Tregitopes Induce Active Tolerance in Autoimmune Diabetes & Allergy

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Director, Institute for Immunology and Informatics
Professor (Research) Univ. Rhode Island

March 2019

EpiVax

JDRF IMPROVING LIVES. CURING TYPE 1 DIABETES.

NATIONAL INSTITUTES OF HEALTH

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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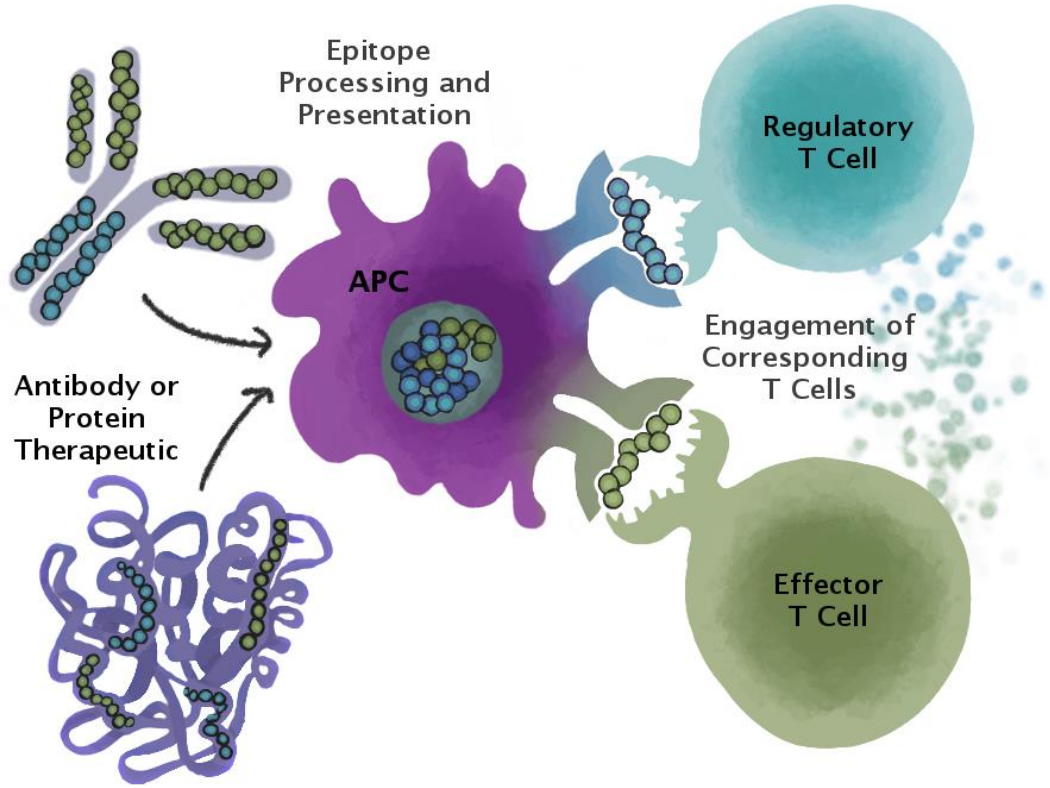
What are Tregitopes?

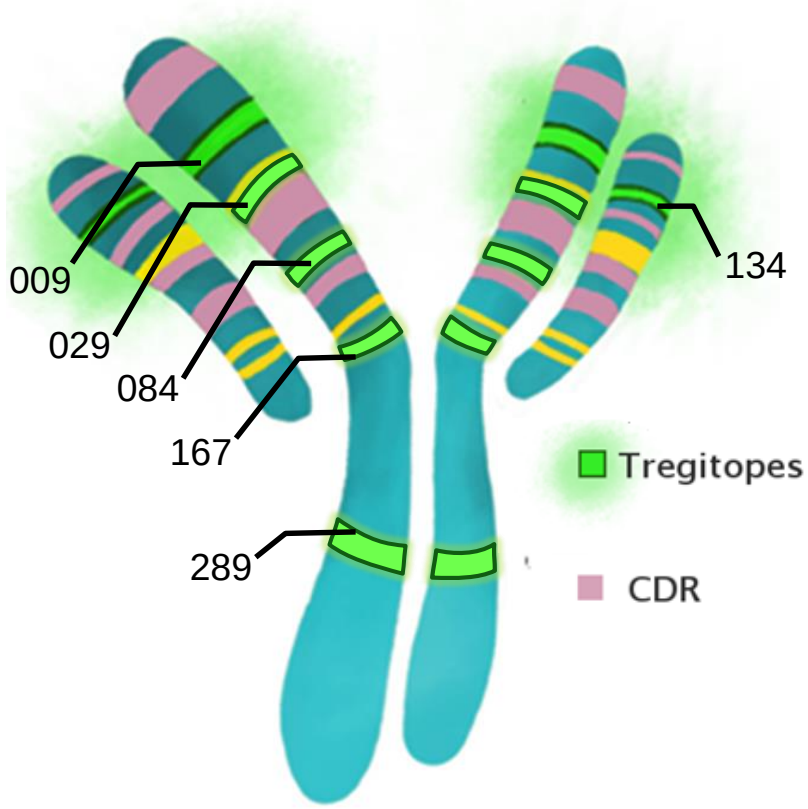


- Short, linear peptide sequences that bind to HLA and activate *regulatory* T cells
- Identified by immunoinformatics searching epitopes that are homologous to human genome at the TCR face
- Discovered and patented by Annie De Groot and Bill Martin at EpiVax
- Can be co-formulated or attached to proteins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

http://bit.ly/Treg1

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://tinyurl.com/ASDeGroot-Blood-2008

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

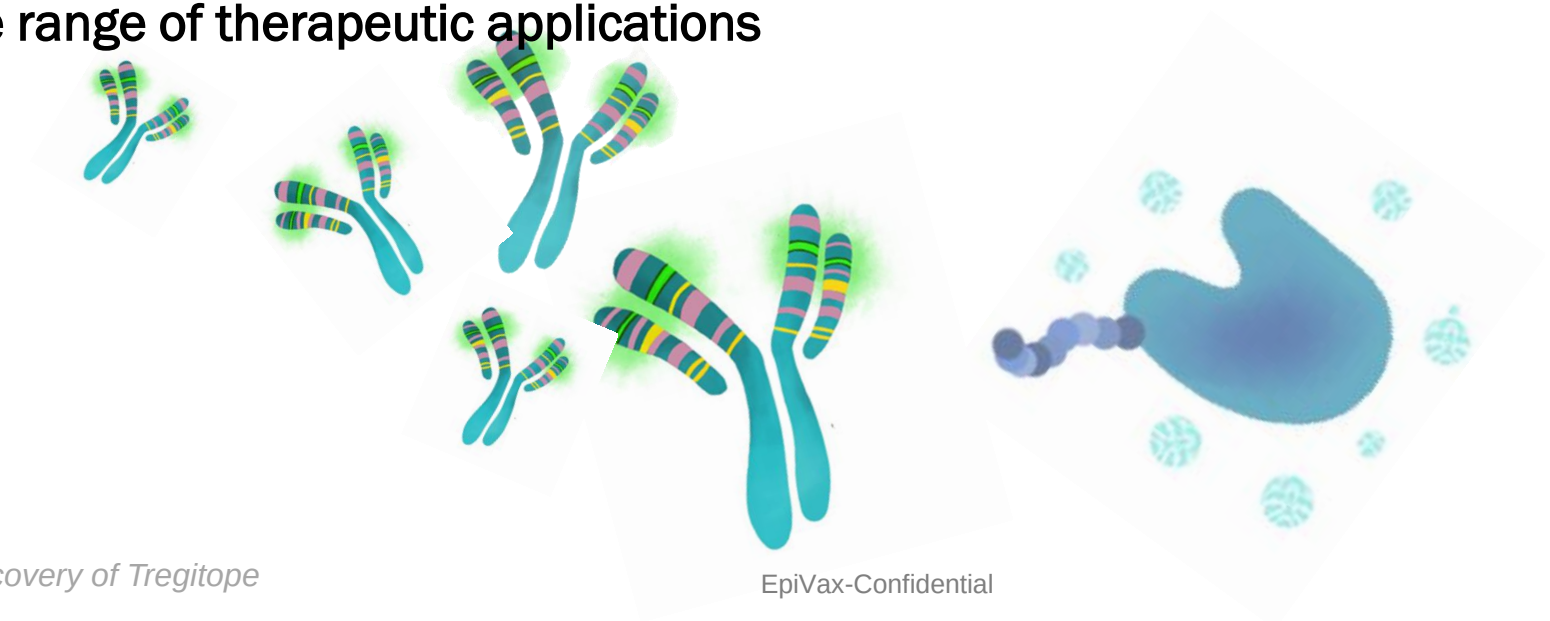
We have identified at least 2 highly promiscuous major histocompatibility complex class II T-cell epitopes in the Fc fragment of IgG that are capable of specifically activating $CD4^+CD25_{Hi}FoxP3^+$ natural regulatory T cells (nT_{Regs}). Coincubation of these regulatory T-cell epitopes or "Tregitopes" 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_{Regs} such as FoxP3. In vivo administration of the murine homologue of the Fc region Tregitope resulted in suppression of immune response 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_{Regs} that tips the resulting immune response toward tolerance rather than immunogenicity. (Blood. 2008;0:000-000)

http://bit.ly/Tregitope_API

What's the Excitement About?

