Tregitopes – Therapy for Autoimmunity, Allergy and also Relevant to Vaccines and Therapeutics

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

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Outline

- ✓Tregitopes – what are they?
- ✓Pre-clinical studies with Tregitopes…
- ✓Have Tregitopes been in the clinic?
- ✓What's the EpiVax plan for Tregitopes?

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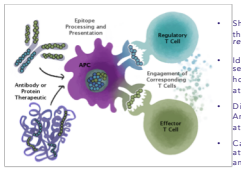
What are Tregitopes?

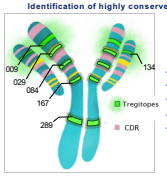


- Short, linear peptide sequences that bind to HLA and activate regulatory T cells
- Identified by immunoinformatics searching epitopes that are homologous to human genome at the TCR face
- Discovered and patented by Annie De Groot and Bill Martin at EpiVax
- Can be co-formulated or attached to proteins to provide antigen-specific tolerance
- Wide range of therapeutic applications

http://bit.ly/Tmp1

Tregitope Technology=A Set of peptides

Identification of highly conserved epitopes while screening Mabs

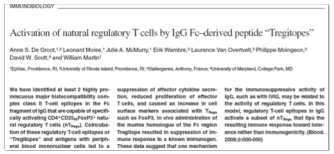


- 15-20 mer peptides in conserved regions
- Strong signals for T cells ("EpiBars")
- Highly conserved among IgG molecules
- Not conserved across Ig subtypes (IgG -> IgA)
- Relatively conserved across species (e.g. mouse)

De Groot A.S., et al., Activation of Natural Regulatory T cells by IgG Fc-derived Peptide "Tregitopes". Blood, 2008;112: 3303 http://bloodjournal.hematologylibrary.org/cgi/content/abstract/112/8/3303

Original Publication

Published in Blood, 25 July 2008
Reprints available on request



IMMUNOBIOLOGY

Activation of natural regulatory T cells by IgG Fc–derived peptide "Tregitopes"

Anne S. De Groot,[1,2] Leonard Moise,[1] Julie A. McMurry,[1] Erik Wambre,[3] Laurence Van Overtvelt,[3] Philippe Moingeon,[3] David W. Scott,[4] and William Martin[1]

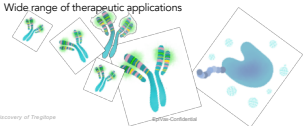
[1]EpiVax, Providence, RI; [2]University of Rhode Island, Providence, RI; [3]Stallergenes, Anthony, France; [4]University of Maryland, College Park, MD

We have identified at least 2 highly promiscuous major histocompatibility complex class II T-cell epitopes in the Fc fragment of IgG that are capable of specifically activating CD4+CD25$_{hi}$FoxP3+ naturel regulatory T cells (nT_{reg}). Coincubation of these regulatory T-cell epitopes or "Tregitopes" and antigens with peripheral blood mononuclear cells led to a suppression of effector cytokine secretion, reduced proliferation of effector T cells, and caused an increase in cell surface markers associated with T_{reg} such as FoxP3. In vivo administration of the murine homologue of the Fc region Tregitope resulted in suppression of immune responses to a known immunogen. These data suggest that one mechanism for the immunosuppressive activity of IgG, such as with IVIG, may be related to the activity of regulatory T cells. In this model, regulatory T-cell epitopes in IgG activate a subset of nT_{reg} that ligs the resulting immune response toward tolerance rather than immunogenicity. (Blood. 2008;0:000-000)

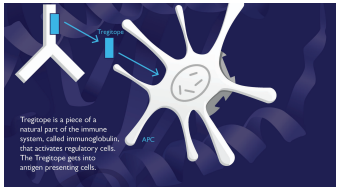
http://bit.ly/Tregitopes_AJCI

What's the Excitement About?

- Tregitopes belong to a novel class of immunotherapeutics
- They induce **expansion and activation of Tregs** and can be used to teach the immune system to tolerize against immunogenic proteins
- Tregitopes are a new therapy for **autoimmune disease** with additional applications in **transplant and allergy**
- Can be co-formulated or attached to proteins to provide antigen-specific tolerance
- Wide range of therapeutic applications



Tregitope – Simplified Mechanism of Action (MOA)



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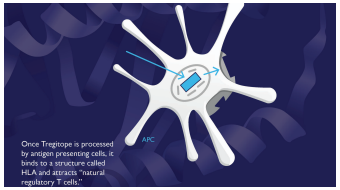

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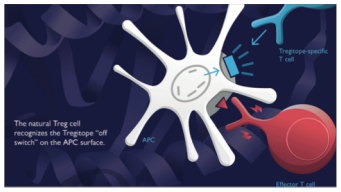

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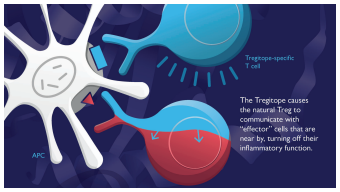
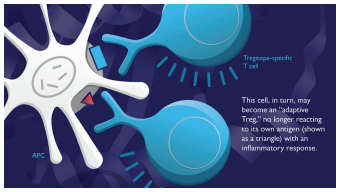


Tregitope – Simplified Mechanism of Action (MOA)



Tregitopes may explain (one) IVIG mechanism of action

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Kaveri and Bayry, Trends in Immunology IVIg Review
Tregitope as one of the MOA of IVIG

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Trends in Immunology

CellPress REVIEWS

Forum

Circulating Normal IgG as Stimulator of Regulatory T Cells: Lessons from Intravenous Immunoglobulin

Mohan S Maddur,[1,2,3,5] Srini V. Kaveri,[1,2,3,4] and Jagadeesh Bayry[1,2,3,4,*]

Intravenous immunoglobulin (IVIG),

of defense against invading pathogens. Accordingly, deficiency of IgG, as in the case of common variable immunodeficiency (CVID) or X-linked agammaglobulinemia, leads to increased predisposition to recurrent infections. Paradoxically, immunodeficiencies are also associated with autoimmunity and inflammatory conditions, suggestive of a dysregulated immune status. Intriguingly, replacement therapy with low-dose IVIG (400 mg/kg) consisting of intact IgG molecules purified from the pooled plasma of several thousand healthy blood donors, not only prevents recurrence of infectious diseases but also suppresses autoimmune and inflammatory responses. These observations

Treg cells correlates with its therapeutic benefits both in autoimmune disease patients and experimental models [2,4]. High-dose IVIG therapy exerts sustained effect on Treg cells and despite a gradual decline, the effect on Treg cells goes beyond the half-life of infused IgG in majority of the patients who respond to this therapy [4]. As IVIG is nothing but pooled IgG from normal donors, the effect of IVIG on Treg cells likely represents a primordial function of circulating IgG in regulating immune homeostasis.

Treg Cell Expansion by IVIG: How Many Mechanisms after All?

