and in vivo**. Clin Immunol. **2012** Mar;142(3):320-31. PMID:22222093. http://tinyurl.com/Moise-FVIII-Deimmunization.
2. Leslie P. Cousens, Yan Su, Elizabeth McClaine, Xin Li, Frances Terry, Robert Smith, Jinhee Lee, William Martin, David W. Scott **Anne S. De Groot**. **Application of IgG-derived natural Treg epitopes (IgG Tregitopes) to antigen-specific tolerance induction in a murine model of type 1 diabetes.** Experimental Diabetes Research. In Press April **2013**.
3. van der Marel S, Majowicz A, Kwikkers K, van Logtenstein R, te Velde AA, **De Groot AS**, Meijer SL, van Deventer SJ, Petry H, Hommes DW, Ferreira V. **Adeno-associated virus mediated delivery of Tregitope 167 ameliorates experimental colitis. World Journal of Gastroenterology.** World J Gastroenterol. **2012** Aug 28;18(32):4288-99. PMID: 22969191 http://www.ncbi.nlm.nih.gov/pubmed/22969191
4. Cousens LP, Najafian N, Mingozzi F, Elyaman W, Mazer B, Moise L, Messitt TJ, Su Y, Sayegh M, High K, Khoury SJ, Scott DW, **De Groot AS**. **In Vitro and In Vivo Studies of IgG-Derived Treg Epitopes (Tregitopes): A Promising New Tool for Tolerance Induction and Treatment of Autoimmunity.** J Clin Immunol. 2013**. January; 33(1): 43–49.** PMID:22941509**.** http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3538121/
5. Hidefumi Inaba, Leonard Moise, William Martin, Anne S. De Groot, Joe Desrosiers, Ryan Tassone, George Buchman, Takashi Akamizu, and Leslie J. De Groot. **Epitope recognition in HLA-DR3 transgenic mice immunized to TSH-R protein or peptides.** Accepted for publication. Endocrinology. March 2013.

Vaccines 2012-2013

6. **De Groot AS**, Levitz L, Ardito MT, Skowron G, Mayer KH, Buus S, Boyle CM, Martin WD. **Further progress on defining highly conserved immunogenic epitopes for a global HIV vaccine: HLA-A3-restricted GAIA Vaccine epitopes.** Hum Vaccin Immunother. **2012** Jul 1;8(7). PMID: 22777092. http://tinyurl.com/GAIA-HLA-A3
7. Levitz L, Koita OA, Sangare K, Ardito MT, Boyle CM, Rozehnal J, Tounkara K, Dao SM, Koné Y, Koty Z, Buus S, Moise L, Martin WD, **De Groot A. Conservation of HIV-1 T cell epitopes across time and clades: Validation of HLA-A2 epitopes selected for the GAIA HIV vaccine.**

Vaccine. 2012 Oct 23. pii: S0264-410X(12)01488-0. doi:10.1016/j.vaccine.2012.10.042. PMID:23102976

http://www.sciencedirect.com/science/article/pii/ S0264410X12014880

8. Elfaki ME, Khalil EA, **De Groot AS**, Musa AM, Gutiérrez Núñez A, Younis BM, Salih KA, El-Hassan AM.. **Immunogenicity and immune modulatory effects of in silico predicted L-donovani candidate peptide vaccines.** Human Vaccines and Immunotherapy. **Hum Vaccin Immunother.** 2012 **Aug 24;8(12). PMID:22922767.**

http://www.psychepharmaceuticals.com/journals/vaccines/toc/volume/8/issue/7/

9. Ruicheng Wei, Chunfu Yang, Mei Zeng, Frances Terry, Qinsong Pan, Kai Zhu, Chunhui Yang, Chaoyang Deng Ralf Altmeyer, William Martin, Anne S. De Groot and Qibin Leng. **A Dominant EV71-specific CD4+ T cell epitope is Highly Conserved Among Human Enteroviruses**. PLoS One. 2012;7(12):e51957. doi: 10.1371/journal.pone.0051957. Epub 2012 Dec 14. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3522610/

10. Lenny Moise, Andres H. Gutierrez, Chris Bailey-Kellogg, Frances Terry, Qibin Leng, Karim M. Abdel Hady, Nathan VerBerkmoes, Marcelo B. Sztein, Phyllis Losikoff, William D. Martin, Alan Rothman, Anne S. De Groot. **The Two-Faced T cell Epitope: Examining the Host-Microbe Interface with JanusMatrix.** Human Vaccines and Immunotherapy. In Press. April 2013.