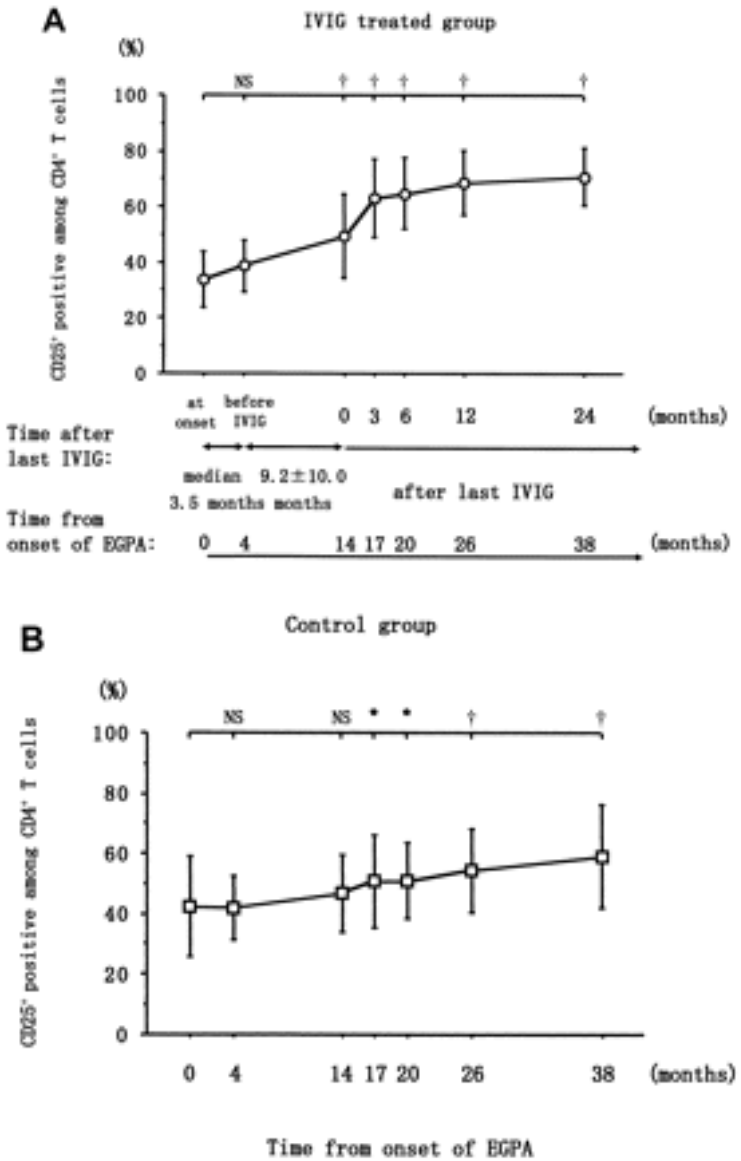
EpiVax

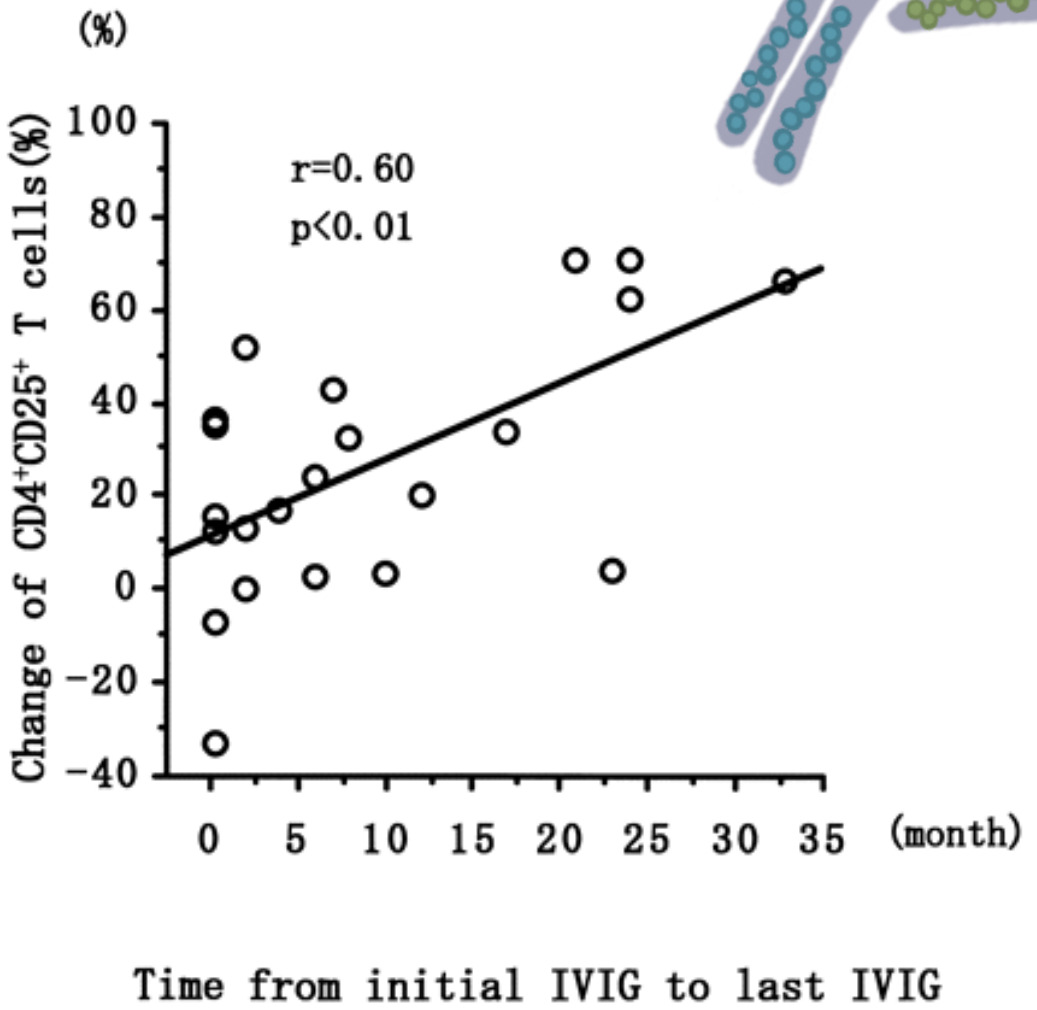
- Tregitopes belong to a novel class of immunotherapeutics
- They induce **expansion and activation of Tregs** and can be used to teach the immune system to tolerize against immunogenic proteins
- Tregitopes are a new therapy for **autoimmune disease** with additional applications in **transplant and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



Tregitopes may explain (one) IVIG mechanism of action

IVIG expands Tregs in Human Diseases = Tregitope effect





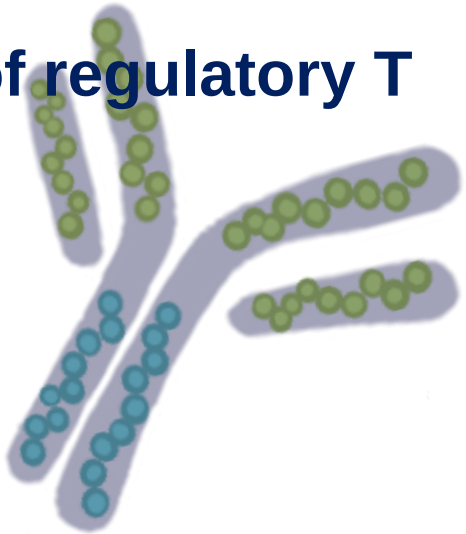
High-dose Intravenous Immunoglobulin Treatment Increases Regulatory T Cells in Patients with Eosinophilic Granulomatosis with Polyangiitis

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

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

High dose but not low dose IVIG expands Tregs in Human Diseases

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

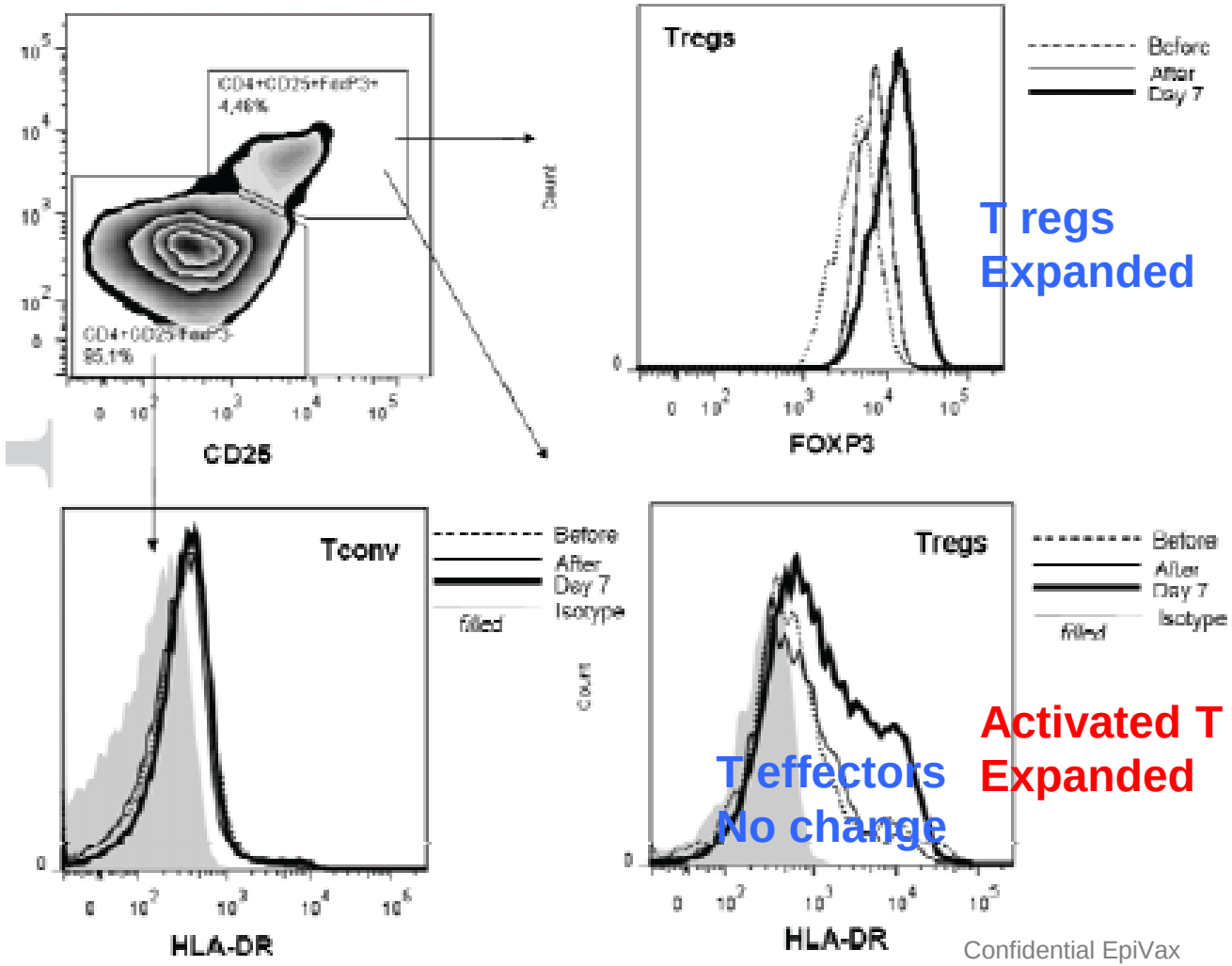


Clinical and Experimental Immunology (2013)

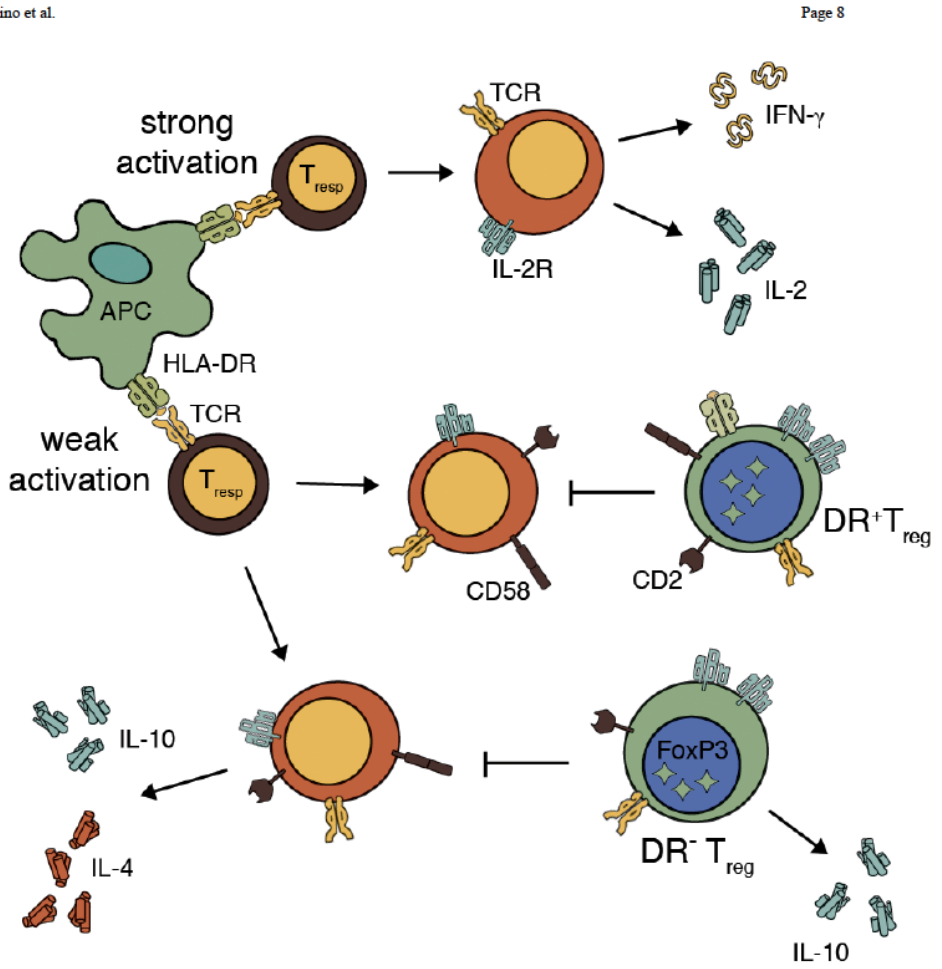
Authors:

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)

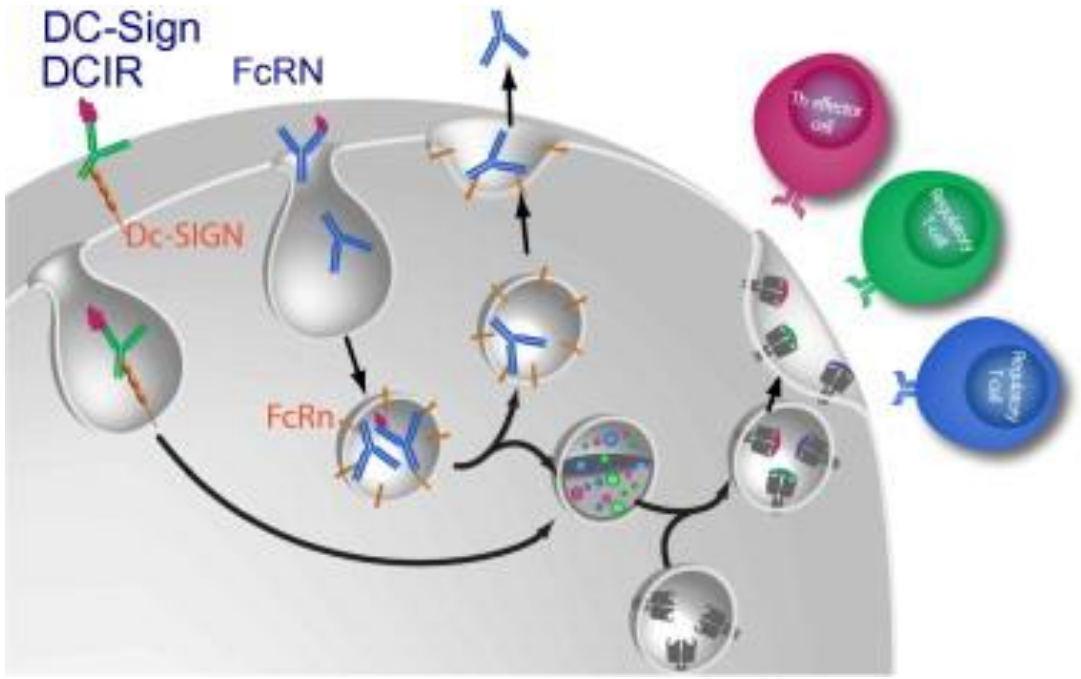


Tjon et al. 2013



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.