Tregitope as (one of the) mechanism of action by IVIG
Kaveri and Bayry, Trends in Immunology 2019

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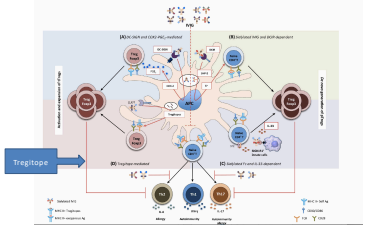
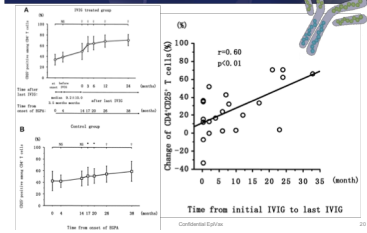


IVIG expands Tregs in Human Diseases = Tregitope effect

EpiVax



High-dose Intravenous Immunoglobulin Treatment Increases Regulatory T Cells in Patients with Eosinophilic Granulomatosis with Polyangiitis

NAOMI TSURIKISAWA, HIROSHI SAITO, CHIYAKO OSHIKATA, TAKAHIRO TSUBURAI and KAZUO AKIYAMA

http://www.jrheum.org/content/39/5/1019.long

IVIG expands Tregs in Human Diseases = Tregitope effect

EpiVax

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

Clinical and Experimental Immunology (2013)

Authors:

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

High dose (but not low dose) IVIG expands Tregs in Humans (Tjon et al)

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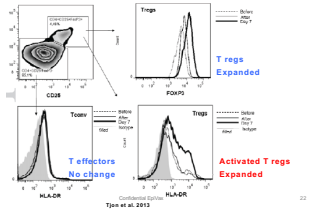
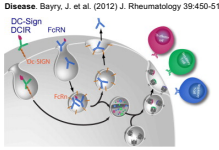


Tjon et al. 2013

Tregitopes as the active principle in IVIG and Fc-based biologics

Patients treated with high-dose intravenous immunoglobulin show selective activation of regulatory T cells. Tjon, A.S.W. et al. (2013) Clin. Exp. Immunol. 173:259-267.

Intravenous Immunoglobulin Expands Regulatory T Cells in Autoimmune Rheumatic Disease. Bayry, J. et al. (2012) J. Rheumatology 39:450-51.



Cousens, L. et al Hum Immunol. 2014;12:1139-46.

Intravenous immunoglobulins promote skin allograft acceptance by triggering functional activation of CD4+Foxp3+ T cells. Tha-In T. et al. (2010) Transplantation 89:1446-55.

IVIG prevents inflammation in vivo: experimental autoimmune encephalomyelitis and Herpes Simplex Virus-induced encephalitis. Ephrem et al. Blood 2008 and Ramakrishna Plos Pathogens 2011

IVIG activates Tregs in murine and Human studies = Tregitope effect

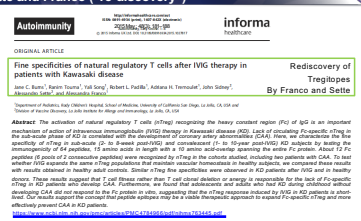
IVIG can expand Tregs in patients.
Bayry, J. Rheumatology 2012

IVIG activates human and mouse CD4+ CD25+ FOXP3+ Tregs *in vitro* and increases their ability to suppress allogeneic T cell proliferation. *Tha-In T et al. Transplantation 2010.*

IVIg prevents graft rejection in a fully mismatched skin transplant model by triggering functional activation of Tregs.
Tha-In T et al. Transplantation 2010

IVIg prevents mice from developing experimental autoimmune encephalomyelitis and Herpes Simplex Virus-induced encephalitis.
Ephrem et al. Blood 2008 and Ramakrishna Plos Pathogens 2011.

External validation of Tregitopes (IVIG) from Sette and Franco ("re-discovery")



Autoimmunity

informa healthcare

ORIGINAL ARTICLE

Fine specificities of natural regulatory T cells after IVIG therapy in patients with Kawasaki disease

Rediscovery of Tregitopes By Franco and Sette

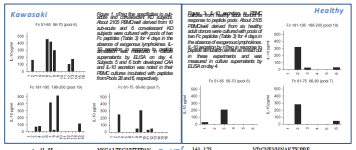
Jane C. Burns[1], Ramin Toumi[1], Yali Song[1], Robert L. Padilla[1], Adriana H. Tremoulet[1], John Sidney[2], Alessandro Sette[2] and Alessandro Franco[1]

[1]Department of Pediatrics, Rady Children's Hospital, School of Medicine, University of California San Diego, La Jolla, CA, USA and [2]Division of Vaccine Discovery, La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA

Abstract *The activation of natural regulatory T cells (nTreg) recognizing the heavy constant region (Fc) of IgG is an important mechanism of action of intravenous immunoglobulin (IVIG) therapy in Kawasaki disease (KD). Lack of circulating Fc-specific nTreg in the sub-acute phase of KD is correlated with the development of coronary artery abnormalities (CAA). Here, we characterize the fine specificity of nTreg in sub-acute (2- to 9-week post-IVIG) and convalescent (1- to 10-year post-IVIG) KD subjects by testing the immunogenicity of 64 peptides, 15 amino acids in length with a 10 amino acid-overlap spanning the entire Fc protein. About 13 Fc peptides (6 pools of 2 consecutive peptides) were recognized by nTreg in the cohorts studied, including two patients with CAA. To test whether IVIG expands the same nTreg populations that maintain vascular homeostasis in healthy subjects, we compared these results with results obtained in healthy adult controls. Similar nTreg fine specificities were observed in KD patients after IVIG and in healthy donors. These results suggest that T cell fitness rather than T cell clonal deletion or anergy is responsible for the lack of Fc-specific nTreg in KD patients who develop CAA. Furthermore, we found that adolescents and adults who had KD during childhood without developing CAA did not respond to the Fc protein in vitro, suggesting that the nTreg response induced by IVIG in KD patients is short-lived. Our results support the concept that peptide epitopes may be a viable therapeutic approach to expand Fc-specific nTreg and more effectively prevent CAA in KD patients.*

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4784965/pdf/nihms763445.pdf

Sette and Franco IVIg study: Peptide Pools induce IL-10 On inspection: Peptides inducing IL 10 are Tregitopes



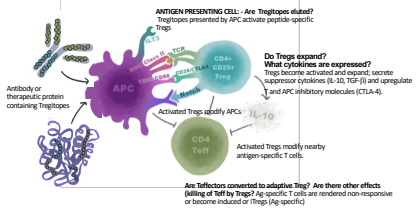
Pool 5	41-55	NSGALTSGVHTFPAV	Pool 17	161-175	VDGVEVHNAKTKPRE
Pool 6	46-60	TSGVHTFPAVLQSSG	Pool 18	166-180	VHNAKTKPREEQYNS
Pool 6	51-65	TFPAVLQSSGLYSLS	Pool 18	171-185	TKPREEQYNSTYRVV
Pool 7	56-70	LQSSGLYSLSSVVTV	Pool 19	176-190	EQYNSTYRVVSVLTV
Pool 7	61-75	LYSLSSVVTVPSSSL	Pool 19	181-195	TYRVVSVLTVLHQDW
Pool 8	66-80	SVVTVPSSSLGTQTY		186-200	SVLTVLHQDWLNGKE
Pool 8	71-85	PSSSLGTQTYICNVN			
	76-90	GTQTYICNVNHKPSN			