Clinical Care

11. Ahmed Eldakrourey, Ericka Olivera, Rebecca Martin, Anne S. De Groot. **Adherence to American Diabetes Association Guidelines in a Volunteer-run Free Clinic for the Uninsured in Providence, Rhode Island: Comparison with Standards Achieved by Clinics for Insured Patients.** Journal of Medicine and Health, Rhode Island. **Rhode Island Medical Journa**l. 2013 Jan;96(1):25-9

12. Danielle Poole, Kathleen Tracy, Lauren Levitz, Emily Kossow, Tonhu Huang, Ali Bicki, Mali Rochas, Kotou Sangare, Shahla Yetka, Ibrahima Teguete, Karamoko Tounkara, Ben Aboubacar, Ousmane Koita, Mark Lurie, Don Operario, Anne S. De Groot. **HPV vaccine acceptability and willingness to vaccinate in Bamako, Mali.** Accepted, In Press December **2012 (Plos One).**

Reviews / Chapters / Proceedings Published 2012

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13. Proceedings. De Groot A.S., Cohen TC, Moise, L, Martin WD. **Reducing Protein Immunogenicity by Design: Deimmunization and Tolerance Induction**. Proceedings of the 21st Annual Meeting of the European Society for Animal Cell Technology (ESACT), Dublin, Ireland, June 7-10, 2009 . ESACT Proceedings, 2012, Volume 5, Part 6, 525-534, DOI: 10.1007/978-94-007-0884-6_90. **Published on line in 2012** at http://www.springerlink.com/content/w086t8065l666g74/?MUD=MP.

14. Chapter. Vibha Jawa; Leslie Cousens and Anne S. De Groot. **Immunogenicity of Therapeutic Fusion proteins: Contributory Factors and Clinical Experience.** Chapter in: Fusion Protein Technologies for Biopharmaceuticals: Applications and Challenges, John Wiley and Sons, Inc.

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15. Proceedings. Andres H. Gutierrez, Leonard Moise, Frances Terry, Kristen Dasilva, Chris Bailey-Kellogg, William Martin, Anne S. De Groot **Immunoinformatic Analysis of Chinese Hamster**

Ovary (CHO) Protein Contaminants in Therapeutic Protein Formulations, J. Immunologic Methods, BCB '12 Proceedings of the ACM Conference on Bioinformatics, Computational Biology and Biomedicine, (ICIW) Pages 637-642.

16. Point of View. Gutiérrez AH, Moise L, **De Groot AS**, Of [Hamsters] and Men: **A New Perspective on Host Cell Proteins**. Hum. Vaccin. Immunother. 2012 8 (9). **2012**. PMID: 23124469 **http://www.landesbioscience.com/journals/vaccines/article/22378/** http://www.slideshare.net/AnnieDG/cho-hcp-immunogenicity-iciw-bailey-kellog

Autoimmunity-Tregitope

17. Review. Cousens LP, Tassone R, Mazer BD, Ramachandiran V, Scott DW, **De Groot AS**. **Tregitope Update: Mechanism of Action Parallels IVIg**. Autoimmun Rev. 2012 Aug 28. PMID:22944299 **http://tinyurl.com/Cousens-Tregitope-Autoimmunity.**
18. Review. Cousens LP, Mingozzi F, van der Marel S, Su Y, Garman R, Ferreira V, Martin W, Scott DW, **De Groot AS.** Teaching Tolerance: **New Approaches to Enzyme Replacement Therapy for Pompe Disease.** Hum. Vaccin. Immunother. **2012** Oct;8 (10). **PMID:23095864 2012. http://tinyurl.com/De-Groot-Tregitope-Pompe**

Vaccines

19. Proceedings. He Y, Cao Z, De Groot AS, Brusic V, Schönbach C, Petrovsky N. **Computational vaccinology and the ICoVax 2012 workshop.** BMC Bioinformatics. 2013;14 Suppl 4:I1. doi: 10.1186/1471-2105-14-S4-I1. Epub 2013 Mar 8.Review.
20. Review. Gutiérrez AH, Spero D, Gay C, Zimic M, **De Groot AS. New vaccines needed for pathogens infecting animals and humans: One Health**. Hum Vaccin Immunother. **2012** Jul 1;8(7). PMID:22485046.
21. Review. Leonard Moise, Steven F. Moss and Anne S. De Groot. **Moving H. pylori Vaccine Development Forward with Bioinformatics and Immunomics**. Invited Editorial for Expert Rev Vaccines. **2012** Sep;11(9):1031-3. doi: 10.1586/erv.12.80. PMID: 23151160
22. Sanou MP, De Groot AS, Murphey-Corb M, Levy JA, Yamamoto JK **HIV-1 Vaccine Trials: Evolving Concepts and Designs.** Open AIDS J. 2012;6:274-88. doi: 10.2174/1874613601206010274. Epub 2012 Nov 30. 2012.

Publications Submitted or in Preparation as of March 2013

1. Daniel J Hui, Etiena Basner-Tschakarjan, Yifeng Chen, Robert J Davidson, George Buchlis, Mustafa Yazicioglu, Gary C Pien, Jonathan D Finn, Virginia Haurigot, Alex Tai, David W Scott, Leslie P Cousens, Shangzhen Zhou, Annie S De Groot, Federico Mingozzi. **Modulation of CD8+ T cell responses to AAV vectors in vitro and in vivo with IgG-derived MHC class II epitopes**. Submitted (Blood). 2013
2. Yan Su, Robert Rossi, Anne S. De Groot, and David W. Scott**. Regulatory T cell epitopes** (**Tregitopes) in IgG induce tolerance in vivo and lack immunogenicity per** se. J. Leukocyte Biology. Accepted for publication April 2013.
3. De Groot AS, Artidito, M., Terry, F. Levitz L., Ross T., Moise L., Martin B. **Rapid Assessment of H7N9 Immunogenicity for Humans: Implication for Influenza Vaccine Design.** Hum Vaccin Immunother.