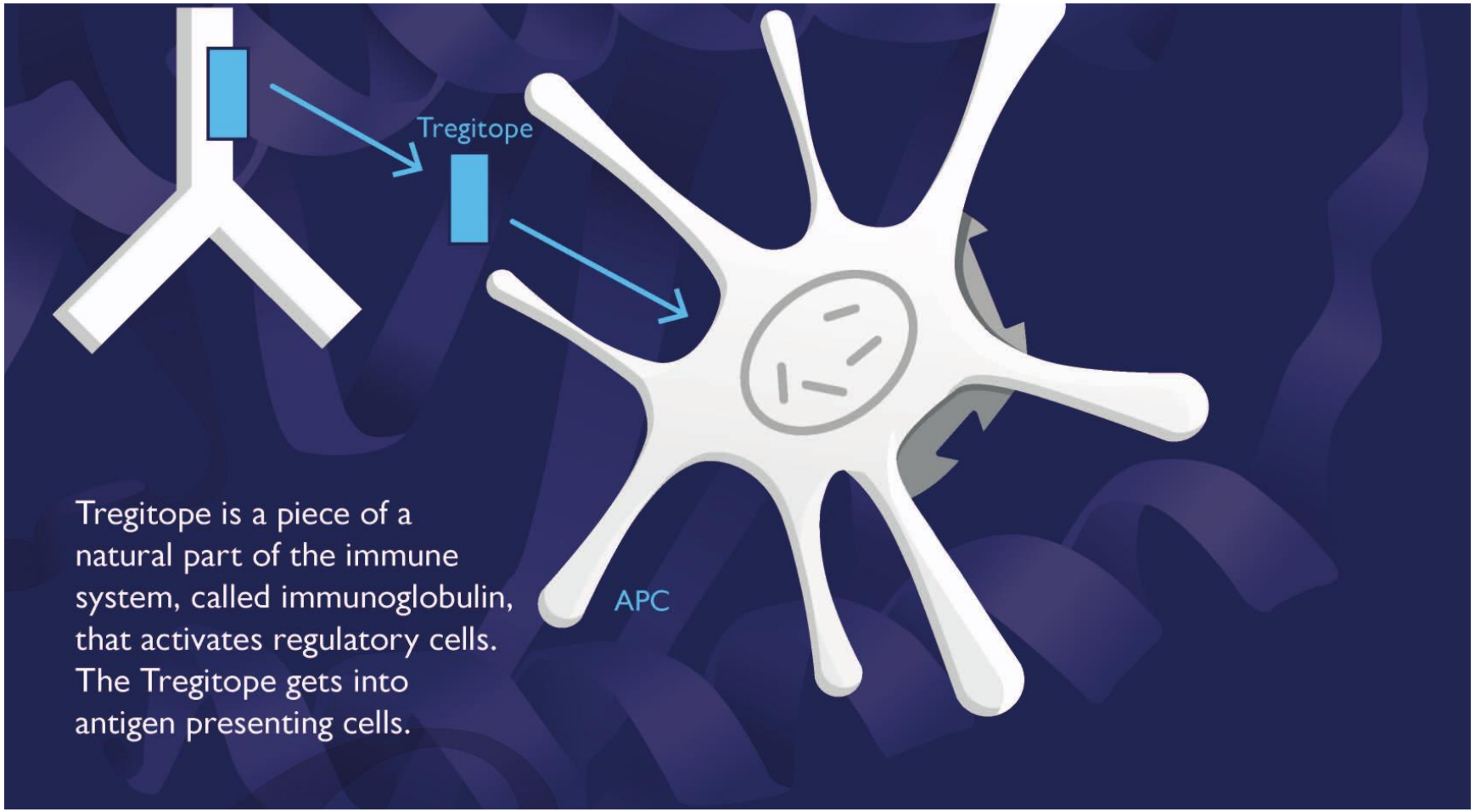
IVIG prevents inflammation in vivo: experimental autoimmune encephalomyelitis and Herpes Simplex Virus-induced encephalitis. Ephrem et al. Blood 2008 and Ramakrishna Plos Pathogens 2011

Dendritic cell immunoreceptor: A novel receptor for intravenous immunoglobulin mediates induction of regulatory T cells Massoud, et al, www.jacionline.org/article/S0091-6749(13)01476-0/fulltext

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?**

Tregitope – Simplified Mechanism of Action (MOA)



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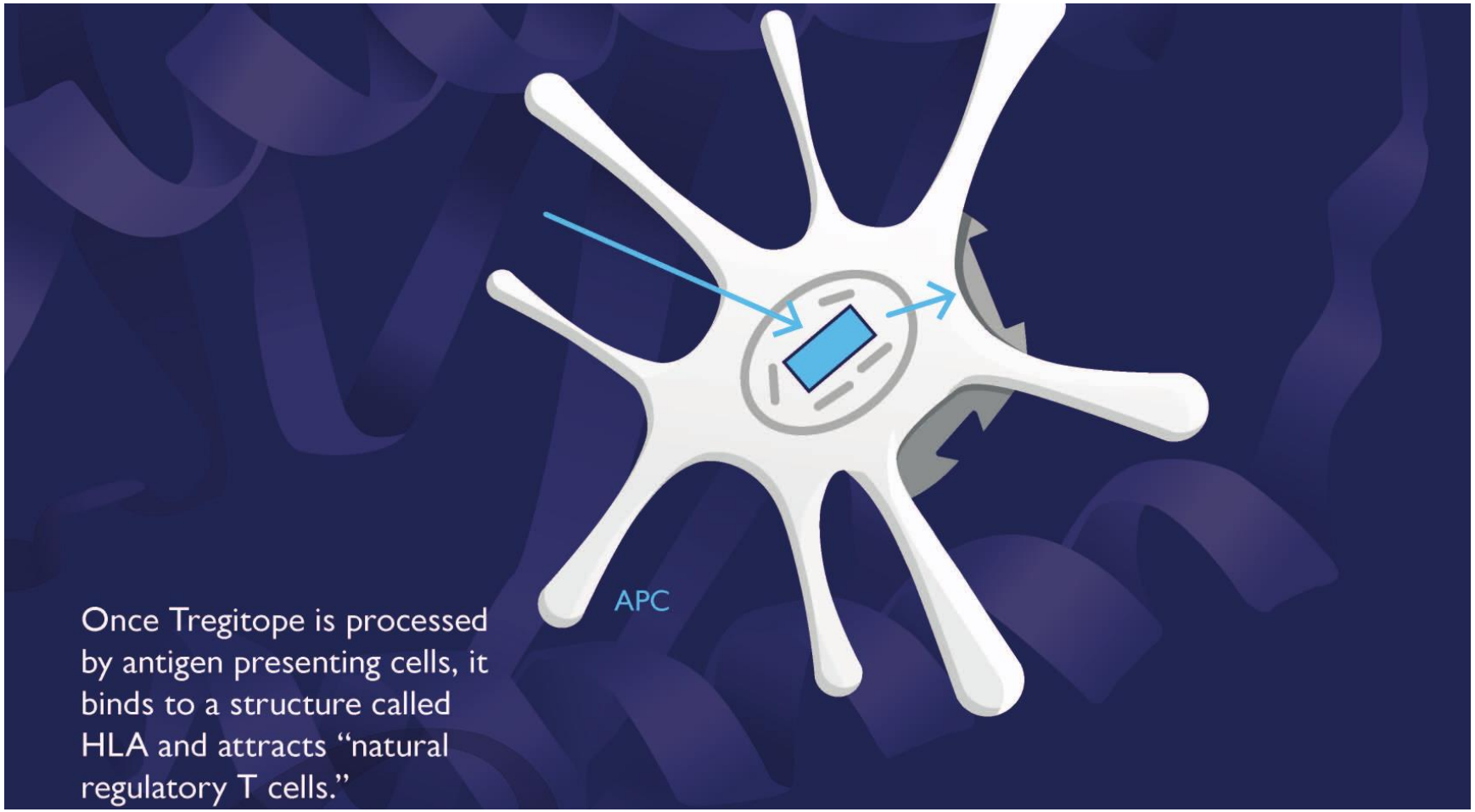

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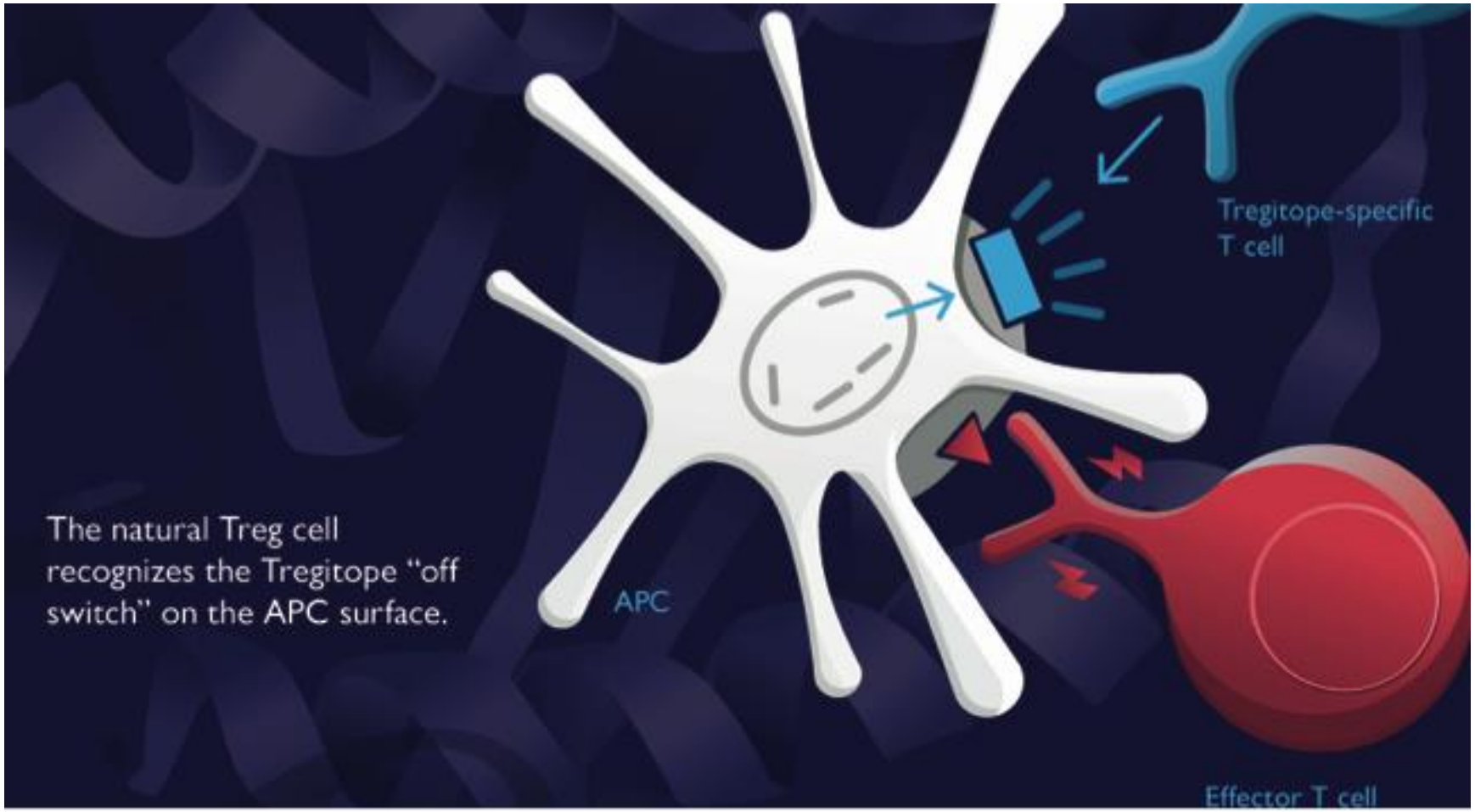