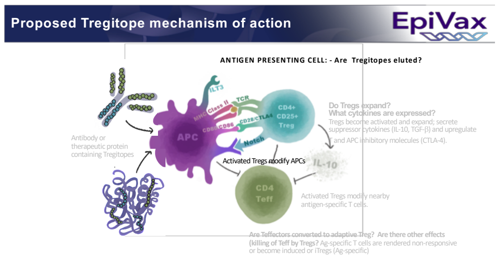
Green underline indicates Tregitope 9-mer (Tregitope 167 in pools 6 and 7, Tregitope 289 in pool 18 and 19)

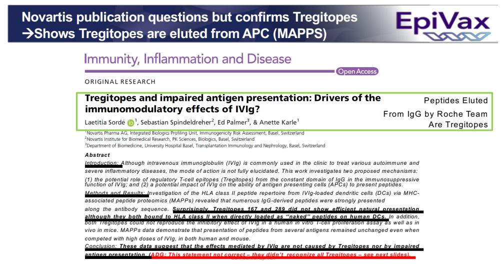
Outline

- ✓ **Tregitopes – what are they?**
  - ✓ **Mechanism of Action**
- ✓ Pre-clinical studies with Tregitopes…
- ✓ Have Tregitopes been in the clinic?
- ✓ What's the EpiVax plan for Tregitopes?

Proposed Tregitope mechanism of action







Immunity, Inflammation and Disease

Open Access

ORIGINAL RESEARCH

Tregitopes and impaired antigen presentation: Drivers of the immunomodulatory effects of IVIg?

Laetitia Sordé[1], Sebastian Spindeldreher[2], Ed Palmer[3], & Anette Karle[1]

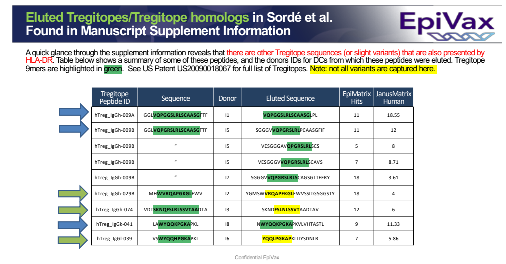
[1]Novartis Pharma AG, Integrated Biologics Profiling Unit, Immunogenicity Risk Assessment, Basel, Switzerland
[2]Novartis Institute for Biomedical Research, PK Sciences, Biologics, Basel, Switzerland
[3]Department of Biomedicine, University Hospital Basel, Transplantation Immunology and Nephrology, Basel, Switzerland

Abstract

Introduction: Although intravenous immunoglobulin (IVIg) is commonly used in the clinic to treat various autoimmune and severe inflammatory diseases, the mode of action is not fully elucidated. This work investigates two proposed mechanisms: (1) the potential role of regulatory T-cell epitopes (Tregitopes) from the constant domain of IgG in the immunosuppressive function of IVIg; and (2) a potential impact of IVIg on the ability of antigen presenting cells (APCs) to present peptides.

*Methods and Results: Investigation of the HLA class II peptide repertoire from IVIg-loaded dendritic cells (DCs) via MHC-associated peptide proteomics (MAPPs) revealed that numerous IgG-derived peptides were strongly presented along the antibody sequence. **Surprisingly, Tregitopes 167 and 289 did not show efficient natural presentation although they both bound to HLA class II when directly loaded as "naked" peptides on human DCs.** In addition, both Tregitopes could not reproduce the inhibitory effect of IVIg in a human in vitro T-cell proliferation assay as well as in vivo in mice. MAPPs data demonstrate that presentation of peptides from several antigens remained unchanged even when competed with high doses of IVIg, in both human and mouse.*

*Conclusion: **These data suggest that the effects mediated by IVIg are not caused by Tregitopes nor by impaired antigen presentation.** (ADG: This statement not correct – they didn't recognize all Tregitopes – see next slides).*



A quick glance through the supplement information reveals that there are other Tregitope sequences (or slight variants) that are also presented by HLA-DR. Table below shows a summary of some of these peptides, and the donors IDs for DCs from which these peptides were eluted. Tregitope 9mers are highlighted in green. See US Patent US20090018067 for full list of Tregitopes. Note: not all variants are captured here.

Tregitope Peptide ID	Sequence	Donor	Eluted Sequence	EpiMatrix Hits	JanusMatrix Human
hTreg_IgGh-009A	GGLVQPGGSLRLSCAASGFTF	I1	VQPGGSLRLSCAASGLPL	11	18.55
hTreg_IgGh-009B	GGLVQPGRSLRLSCAASGFTF	I5	SGGGVVQPGRSLRLPCAASGFIF	11	12
hTreg_IgGh-009B	"	I5	VESGGGAVQPGRSLRLSCS	5	8
hTreg_IgGh-009B	"	I5	VESGGGVVQPGRSLRLSCAVS	7	8.71
hTreg_IgGh-009B	"	I7	SGGGVVQPGRSLRLSCAGSGLTFERY	18	3.61
hTreg_IgGh-029B	MHWVRQAPGKGLEWV	I2	YGMSWVRQAPEKGLEWVSSITGSGGSTY	18	4
hTreg_IgGh-074	VDTSKNQFSLRLSSVTAADTA	I3	SKNDFSLNLSSVTAADTAV	12	6
hTreg_IgGk-041	LAWYQQKPGKAPKL	I8	NWYQQKPGKAPKVLVHTASTL	9	11.33
hTreg_IgGl-039	VSWYQQHPGKAPKL	I6	YQQLPGKAPKLLIYSDNLR	7	5.86

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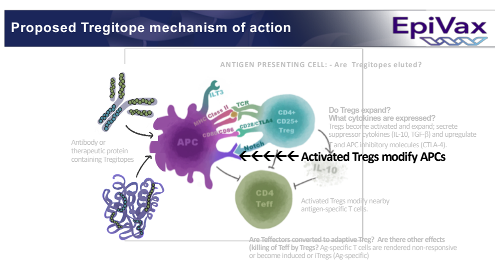
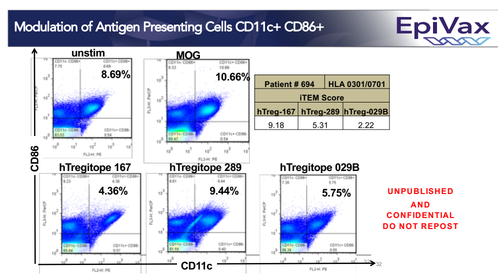
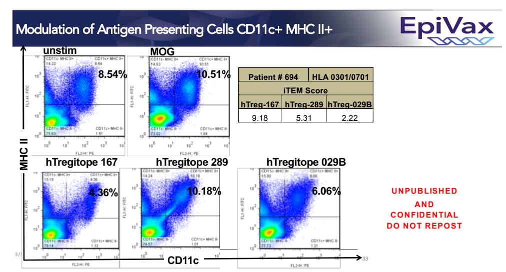