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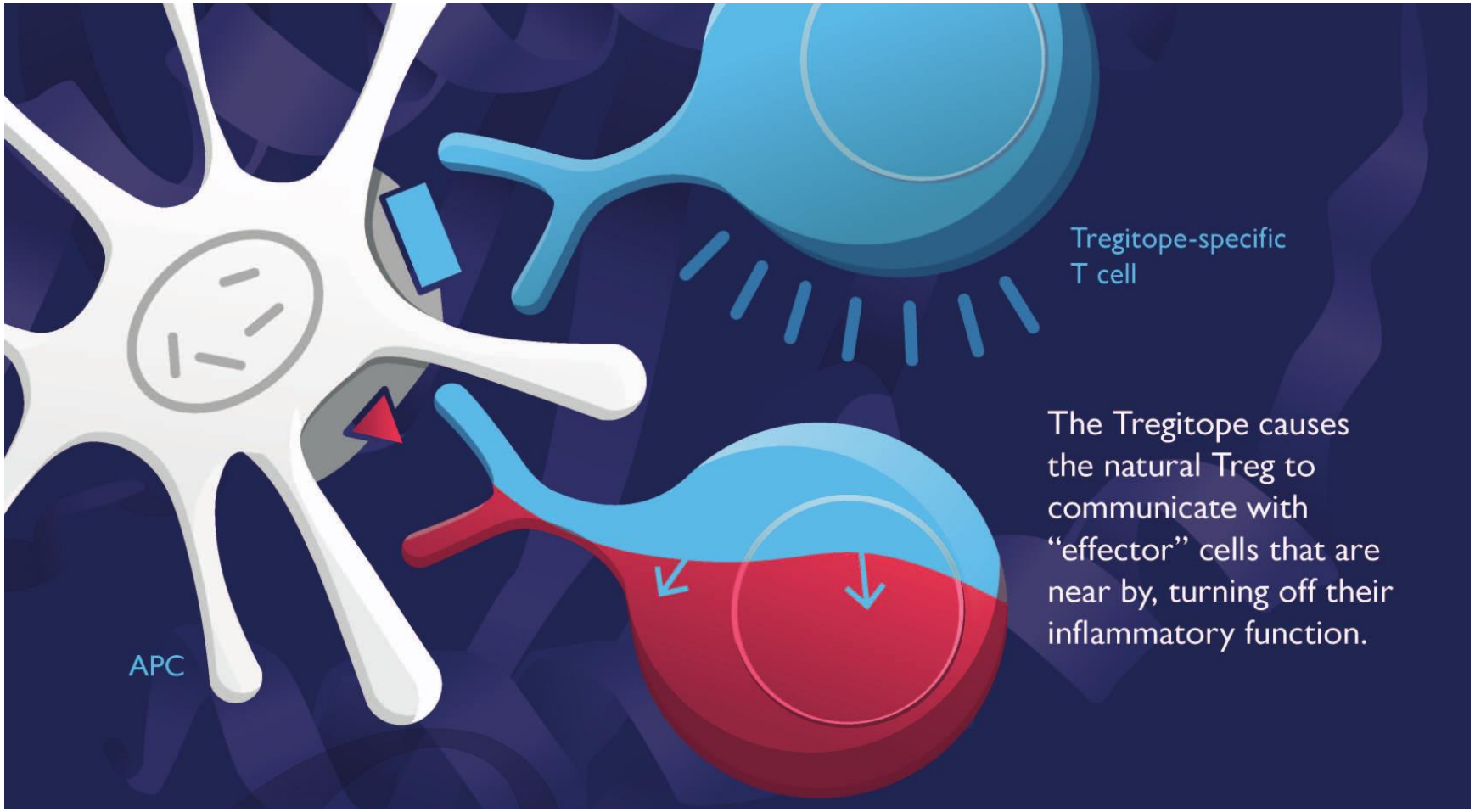

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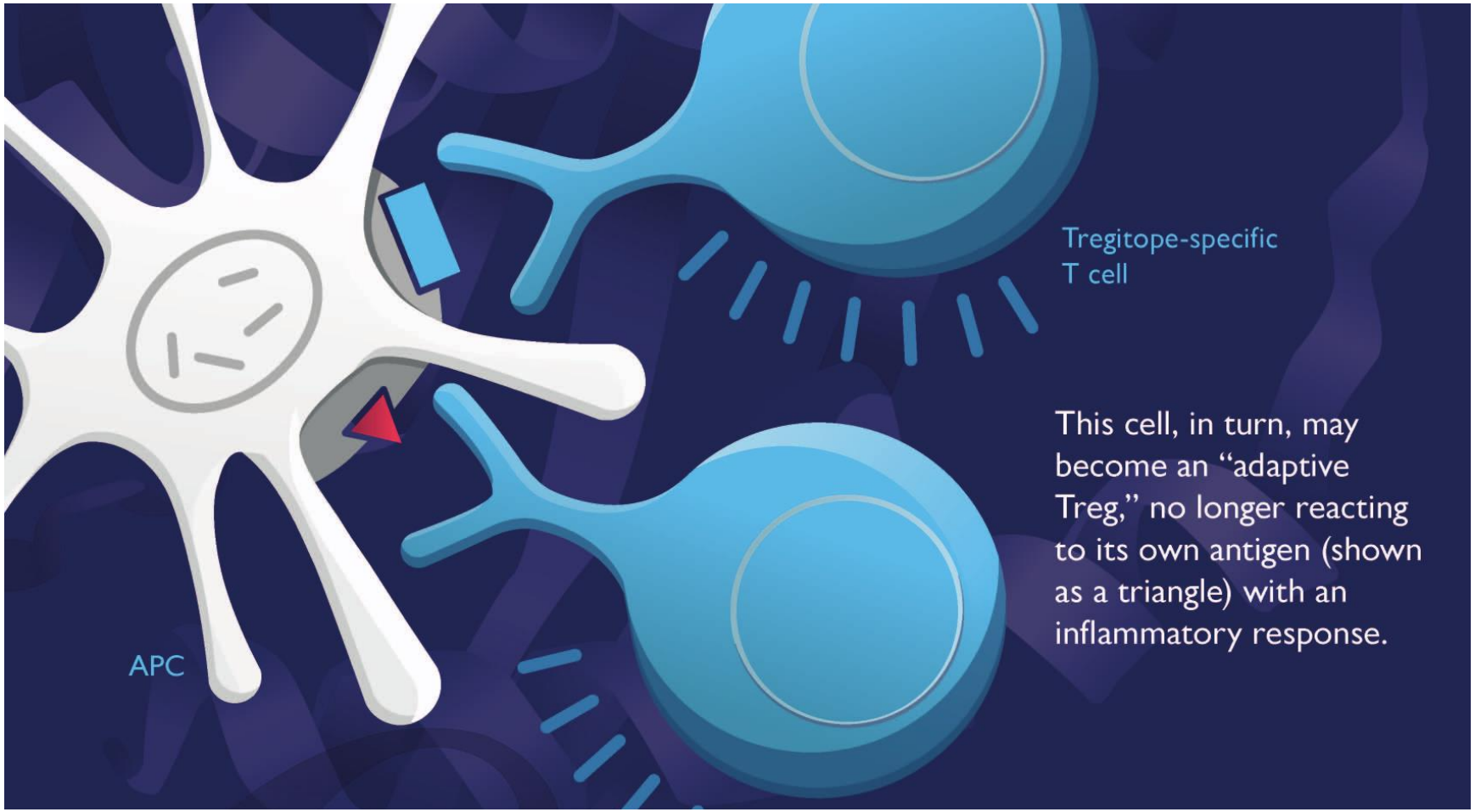
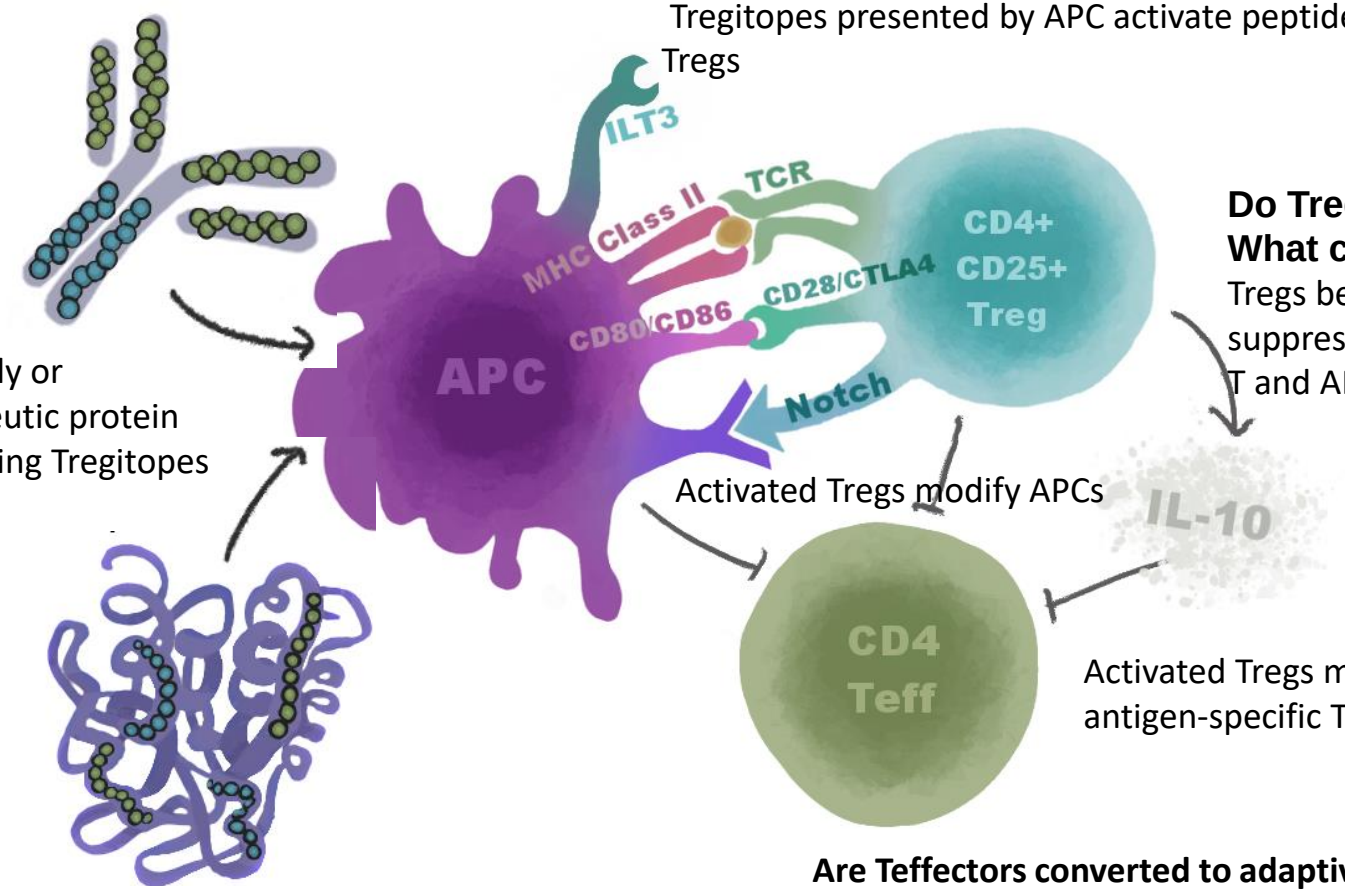


Proposed Tregitope mechanism of action

ANTIGEN PRESENTING CELL: - Are Tregitopes eluted?
Tregitopes presented by APC activate peptide-specific Tregs



Antibody or therapeutic protein containing Tregitopes

Activated Tregs modify APCs

Do Tregs expand?
What cytokines are expressed?
Tregs become activated and [may] expand; secrete suppressor cytokines (IL-10, TGF-β) and upregulate T and APC inhibitory molecules (CTLA-4).

Activated Tregs modify nearby antigen-specific T cells.

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)

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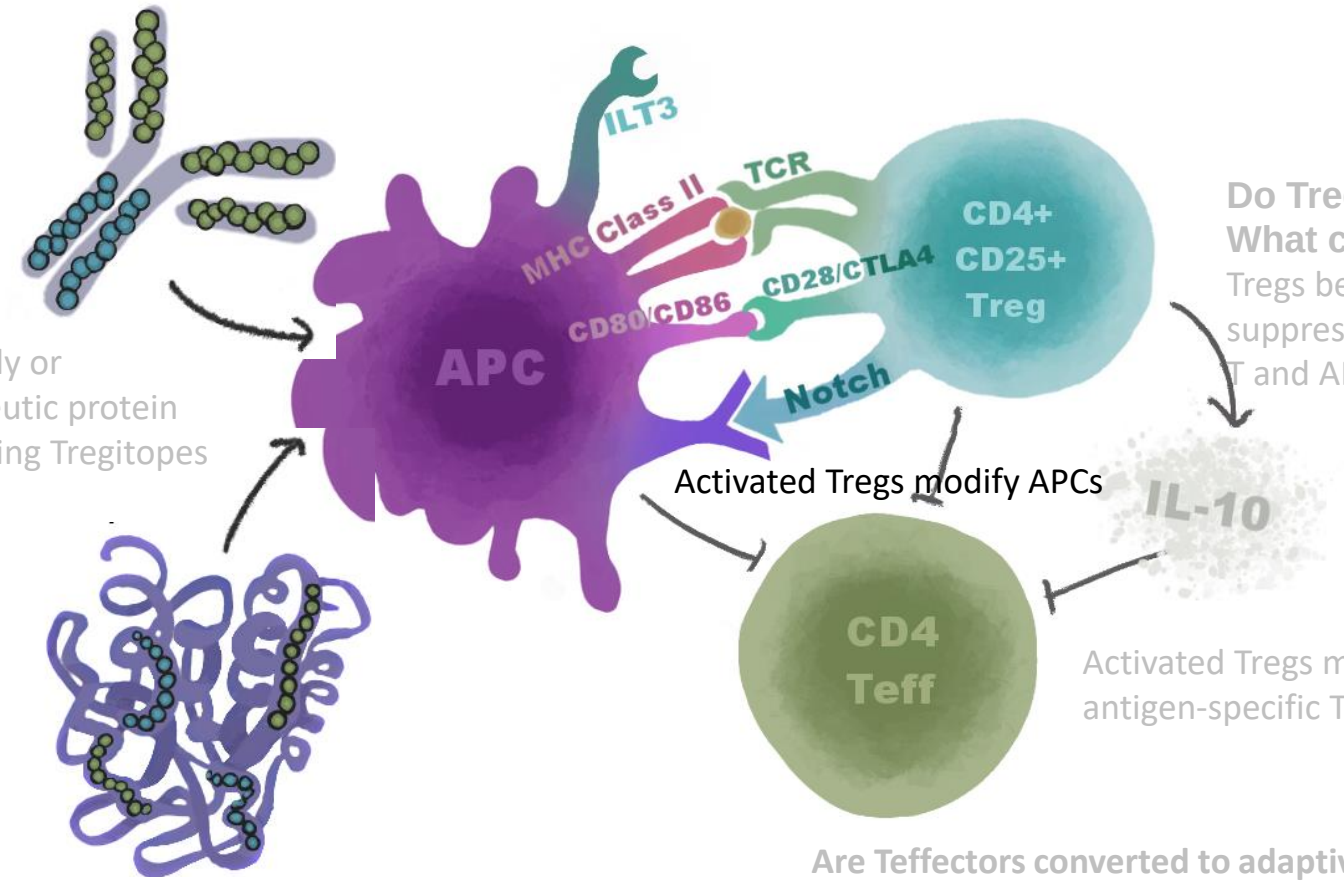

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Novartis publication questions but confirms Tregitopes →Shows Tregitopes are eluted from APC (MAPPS)

Immunity, Inflammation and Disease

Open Access

ORIGINAL RESEARCH

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

Peptides Eluted From IgG by Novartis Team Are Tregitopes

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).***

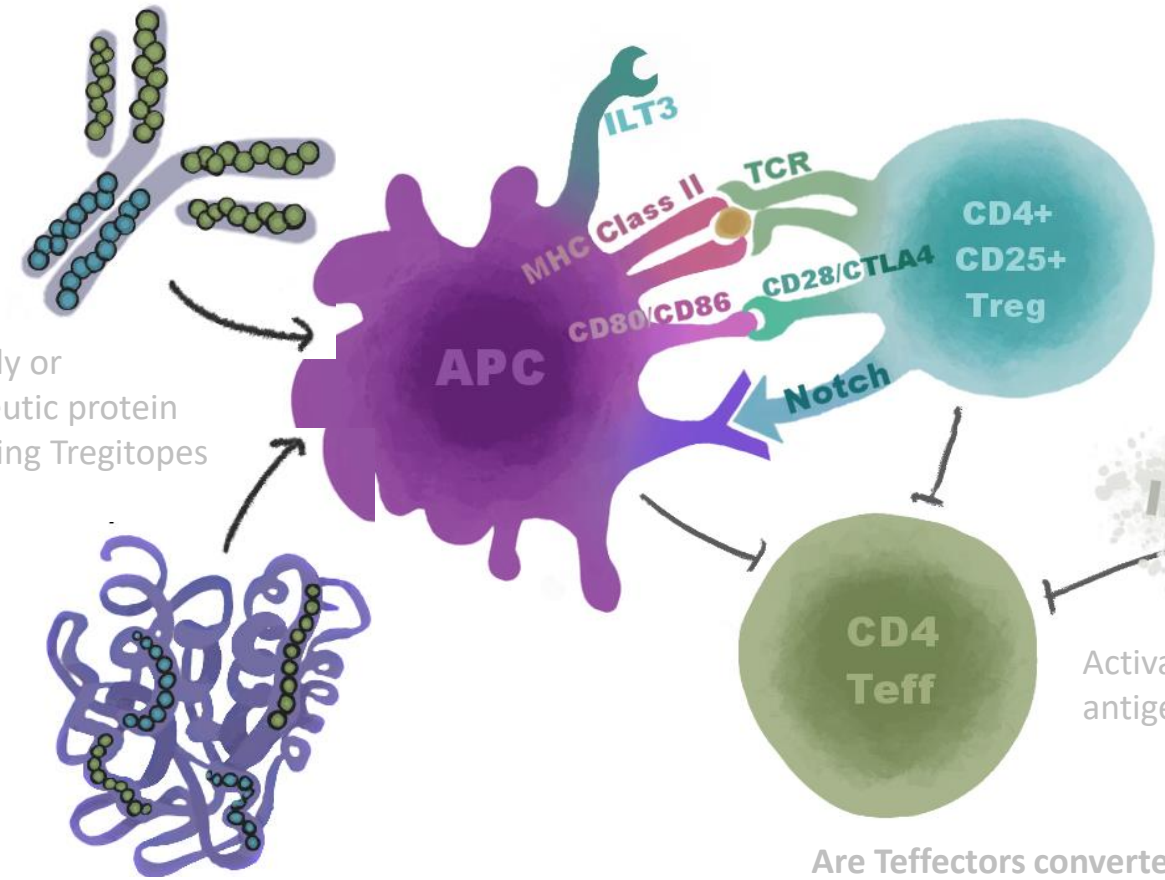
Eluted Tregitopes/Tregitope homologs in Sordé et al. Found in Manuscript Supplement Information

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	GGL**VQPGGSLRLSCAASG**FTF	I1	**VQPGGSLRLSCAASG**LPL	11	18.55
hTreg_IgGh-009B	GGL**VQPGRSLRLSCAASG**FTF	I5	SGGGV**VQPGRSLRL**PCAASGFIF	11	12
hTreg_IgGh-009B	"	I5	VESGGGAV**QPGRSLRL**SCS	5	8
hTreg_IgGh-009B	"	I5	VESGGGV**VQPGRSLRL**SCAVS	7	8.71
hTreg_IgGh-009B	"	I7	SGGGV**VQPGRSLRLS**CAGSGLTFERY	18	3.61
hTreg_IgGh-029B	MH**WVRQAPGKGL**EWV	I2	YGMS**WVRQAPEKGLE**WVSSITGSGGSTY	18	4
hTreg_IgGh-074	VDT**SKNQFSLRLSSVTAA**DTA	I3	SKN**DFSLNLSSVT**AADTAV	12	6
hTreg_IgGk-041	LA**WYQQKPGKA**PKL	I8	N**WYQQKPGKA**PKVLVHTASTL	9	11.33
hTreg_IgGl-039	VS**WYQQHPGKA**PKL	I6	**YQQLPGKAP**KLLIYSDNLR	7	5.86

Proposed Tregitope mechanism of action

ANTIGEN PRESENTING CELL: - Are Tregitopes eluted?



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

Autoimmunity

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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], Ranim Touma[1], Yali Song[1], Robert L. Padilla[1], Adriana H. Tremoulet[1], John Sidney[2], Alessandro Sette[2], and Alessandra 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 8-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 12 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/PMC4784966/pdf/nihms763445.pdf

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

Kawasaki

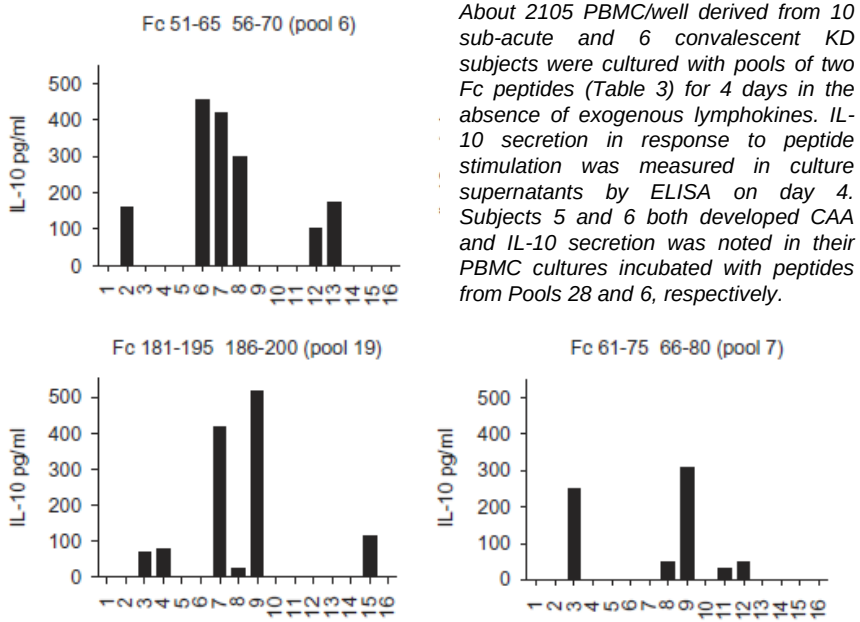
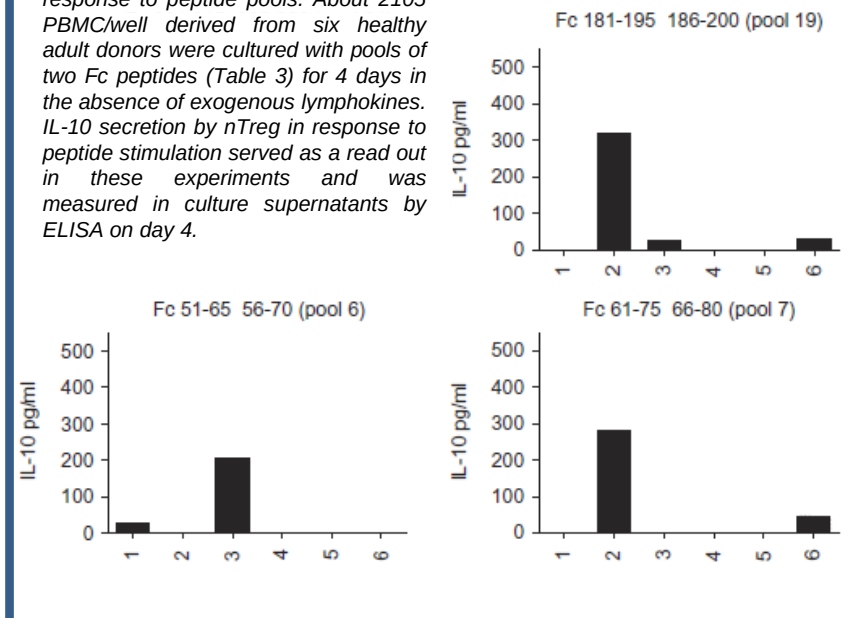


Figure 1. nTreg fine specificities in sub-acute and convalescent KD subjects. About 2105 PBMC/well derived from 10 sub-acute and 6 convalescent KD subjects were cultured with pools of two Fc peptides (Table 3) for 4 days in the absence of exogenous lymphokines. IL-10 secretion in response to peptide stimulation was measured in culture supernatants by ELISA on day 4. Subjects 5 and 6 both developed CAA and IL-10 secretion was noted in their PBMC cultures incubated with peptides from Pools 28 and 6, respectively.

Healthy

Figure 3. IL-10 secretion in PBMC cultures from healthy adult donors in response to peptide pools. About 2105 PBMC/well derived from six healthy adult donors were cultured with pools of two Fc peptides (Table 3) for 4 days in the absence of exogenous lymphokines. IL-10 secretion by nTreg in response to peptide stimulation served as a read out in these experiments and was measured in culture supernatants by ELISA on day 4.

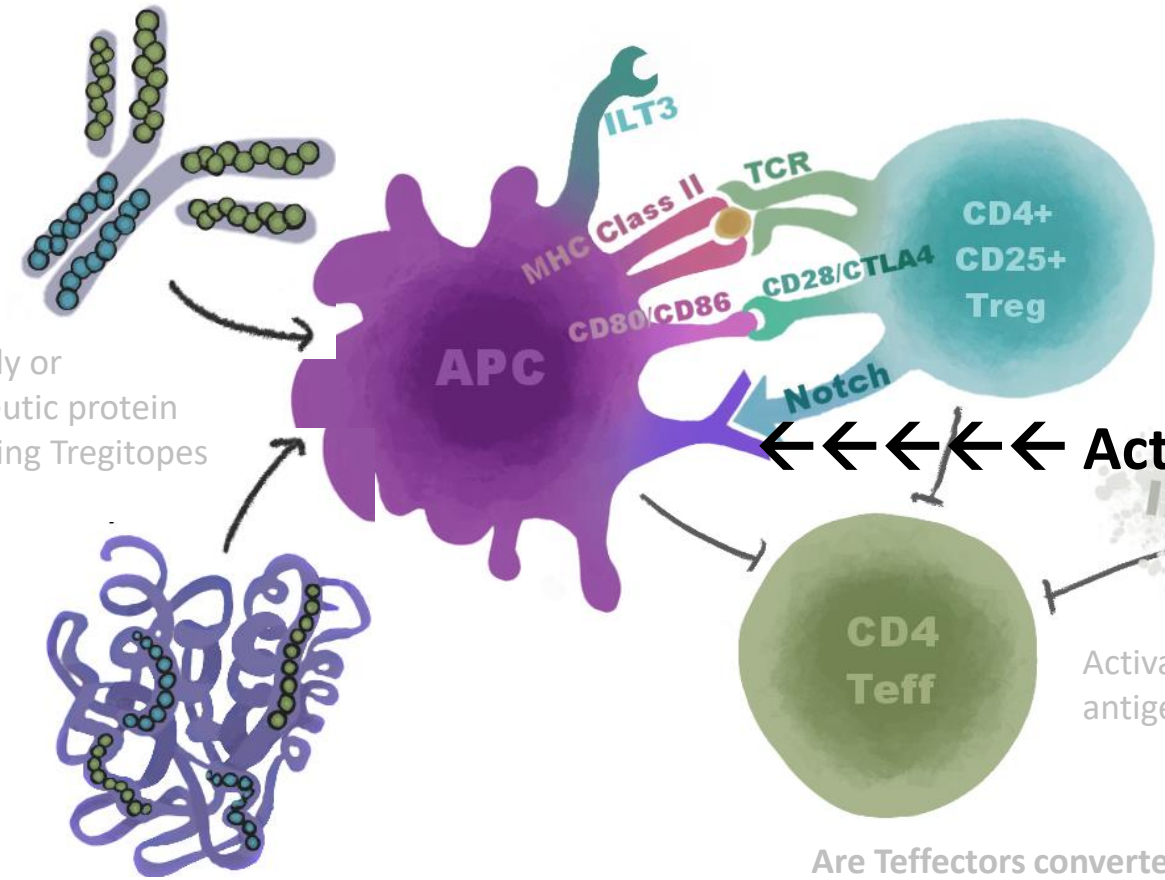


Pool 5	41–55	NSGALTSGVHTFPAV
	46–60	TSGVHTFPAVLQSSG
Pool 6	51–65	TFPAVLQSSGLYSLS
	56–70	LQSSGLYSLSSVVTV
Pool 7	61–75	LYSLSSVVTVPSSSL
	66–80	SVVTVPSSSLGTQTY
Pool 8	71–85	PSSSLGTQTYICNVN
	76–90	GTQTYICNVNHKPSN
Pool 17	161–175	VDGVEVHNAKTKPRE
	166–180	VHNAKTKPREEQYNS
Pool 18	171–185	TKPREEQYNSTYRVV
	176–190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

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

Proposed Tregitope mechanism of action

ANTIGEN PRESENTING CELL: - Are Tregitopes eluted?