Patient # 694	HLA 0301/0701	
iTEM Score		
hTreg-167	hTreg-289	hTreg-029B
9.18	5.31	2.22



Patient # 694	HLA 0301/0701	
iTEM Score		
hTreg-167	hTreg-289	hTreg-029B
9.18	5.31	2.22

Modulation of Antigen Presenting Cells CD11c+ ILT3+

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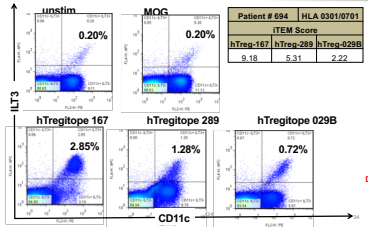

Patient # 694	HLA 0301/0701
iTEM Score	
hTreg-167	hTreg-289	hTreg-029B
9.18	5.31	2.22

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Effect of Tregitope on APC / Tregs c/w Edratide)
Tregitope 029B vs. Edratide (029 tested in SLE patients)

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	EpiVax 029B	Edratide
MHC II	↓	↓
CD80	↓	↓
CD86	↓	↓
IL-T3	↑	No Data
IL-10	↑	↓
IFNγ	No Data	↓
TGFβ	No Data	↑
Tregs	↑	↑
IL-7	No Data	↓
B Cells	No Data	↓

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VI. Edratide, HLA-restricted Tregitope

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Proposed Tregitope mechanism of action

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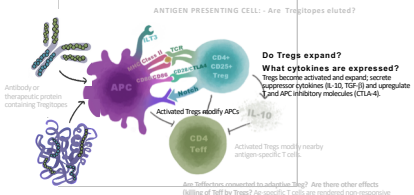

Expansion Reported in Cousens et al.
Backgating subsequently shows these are monocytes

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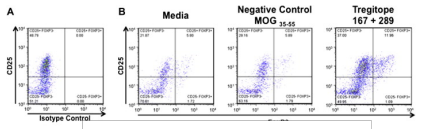

Expansion Reported in Cousens et al.
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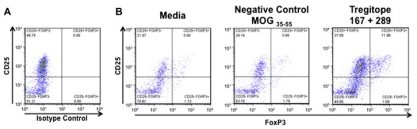

We believe that Natural Regulatory Tregitope specific T cells do not expand in vitro without strong signals.

Effective Proliferation of Human Regulatory T Cells Requires a Strong Costimulatory CD28 Signal That Cannot Be Substituted by IL-2
Andreas A. Hombach, David Kofler, Anja Hombach, Gunter Rappl and Hinrich Abken
J Immunol December 1, 2007, 179 (11) 7924-7931; DOI: https://doi.org/10.4049/jimmunol.179.11.7924

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Proposed Tregitope mechanism of action

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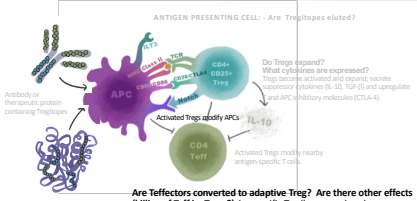
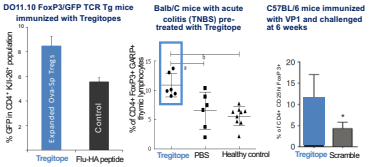
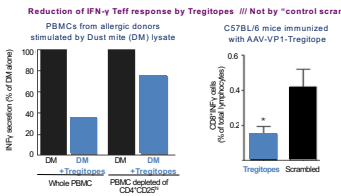


Are Teffectors converted to adaptive Treg? Are there other effects (killing of Teff by Tregs?) Ag-specific T cells are rendered non-responsive or become induced or iTregs (Ag-specific)

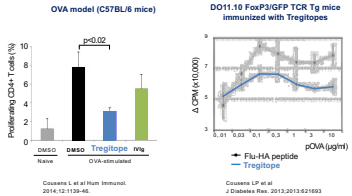
Tregitopes induces expansion of antigen-specific Regulatory T cells in vivo



Tregitopes suppress effector response in vivo



Tregitopes suppress T cell proliferation in vivo In standard (Bl/6) and TCR transgenic models



Tregitope effect in the Tetanus Toxoid Bystander Assay

1. Have we tested a control peptide to compare with Tregitope in HLA-DR binding and TTBSA ?
2. Does Tregitope also have an effect on CD8 T cells? (no MHC competition)
3. Is there any competition for binding of Tregitope for their inhibitory effect?

Addressing concerns about "competition" as potential cause of decreased T effector response

1. Have we tested a control peptide to compare with Tregitope in HLA-DR binding and TTBSA ?
2. Does Tregitope also have an effect on CD8 T cells?
3. Is there any competition for binding of Tregitope for their inhibitory effect?

Addressing concerns about "competition" as potential cause of decreased T effector response

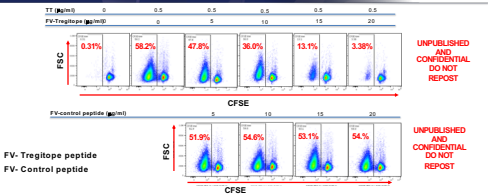
Peptide Name	HLA DRB1 (IC50)						
	*0101	*0301	*0401	*0701	*1101	*1301	*1501
FV-control peptide	2170	78594	20114	7033	4361	2963	2695
FV-Tregitope	691	16	2422	251	1015	2885	104

IC50 (nM)
Non-Binder
>100,000
10,000 -100,000
1,000 -10,000
100 -1,000
<100

FV Tregitope 621 **peptides bind to multiple HLA-DRB1 alleles.** HLA-DRB1 binding of selected putative Tregitope peptides was determined invitro and IC50 values calculated for the FV-control peptide and the Tregitope 621. Both peptides bind to the alleles tested (DRB1*01:01, *03:01, *04:01, *07:01, *11:01, *13:01 and *15:01). Color coding reflects binding affinity (extrapolated to IC50) as determined by performing in a seven-point assay using a validated control.
Note that both FV-Tregitope and FV-control peptide demonstrated high binding and low IC50's to nearly all HLAs tested.

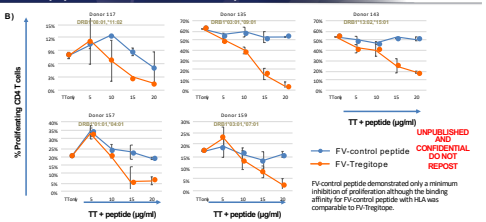
Comparing the HLA-DRB1 binding of Tregitope 621 **and control peptide**

Control peptide does not inhibit CD4 T cell proliferation compared to Tregitope 621 in TTBSA (representative flow data)



Representative flow data comparing FV-Tregitope and FV-control peptide on the inhibition of CD4 recall response in the Tetanus Toxoid (TT) Bystander Suppression Assay.