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←←←← **Activated Tregs modify APCs**

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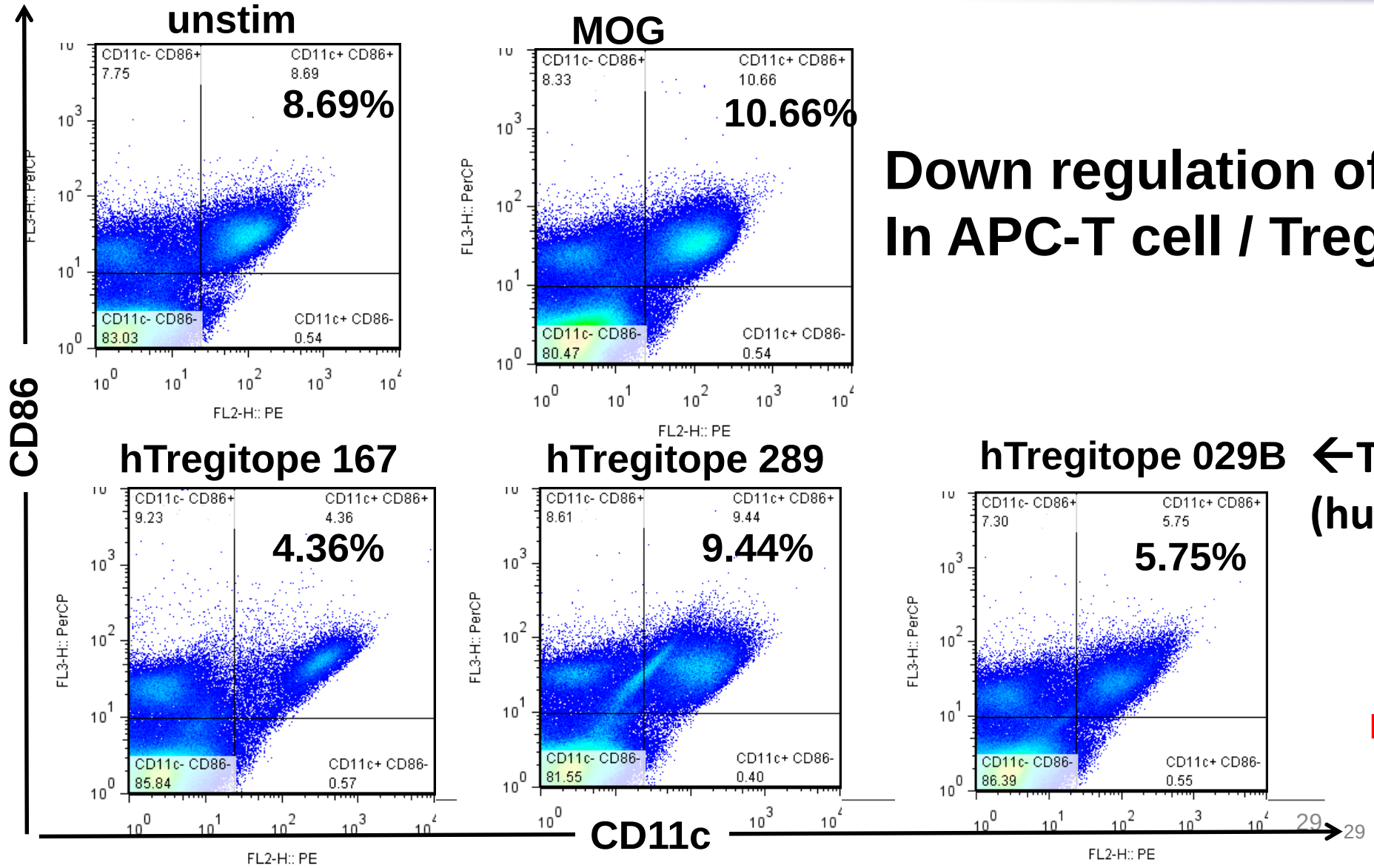
Modulation of Antigen Presenting Cells CD11c+ CD86+

EpiVax



Down regulation of CD86
In APC-T cell / Tregitope co-culture

←Tregitope 029 (human version)

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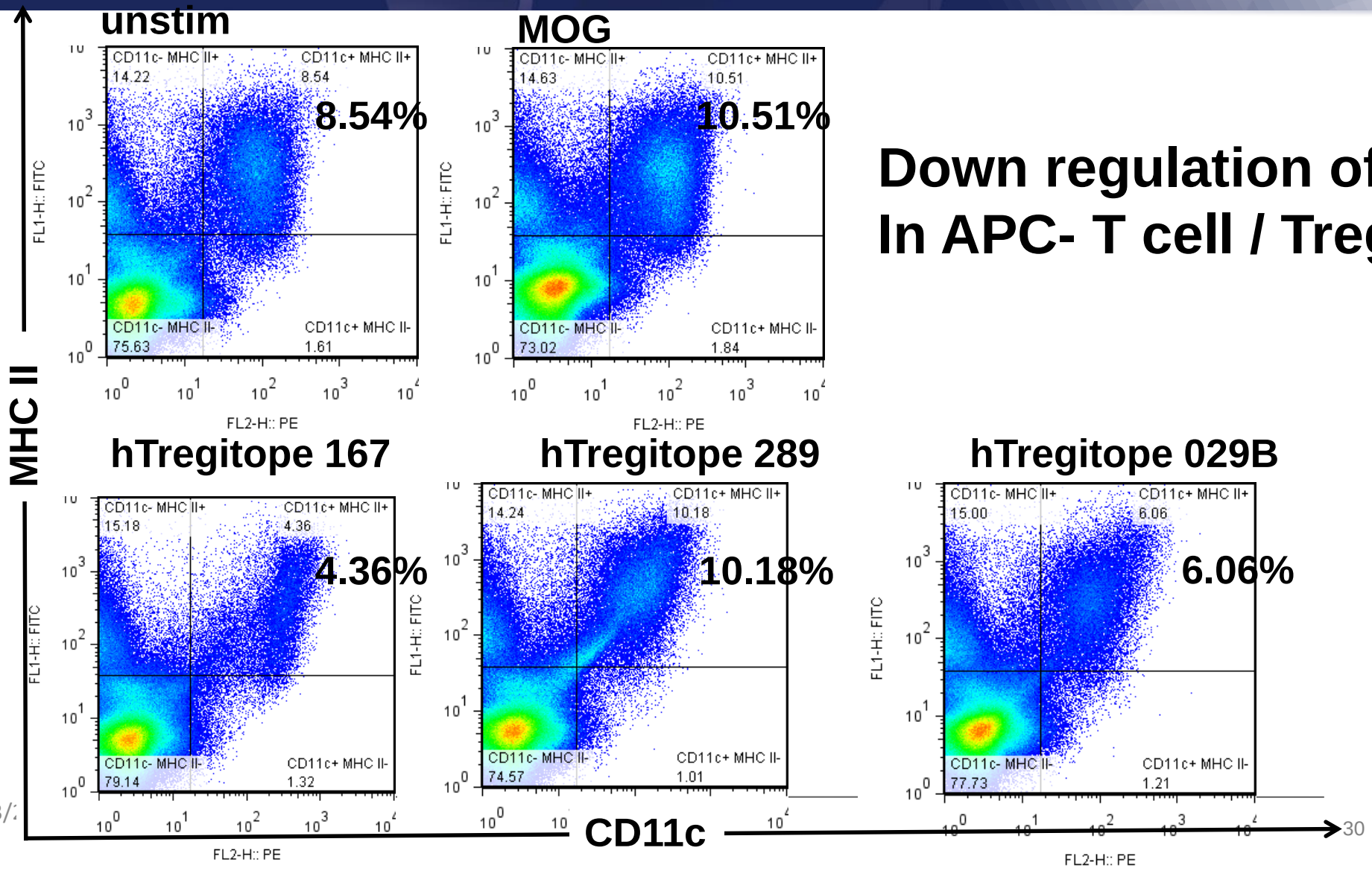
29

Modulation of Antigen Presenting Cells CD11c+ MHC II+

EpiVax



Down regulation of HLA DR In APC- T cell / Tregitope co-culture

←Tregitope 029 (human version)

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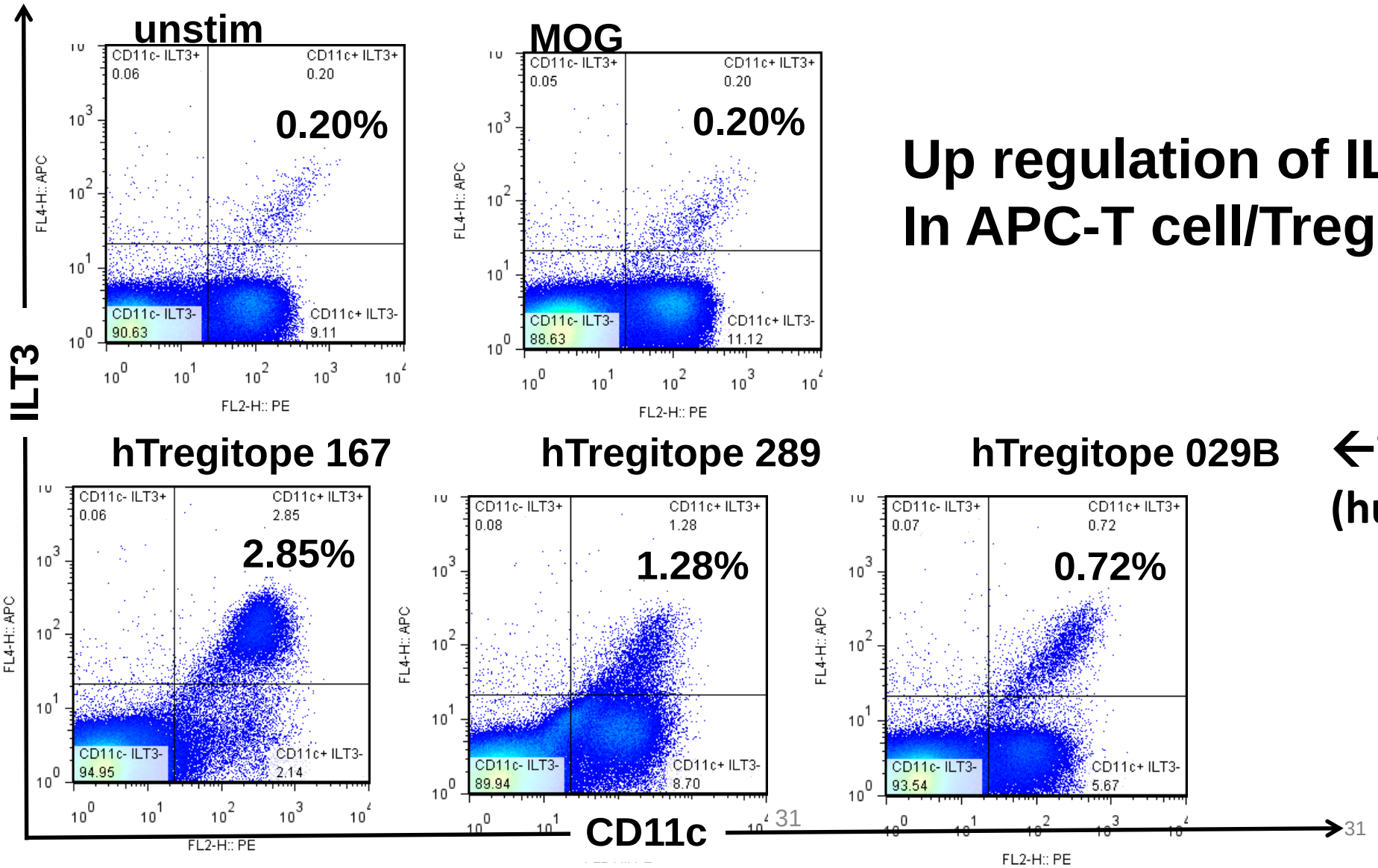
Modulation of Antigen Presenting Cells CD11c+ ILT3+

EpiVax



Up regulation of ILT3
In APC-T cell/Tregitope co-culture

←Tregitope 029 (human version)

Tregitope Homolog in Clinical Studies = Edratide

EpiVax

←Tregitope 029 (humanized anti idiotype from murine IgG)

Clinical trials and drug discovery

LUPUS SCIENCE & MEDICINE™

Safety and efficacy of hCDR1 (Edratide) in patients with active systemic lupus erythematosus: results of phase II study

Murray B Urowitz,[1] David A Isenberg,[2] Daniel J Wallace[3]

To cite: Urowitz MB, Isenberg DA, Wallace DJ. Safety and efficacy of hCDR1 (Edratide) in patients with active systemic lupus erythematosus: results of phase II study. *Lupus Science & Medicine* 2015;**2**: e000104. doi:10.1136/lupus-2015-000104

ABSTRACT

Objective: To evaluate the safety and efficacy of hCDR1 (Edratide) in patients with systemic lupus erythematosus (SLE).