Comparing inhibitory capacity of Tregitope 621 versus the control peptide in TTBSA across multiple donors

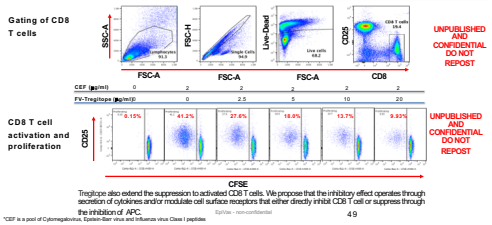


FV-control peptide demonstrated only a minimum inhibition of proliferation although the binding affinity for FV-control peptide with HLA was comparable to FV-Tregitope.

Addressing concerns about "competition" as potential cause of decreased T effector response

1. Have we tested a control peptide to compare with Tregitope in HLA-DR binding and TTBSA ?
2. **Does Tregitope also have an effect on CD8 T cells?**
3. Is there any competition for binding of Tregitope for their inhibitory effect?

Tregitope 621 inhibits activated effector CD8 T cells proliferation upon stimulation with CEF* Class I peptides



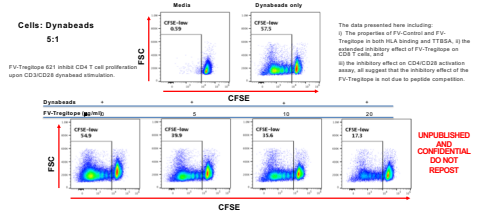
Tregitope also extend the suppression to activated CD8 T cells. We propose that the inhibitory effect operates through secretion of cytokines and/or modulate cell surface receptors that either directly inhibit CD8 T cell or suppress through the inhibition of APC.

*CEF is a pool of Cytomegalovirus, Epstein-Barr virus and Influenzas virus Class I peptides

Addressing concerns about "competition" as potential cause of decreased T effector response

1. Have we tested control peptide to compare with Tregitope in HLA-DR binding and TTBSA ?
2. Does Tregitope also have an effect on CD8 T cells?
3. **Is there any competition for binding of Tregitope for their inhibitory effect?**

Tregitope 621 inhibits low-dose CD3/CD28- dynabead mediated stimulation of CD4 T cell proliferation at Day 7 (no MHC competition)



The data presented here including:
i) The properties of FV-Control and FV-Tregitope in both HLA binding and TTBSA. ii) the extended inhibitory effect of FV-Tregitope on CD8 T cells, and
iii) the inhibitory affect on CD4/CD28 activation assay, all suggest that the inhibitory effect of the FV-Tregitope is not due to peptide competition.

Outline

✓ Tregitopes – what are they?

✓ **Pre-clinical studies with Tregitopes…**

✓ Have Tregitopes been in the clinic?

✓ What's the EpiVax plan for Tregitopes?

Clinical applications of Tregitopes

Model systems

1. Autoimmune diseases
2. Transplantation
3. Inflammatory Bowel Disease
4. Enzyme Replacement Therapy
5. ASATI : Allergy

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Tregitope validation in vivo

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In vivo Model	Immunogen	Delivery	Clinical application	Findings	Publication
C57BL/6	OVA	DMSO	Tolerance induction	Suppress Ag-specific T cell proliferation	Cousens et al Human immunology 2014
C57BL/6	MOG	CFA	MS	Induce Tregs, Reduce of EAE symptoms	Elyaman et al Neur Res Int 2011
NOD /ShiLtJ	PPI	Liposome	T1D	Reduce incidence of Type 1 diabetes when co administered with PPI	Cousens et al J of diabetes Research 2013
Balb/C	none	IFA	T1D	Suppress CD4+ response and are not immunogenic	Su et al JLB 2012
NOD /ShiLtJ	none	IFA	T1D	Reduce Type 1 diabetes after onset	Cousens et al J of diabetes Research 2013
D011.10 TCR Tg	OVA	IFA	ERT	Suppress Ag-specific T cell proliferation and induce Ag specific Treg	Cousens et al J of diabetes Research 2013
ABM TCR tg	bm12	DMSO	Tolerance induction	Induce Ag-specific Tregs	Cousens et al Human immunology 2014
HLADR4 Tg	HDM	saline	Allergy	Suppress immune response to the antigen	De Groot et al Blood 2008
C57BL/6	AAV	encoded	gene therapy	Reduce immune response to AAV capsid	Hui et al Mol Ther 2013
Balb/C	AAV	encoded	inflammatory colitis	Reduce severity of the disease, increase Treg infiltrates in colon	Van der Marel et al,World J Gastroenterol 2012
C57BL/6	OVA	DMSO	asthma	Reduction airway reactivity, Treg induction	Mazer and Massoud, Not published
NOD /ShiLtJ	N/A	HSA-fusion	T1D	Decrease T1D associated mortality when co-administered with PPI	Not Published

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Tregitope validation checklist

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

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Tregitopes compared to IVIG in MS (EAE)

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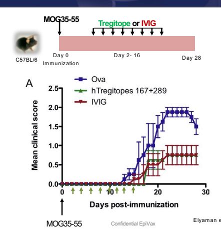

Elyaman et al

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Review Article

Potential Application of Tregitopes as Immunomodulating Agents in Multiple Sclerosis

Wassim Elyaman,[1] Samia J. Khoury,[1] David W. Scott,[2] and Anne S. De Groot[3]

In Vitro and In Vivo Studies of IgG-derived Treg Epitopes (Tregitopes): A Promising New Tool for Tolerance Induction and Treatment of Autoimmunity

Tregitopes prolongs graft survival in Transplant

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Human Immunology 75 (2014) 1139–1146

Contents lists available at ScienceDirect

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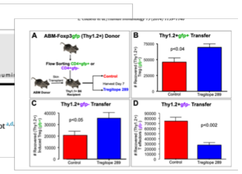
Tregitope: Immunomodulation Powerhouse

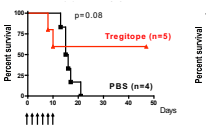
Leslie Cousens [a], Nader Najafian [b,c], William D. Martin [a], Anne S. De Groot [a,d]

[a] EpiVax, Inc., Providence, RI, USA
[b] Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA
[c] Cleveland Clinic, Weston, FL, USA
[d] Institute for Immunology and Informatics, University of Rhode Island, Providence, RI, USA

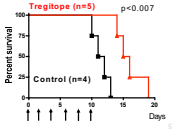


Tregitopes prolongs graft survival in Transplant

BalbC heart transplant into CD28ko B6



Bm12 skin transplant into B6

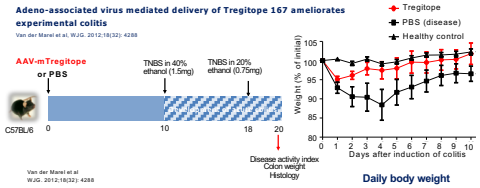


Nader Najafian, De Groot (published)

Tregitopes in an in vivo model of acute colitis

Adeno-associated virus mediated delivery of Tregitope 167 ameliorates experimental colitis

Van der Marel et al, WJG. 2012;18(32): 4288



Van der Marel et al
WJG. 2012;18(32): 4288

Tregitopes reduce immunogenicity of AAV Gene Therapy

Modulation of CD8+ T cell responses to AAV vectors with IgG-derived MHC class II epitopes

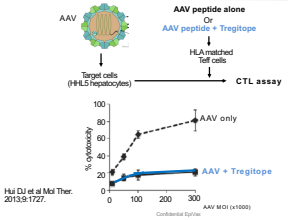
Hui et al, Mol Ther. 2013;21(9):1727

AAV: Adeno-associated virus

- ✓ Do not cause disease
- ✓ Used in gene therapy to express protein therapeutics
- ✓ Main drawback: AAV capsid is immunogenic.