Methods: Patients (n=340) with SLE ≥4 ACR criteria (4–11, mean 7) with active disease (SLEDAI-2K of 6–12). Patients were on average 7.1 years post-diagnosis and their organ involvement was mainly musculoskeletal, mucocutaneous and haematologic. Placebo or Edratide was administered subcutaneously weekly at doses of 0.5, 1.0 or 2.5 mg. The co-primary endpoints were SLEDAI-2K SLE Disease Activity and Adjusted Mean SLEDAI (AMS) reduction in patients compared with controls using a landmark analysis. Secondary outcomes were improvement in British Isles Lupus Assessment Group (BILAG) Responder Index and medicinal flare analysis.

Results: Edratide was safe and well tolerated. The primary endpoints based solely on SLEDAI-2K and AMS were not met. The secondary predefined endpoint, BILAG, was met for the 0.5 mg Edratide arm in the intention to treat (ITT) cohort (N=316) (OR=2.09, p=0.03) with trends in the 1.0 and 2.5 mg

KEY MESSAGES

- Edratide demonstrated efficacy in one and possibly more clinically meaningful endpoints.
- Dose ranging studies demonstrated the 0.5 mg subcutaneous weekly was the most effective dose.
- There were no safety signals in this 26 week study.

Due to the complex nature of the disease, progress in developing new SLE treatment modalities has been slow.[9] [10] Until the approval by the Food and Drug Administration in 2011 of belimumab,[11] no new drugs had been approved for the treatment of SLE since 1955. There is therefore a clear need for new therapeutic agents.[12]

hCDR1 (Edratide) is a novel synthetic peptide of 19 amino acid residues (H-G-Y-Y-W-S-W-I-R-Q-P-P-G-K-G-E-E-W-I) based

▸ Additional material is available. To view please visit the journal (http://dx.doi.org/10.1136/lupus-2015-000104).

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

EpiVax

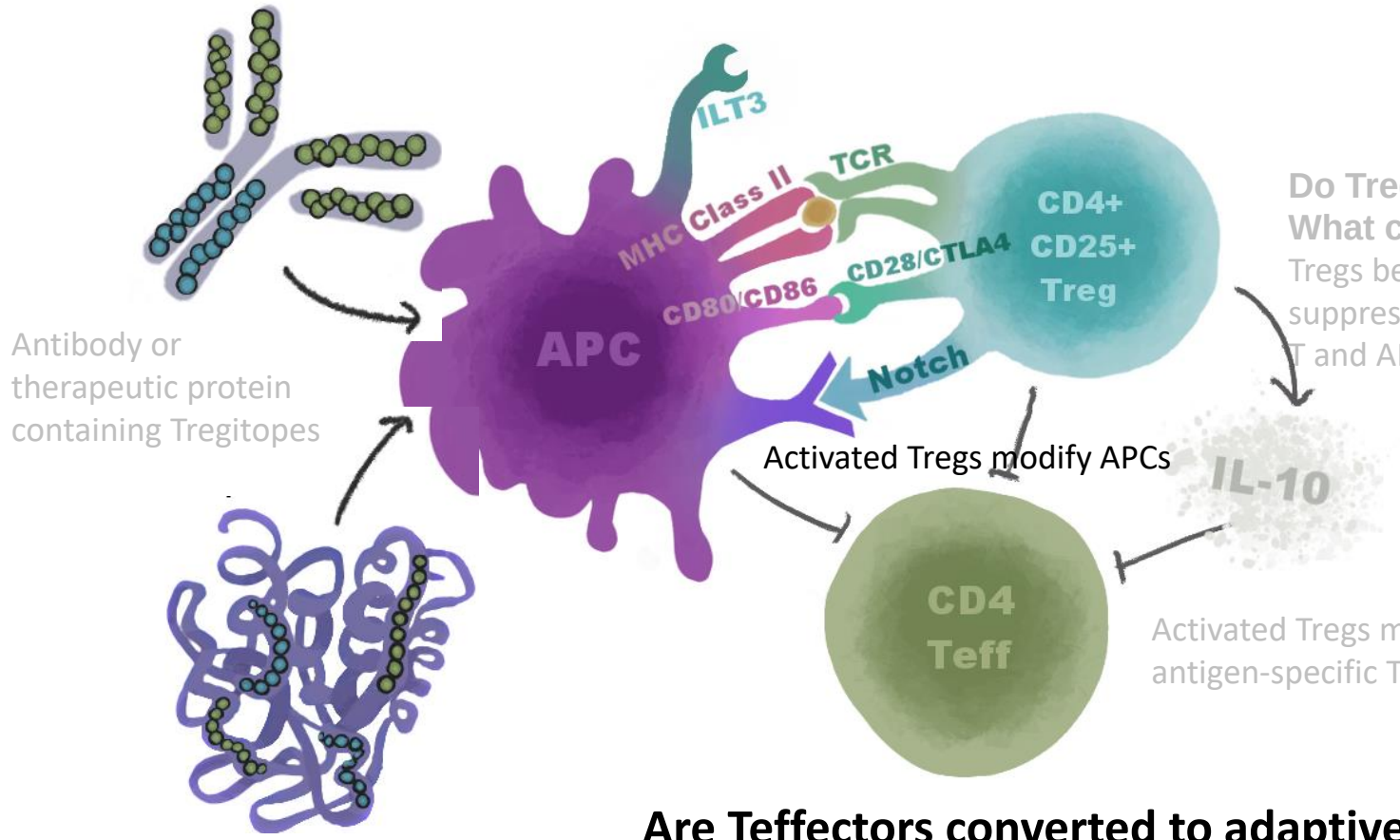
	EpiVax 029B	Edratide
MHC II	↓	↓
CD80	↓	↓
CD86	↓	↓
IL-T3	↑	No Data
IL-10	↑	↓
IFNγ	No Data	↓
TGFβ	No Data	↑
Tregs	↑	↑
IL-7	No Data	↓
B Cells	No Data	↓

←Tregitope 029 /APC

EpiVax = Human Tregitope,
Edratide = Idiotype Tregitope

←Tregitope 029 /T cells

Proposed Tregitope mechanism of action

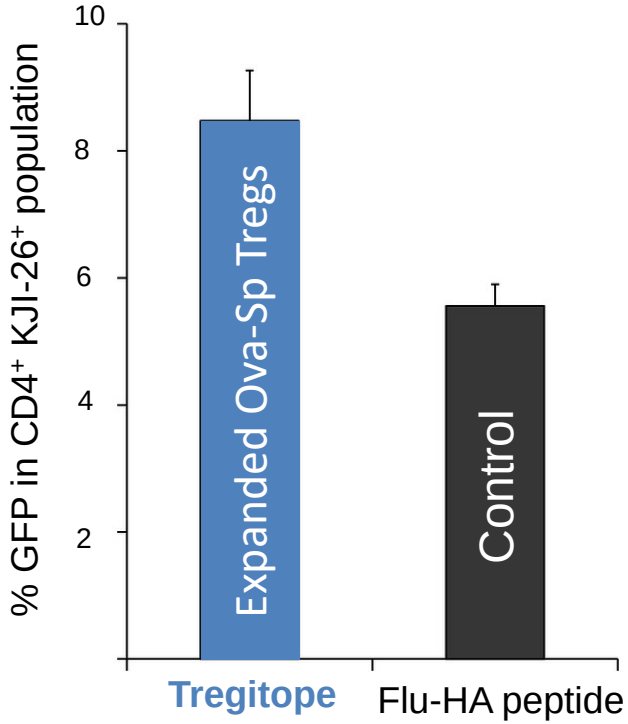


Are Teffectors converted to adaptive Treg? Are there other effects (killing of Teff by Tregs?) Ag-specific T cells are rendered non-responsive or become induced or iTregs (Ag-specific)

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

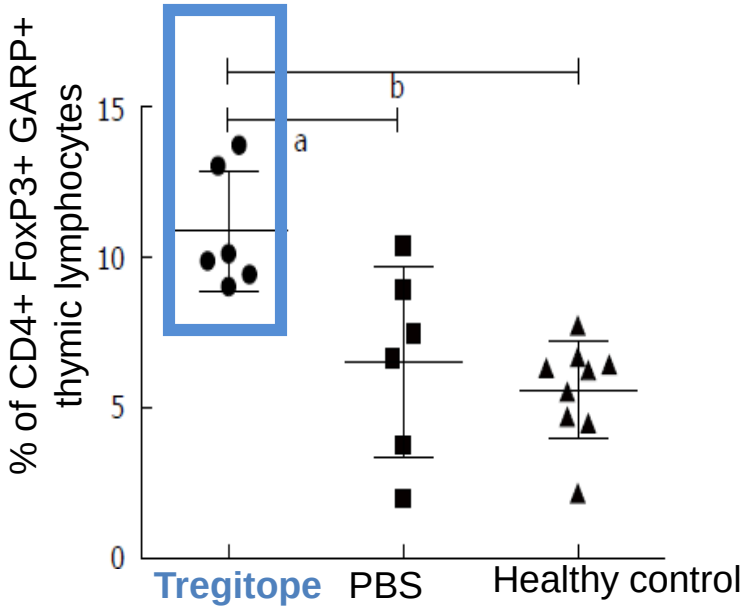
EpiVax

DO11.10 FoxP3/GFP TCR Tg mice immunized with Tregitopes



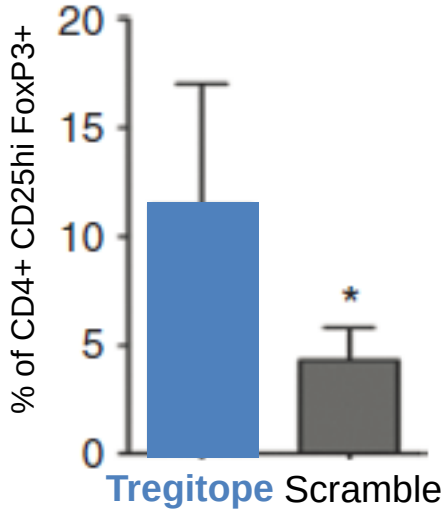
Cousens LP et al
J Diabetes Res. 2013;2013:621693