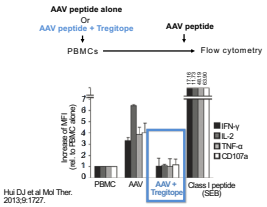
Ex: Glybera (Uniqure) for LPLD: AAV1 to deliver human LPL. Requires immunosuppression from three days prior to and for 12 weeks following administration because of AAV1 immunogenicity

Tregitopes reduce CD8+ T cell response to AAV Gene Therapy



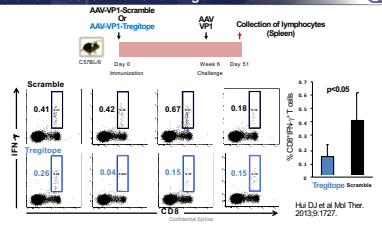
Hui DJ et al Mol Ther. 2013;9:1727.

Tregitopes Reduce Cytokine Response Mice given AAV w/ or w/o Tregitope



Hui DJ et al Mol Ther. 2013;9:1727.

Tregitopes Downregulate IFN-γ response by AAV-Specific CD8+ T cells After Challenge in vivo



Hui DJ et al Mol Ther. 2013;9:1727.

Tregitopes in AAV or other Gene/Enzyme Replacement Therapy

Antigen Specific Adaptive Tolerance Induction (ASATI)

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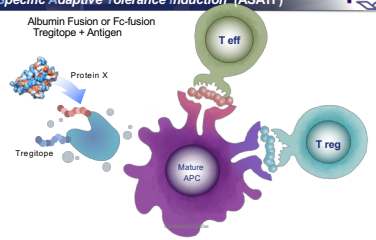

Tregitopes in AAV or other Gene/Enzyme Replacement Therapy

Antigen Specific Adaptive Tolerance Induction (ASATI)

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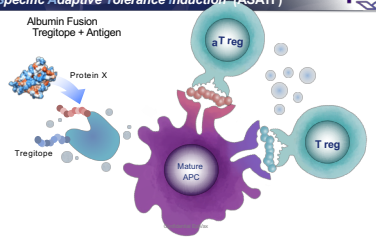


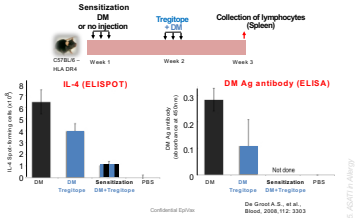
Immunomodulatory properties of Tregitopes in a mouse model of asthma (allergic airway disease - AAD)

Dr. Bruce Mazer
McGill University
Epivax Update 20180227

EpiVax

Tregitopes Reduce IL4 and Antibody Responses in an Allergy Model

EpiVax



Intravenous Immunoglobulin G (IVIg) reduces steroid dependency in asthma

Introduction

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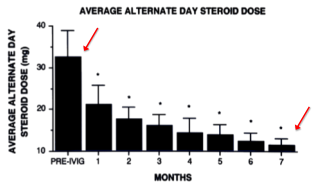
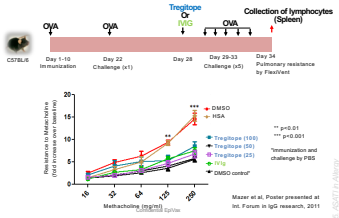


Mazer BD and Gelfand EW, J Allergy Clin Immunol 1991; 87:976-83.

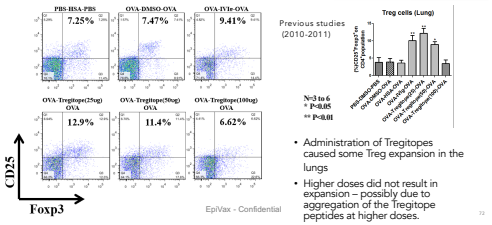
Tregitopes in a Mouse Model of Asthma

EpiVax



Mazer et al. Poster presented at Int. Forum in IgG research, 2011

OVA/IVIg or OVA/Tregitopes increase % CD4+CD25+Foxp3+ Tregs in lung



Previous studies (2010-2011)

N=3 to 6
* P<0.05
** P<0.01

- Administration of Tregitopes caused some Treg expansion in the lungs
- Higher doses did not result in expansion – possibly due to aggregation of the Tregitope peptides at higher doses.

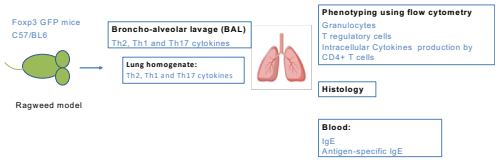
Treg transfer

Tregitope treatment with Allergen
Do Tregitopes abrogate allergy responses?

Marieme Dembele PhD
Bruce Mazer MD
McGill University

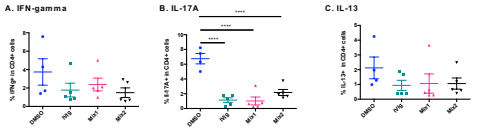
Ragweed

Ragweed-induced model of asthma



Ragweed

Ragweed model: Suppression of cytokine production by CD4+ T cells in lung



Percentage of CD4+ T cells expressing IFN-gamma, IL-17A and IL13

CD4+ T cells
Cells extracted from cells stimulated with PMA inonomycin and use of flow to gate on CD4+ T cells and cytokines (intracellular staining).

Mix 1: hTregitope289 + hTregitope084
Mix 2: hTregitope167 + hTregitope289

UNPUBLISHED AND CONFIDENTIAL DO NOT REPOST

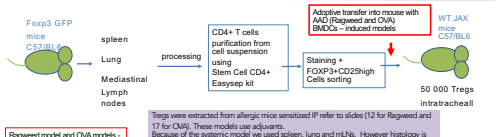
Treg transfer

Treg Transfer Experiment
Test for antigen-specificity of Tregs *in-vivo*

UNPUBLISHED AND CONFIDENTIAL DO NOT REPOST

Treg transfer

Antigen specificity of Tregs



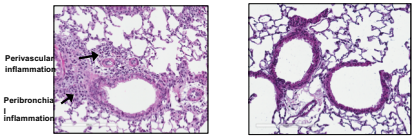
Ragweed model and OVA models - Systemic sensitization and treatments
1. DMSO
2. Mix 1 hTregitopes
3. Mix 2 hTregitopes

Tregs were extracted from allergic mice sensitized IP refer to slides (12 for Ragweed and 17 for OVA). These models use adjuvants.
Because of the systemic model we used spleen, lung and mLNs. However histology is not great with this model in our C57 mice.

To better assess histological changes we used a BMDCs driven allergic model. In this model, BMDCs are treated with the antigen (ragweed or OVA) for 24 hours, 1 million BMDCs are transferred to the lungs of WT mice (n=3). After 10 days, Tregs from ragweed-DMSO, ragweed-mix2 and ragweed-mix 1 were transferred into ragweed-BMDCs sensitized mice. Tregs from ragweed-DMSO, ragweed-mix2 and ragweed-mix 1 or Tregs from ova-DMSO, ova-mix2 and ova-mix1 in OVA-BMDCs sensitized mice.

Treg transfer

H&E staining: Inflamed vs non-inflamed lung



Scanned H&E stained lung from an OVA-BMDC (no Treg) allergic mouse (200X) .

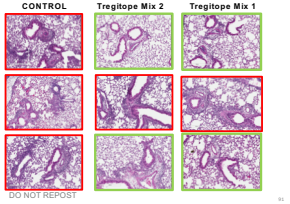
Scanned H&E stained lung from a PBS-BMDC (no Treg) non- allergic mouse (200X).

To assess peribronchial and perivascular inflammation, look at the percentage inflamed bronchi, alveoli and vessels and at infiltrates quality (thickness).

Treg transfer: RW- Tregs to OVA mice

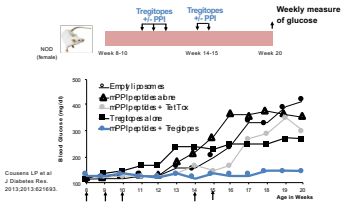
H&E staining of sensitized mouse lungs after adoptive transfer of Tregs

Tregs isolated from donor mouse	Adoptive transfer into lungs of recipient mouse
Ragweed-specific Tregs	Ragweed-allergic mouse
Ragweed-specific Tregs	OVA-allergic mouse
OVA-specific Tregs	OVA-allergic mouse



Mix 1: hTregitope289 + hTregitope084
Mix 2: hTregitope167 + hTregitope289

Tregitopes to Prevent Type 1 Diabetes – Co-administered with PPI in Liposomes

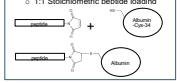


Cousens LP et al J Diabetes Res. 2013;2013:821693.

T1D ASATI with Albumin-Tregitope Fusions

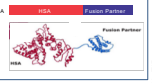
Bioconjugation

- Chemically modify peptide to allow covalent attachment to albumin molecule:
  - Lysine
  - Thyrosine
  - Free Thiol (SH)
- Free thiol is the most widely used conjugation route:
  - Specifically reactive with maleimide groups
  - 1:1 Stoichiometric peptide loading



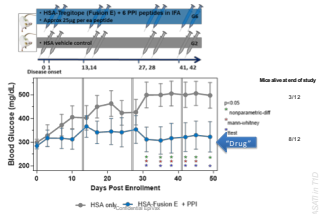
Albumin Fusion

- Contiguous cDNA for target protein/peptide with DNA encoding albumin produces a single protein
- Flexible conjugation options:
  - N or C terminal
  - Combinations
  - Linker molecules
  - Cleavage sites



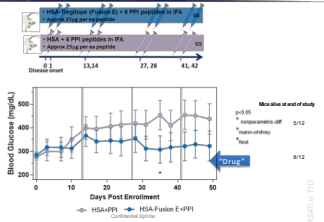
T1D-ASATI: Tregitope-HSA fusion

Comparison of HSA-Fusion E + PPI peptides to HSA-only



T1D-ASATI: Tregitope-HSA fusion

Comparison of HSA-Fusion E + PPI peptides to HSA + PPI



Outline

✓Tregitopes – what are they?

✓Mechanism of Action

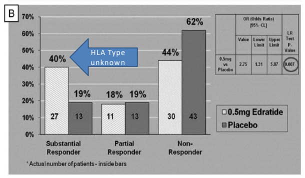
✓Pre-clinical studies with Tregitopes…

✓**Have Tregitopes been in the clinic?**

✓What's the EpiVax plan for Tregitopes?

Phase II clinical study of Edratide (hCDR1) in systemic lupus erythematosus (SLE)

EpiVax



Urowitz MB, Isenberg DA, Wallace DJ. Lupus Science & Medicine 2015

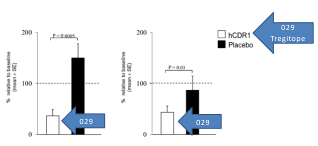
Edratide is a Tregitope with clinical impact in SLE-overview

EpiVax

- Edratide is a **very HLA DR-Restricted Tregitope** peptide, which shares homology with EpiVax's Tregitope 029.
- **EpiVax has Tregitope 029B which has increased binding across multiple alleles, making it less HLA-restrictive**.
- In safety and efficacy study Edratide was administer with Captisol subcutaneously on a weekly basis at several doses (0.5mg, 1.0mg and 2.5 mg).
- Edratide showed favorable safety profile and demonstrated efficacy in several clinical endpoint.
- **In the Phase II study, there was a significant difference in disease activity between Edratide vs. placebo in substantial response measured by BILAG for 0.5 mg dose (with trends for 1.0mg and 2.5 dose groups**). HLA DR type not identified.
- Study demonstrated that Edratide may be more effective in patients receiving lower or no steroids as well as seropositive patients (anti-DNA>30 IU at baseline).

Edratide down regulates in vivo IFN-α in SLE patients

EpiVax



SLE patients were treated (SQ, once a week) with either hCDR1 (0.5, 1, or 2.5 mg) or placebo. Gene expression in blood samples obtained from the patients was determined by real-time RT-PCR. Results are presented as mean percentage of gene expression (6SE) at week 24 compared to the levels at week 0 (defined as 100%; dotted line). Also shown in the Figure is the mean percent reduction in the BILAG score following 24 weeks of treatment with either hCDR1 or placebo as compared to the baseline score (week 0) considered as 100% (dotted line).

Sthoeger Z, Mozes E et al. PLOS ONE 2013

Alignment of Edratide and Tregitope 029

EpiVax

		Identity with Edratide
Edratide peptide:	HGYYWSWIRQPPGKGEEWI	
Our 029 peptide:	WSWVRQPPGRGLEWI	12/19
Our 029B* peptide:	MHWVRQAPGKGLEWV	9/15

029

*Note: All of the EpiVax in vitro experiments to date have been conducted with hTregitope 029B.

Cluster Report – Edratide (hCDR1) peptide

EpiVax

EpiMatrix Cluster Detail Report

File: MOZES_HCDR1 Sequence: HCRD1 Cluster: 1
March 29, 2012 (Epx Ver. 1.2)

Published Edratide sequence in Phase II paper HGYYWSWIRQPPGKGEEWI

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
1	GYYWSWIRQ	9	-1.01	-1.63	-1.79	0.16	0.07	-1.37	-0.74	-0.79	-0.42	0
2	YYWSWIRQP	10	-1.14	-0.15	0.31	0.37	1.11	-0.10	1.06	-0.48	-0.70	0
3	YWSWIRQPP	11	-1.18	0.14	0.52	0.68	0.89	0.80	1.16	0.31	0.40	0
4	WSWIRQPPG	12	-1.08	1.48	-0.31	1.05	0.05	1.19	1.77	0.14	0.15	1
5	SWIRQPPGK	13	-1.41	-0.08	-0.92	-0.50	-0.96	0.55	0.26	0.26	-0.01	0
6	WIRQPPGKG	14	-1.37	2.29	1.27	1.97	1.28	2.62	2.35	0.88	1.26	4
7	IRQPPGKGE	15	-1.66	0.12	0.10	0.08	0.18	0.63	-0.59	0.97	0.03	0
8	RQPPGKGEE	16	-2.54	-2.45	0.29	-2.38	-2.31	-0.21	-1.45	-0.13	-0.65	0
9	QPPGKGEEW	17	-2.14	-1.47	-1.99	-1.62	-1.00	-2.67	-2.50	-2.86	-2.21	0
10	PPGKGEEWI	18	-1.26	-2.63	-1.56	-3.15	-1.36	-1.52	-2.36	-1.54	-2.89	0
11	PGKGEEWIG	19	-1.12	-1.50	-1.15	-1.61	-1.87	-1.80	-0.76	-2.12	-0.89	0

Summarized Results (29-MAR-2012)	DRB1*0101	DRB1*0301	DRB1*0401	DRB1*0701	DRB1*0801	DRB1*1101	DRB1*1301	DRB1*1501	Total
Maximum Single Z score	2.29	1.27	1.97	1.28	2.62	2.35	0.97	1.26	--
Sum of Significant Z scores	2.29	0.00	1.97	0.00	2.62	4.12	0.00	0.00	11.00
Count of Significant Z Scores	1.00	0.00	1.00	0.00	1.00	2.00	0.00	0.00	5.00

Total Assessments Performed: 88	Hydrophobicity: -1.09	EpiMatrix Score: 1.93	EpiMatrix Score (w/o flanks): 1.93
Scores Adjusted for Tregitope:	--	EpiMatrix Score: -7.30	EpiMatrix Score (w/o flanks): -7.30

Z score indicates the potential of a 9-mer frame to bind to a given HLA allele; the strength of the score is indicated by the blue shading. Top 10%* Top 5% Top 1%

All scores in the Top 5% (Z-Score >= 1.64) are considered "Hits". *Scores in the top 10% (shown but not highlighted) are considered elevated, other scores are grayed out for simplicity.

Frames containing four or more alleles scoring above 1.64 are referred to as Epi-Bars and are highlighted in yellow. These frames have an increased likelihood of binding to HLA.

Frames conserved in IgG antibodies and believed to be either passively tolerated or actively regulatory are highlighted in green.

Flanking amino acids, added to stabilize the cluster during in-vitro testing, are presented in blue type face and underlined.

Hydrophobic amino acid sequences scoring above 2.0 can be difficult to synthesize as peptides. Mutated amino acids are indicated in red typeface.

Cluster Report – EpiVax Tregitope 029B Optimized for binding across multiple alleles

EpiVax

EpiMatrix Cluster Detail Report

Accession: IGG_TREGITOPE_CANDIDATES_2011 Sequence: HTREG_IGGH-029B Cluster: 1

June 22, 2011 (Epx Ver. 1.2)

Anticipate that 029B will have increase efficacy in cohort population due to better binding across multiple alleles

Edratide is a Tregitope with clinical impact in SLE- conclusion

EpiVax

- As shown by the EpiMatrix cluster reports of Edratide as compared to Tregitope 029B. Tregitope 029B has a higher EpiMatrix score and is predicted to bind across more HLA-DR1 alleles, making it more druggable to a larger human population.
- EpiVax's Tregitope 029B has been extensively evaluated, demonstrating its ability to tolerize and modulate APCs
- Taken together with current success of Edratide in SLE and the demonstrated effectiveness of Tregitope 029B in our ex vivo studies, we anticipate that Tregitope 029B will be better suited as a drug due to its ability to bind across multiple alleles and be advantageous for a broader and diverse patient population.

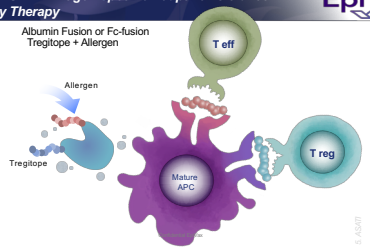
Outline

EpiVax, Inc.

- ✓Tregitopes – what are they?
  - ✓Mechanism of Action
- ✓Pre-clinical studies with Tregitopes…
- ✓Have Tregitopes been in the clinic?
- ✓**What's the EpiVax plan for Tregitopes?**

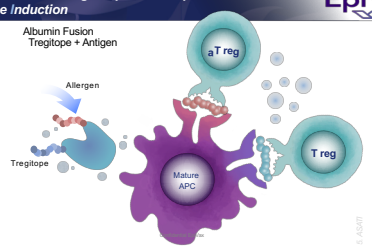
Tregitope ASATI : *Antigen Specific Adaptive Tolerance For Allergy Therapy*

EpiVax



Tregitope ASATI : *Antigen Specific Adaptive Tolerance Induction*

EpiVax

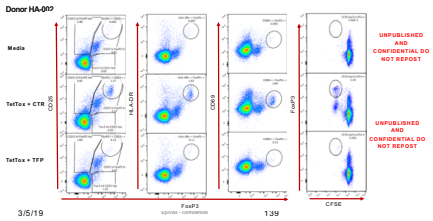


Tregitope-Fusion Plans for Transition to Clinic

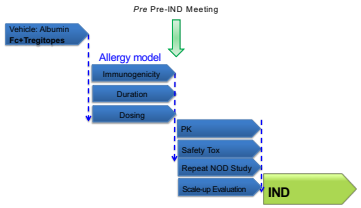
EpiVax

- **Scale-up of HSA-Fusion E** – technical difficulties
  - Glycosylation
  - Truncation of fusion protein
- **Development of Albumin or IgG Tregitope Fusion**
- **And/or individualized peptide allergen co-administration for Allergy**

Effect of TFP on on highly activated Tetanus Tox-specific CD4 T cells



Next Plan Tregitopes . . . Allergy Program In Preparation



Tregitope Applications Translating Antigen-specific tolerance to the clinic

Localized, specific suppression of immune response:

- **Tolerization** of antibodies: introducing Tregitopes into the sequence with minimal point mutations
- **Deimmunization** of protein therapeutics: co-expression or co-administration with immunogenic proteins --- Currently have Option/License for protein fusion
- Autoimmune diseases (type 1 diabetes, multiple sclerosis) --- Currently have Option/License for SLE, other (alopecia areata/derm applications) (for Peptides) – but none for the Alb or Fc Fusion.