Balb/C mice with acute colitis (TNBS) pre-treated with Tregitope



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

C57BL/6 mice immunized with VP1 and challenged at 6 weeks



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

Tregitopes induces antigen-specific Regulatory T cells in vivo

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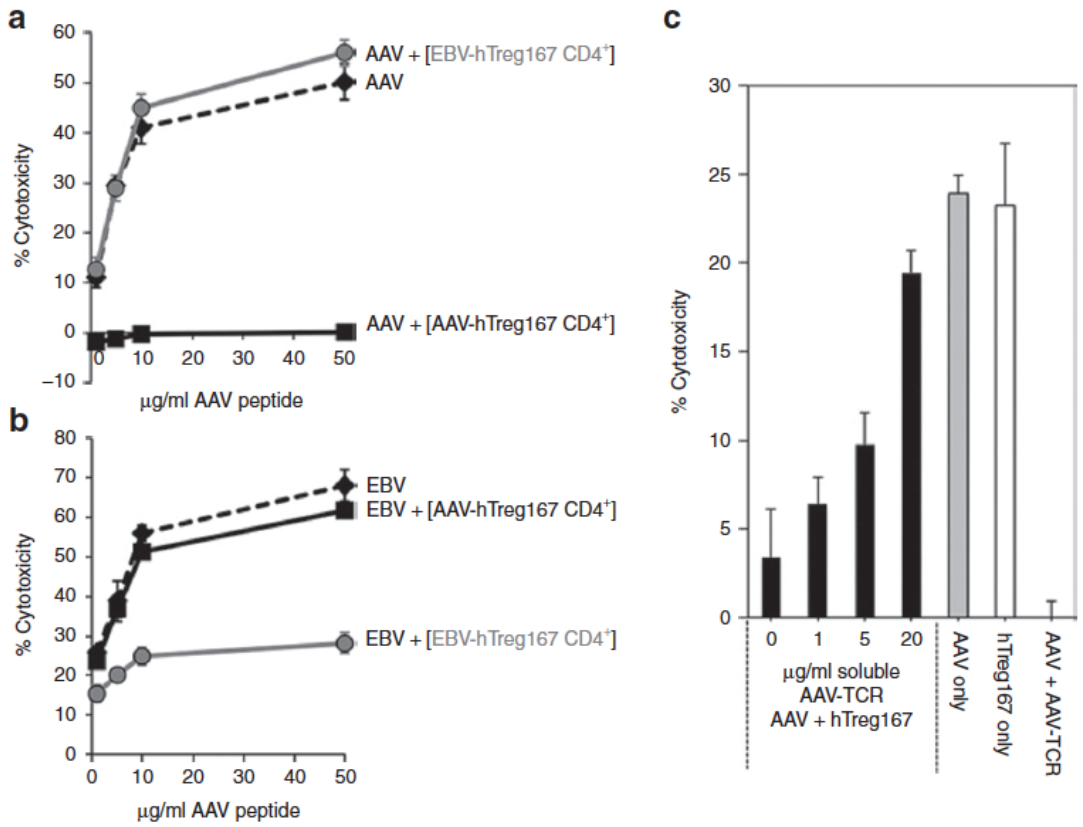
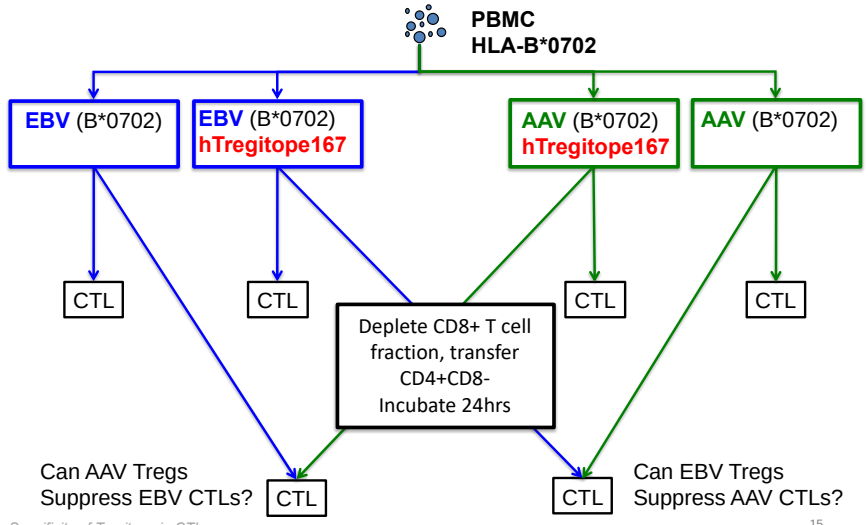


Modulation of CD8+ T cell responses to AAV vectors with IgG-derived MHC class II epitopes

Daniel J Hui[1], Etiena Basner-Tschakarjan[1], Yifeng Chen[1,2], Robert J Davidson[1], George Buchlis[1,3], Mustafa Yazicioglu[1], Gary C Pien[1], Jonathan D Finn[1], Virginia Haurigot[1], Alex Tai[1], David W Scott[4], Leslie P Cousens[5], Shangzhen Zhou[1], Anne S De Groot[5,6] and Federico Mingozzi[1]



Specificity of Tregitope in CTL assay

Figure 5 Antigen-specificity of Tregitopes-induced suppression of CTL responses is mediated by MHC I. (**a**) CTL assay in which target cells were loaded with the MHC I epitope VPQYGYLTL from AAV and incubated with HLA-matched AAV-specific Teff alone (AAV, dashed line), or Teff mixed at a 1:1 ratio with negatively-selected CD4+ T cells from AAV+hTreg167 restimulated PBMC (AAV+[AAV-hTreg167 CD4+], black line), or Teff mixed at a 1:1 ratio with negatively-selected CD4+ T cells from EBV+hTreg167 restimulated PBMC (AAV+[EBV-hTreg167 CD4+], gray line). (**b**) CTL assay

Kaveri and Bayry, Trends in Immunology IVIg Review

Tregitope as one of the MOA of IVIG

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,*]

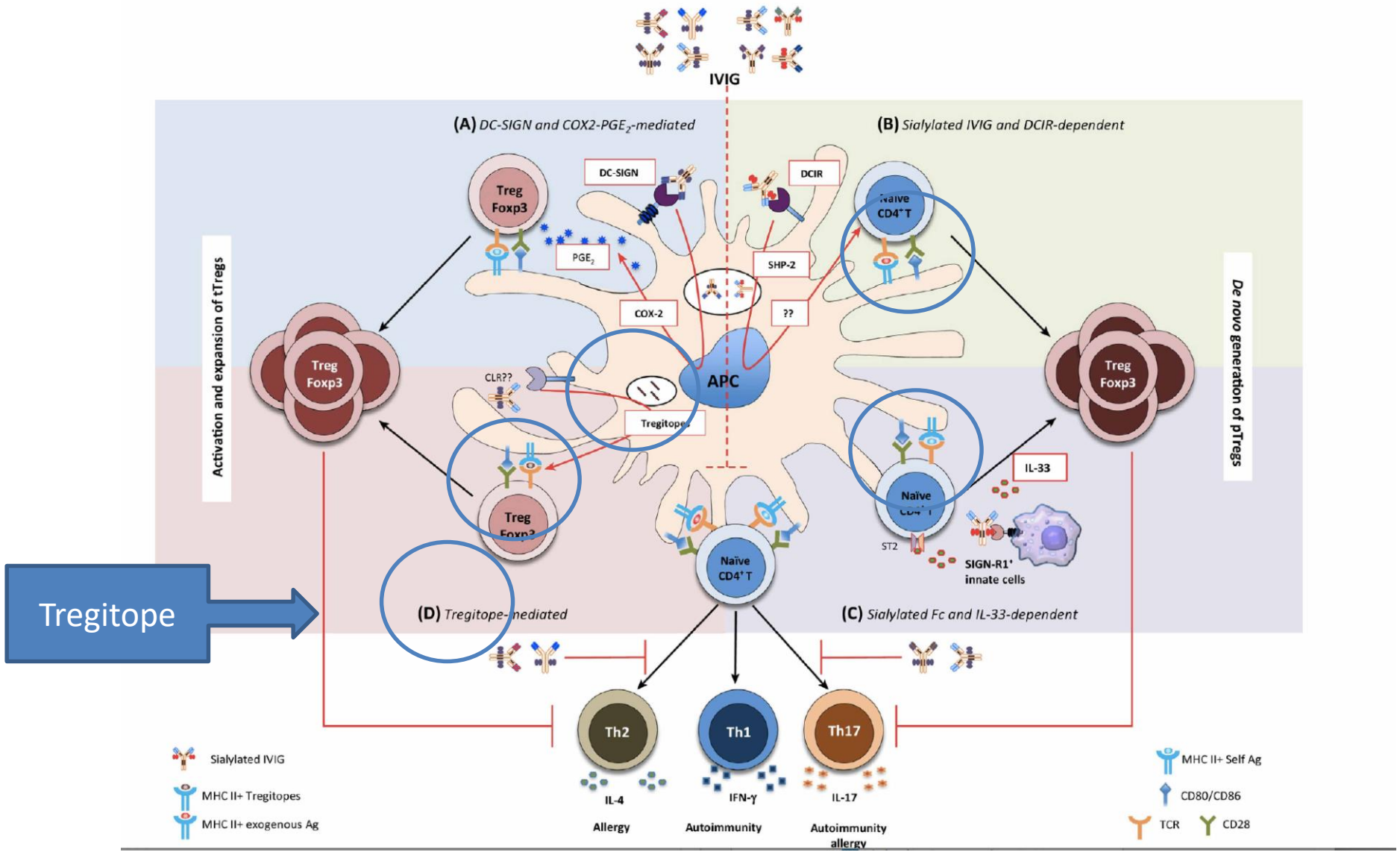
Intravenous immunoglobulin (IVIG),

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



Tregitope

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

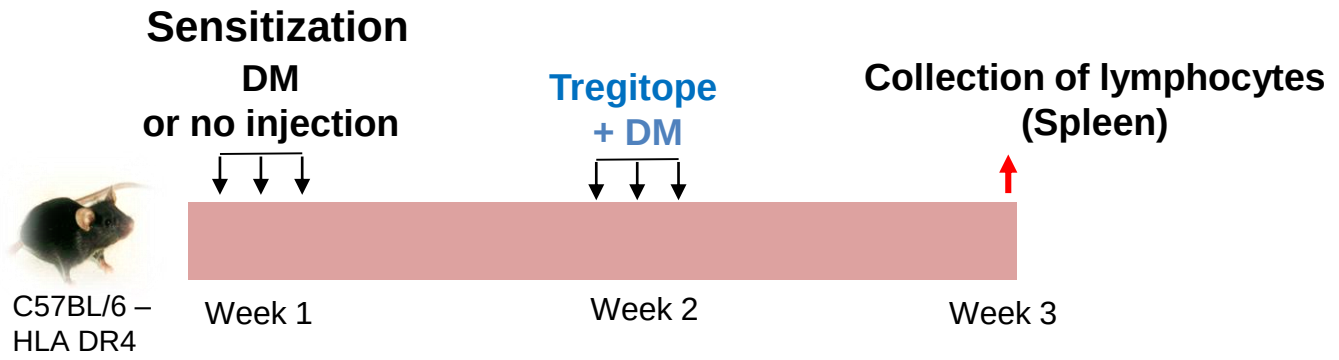
Tregitope validation in vivo

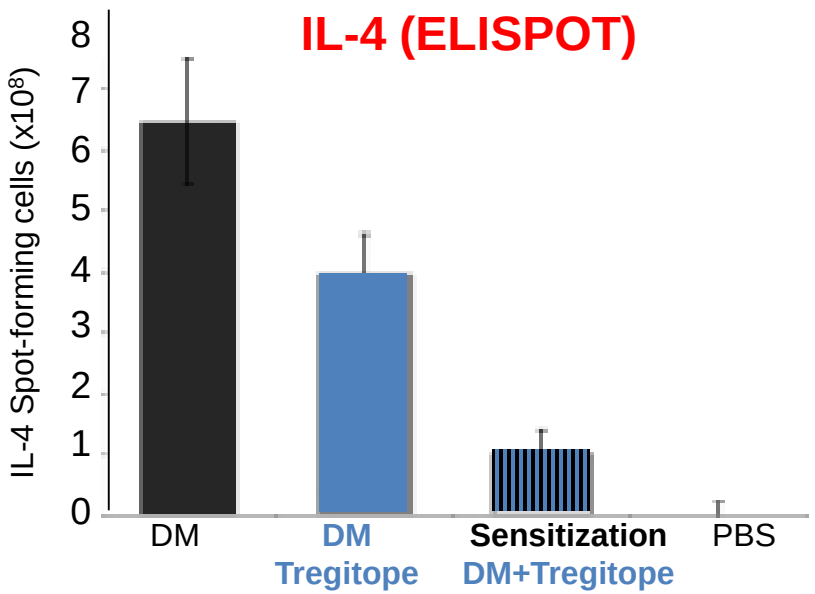
In vivo Model	Immunogen	Delivery	Clinical application	Findings	Publication
C57BL/6	OVA	DMSO	Tolerance induction	Suppress Ag-specific T cell proliferation	Cousens et al Human immunology 2014
C57BL/6	MOG	CFA	MS	Induce Tregs, Reduce of EAE symptoms	Elyaman et al Neur Res Int 2011
NOD /ShiLtJ	PPI	Liposome	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

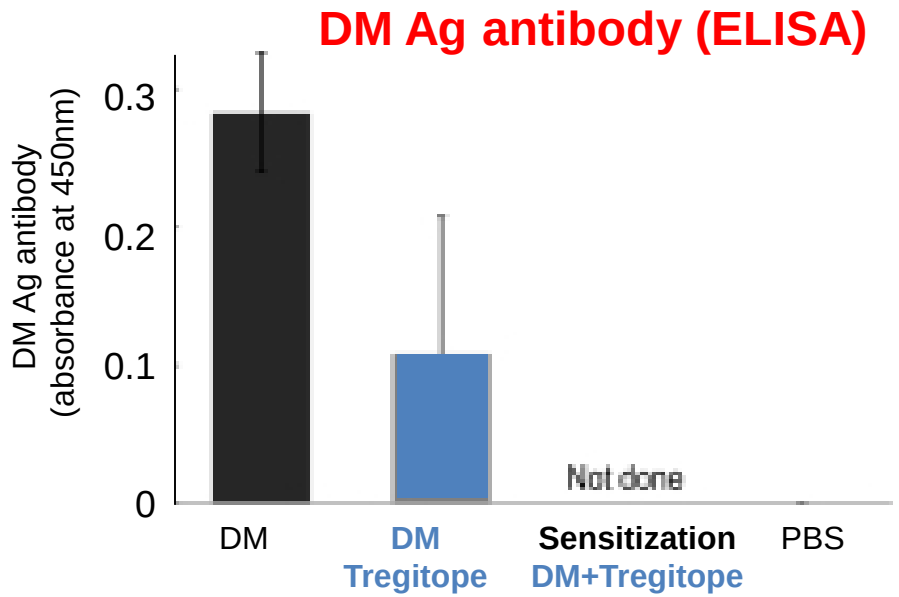
Tregitope validation checklist

- ✓ 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)

Tregitopes Reduce IL4 and Antibody Responses in an Allergy Model







De Groot A.S., et al.,
Blood, 2008,112: 3303

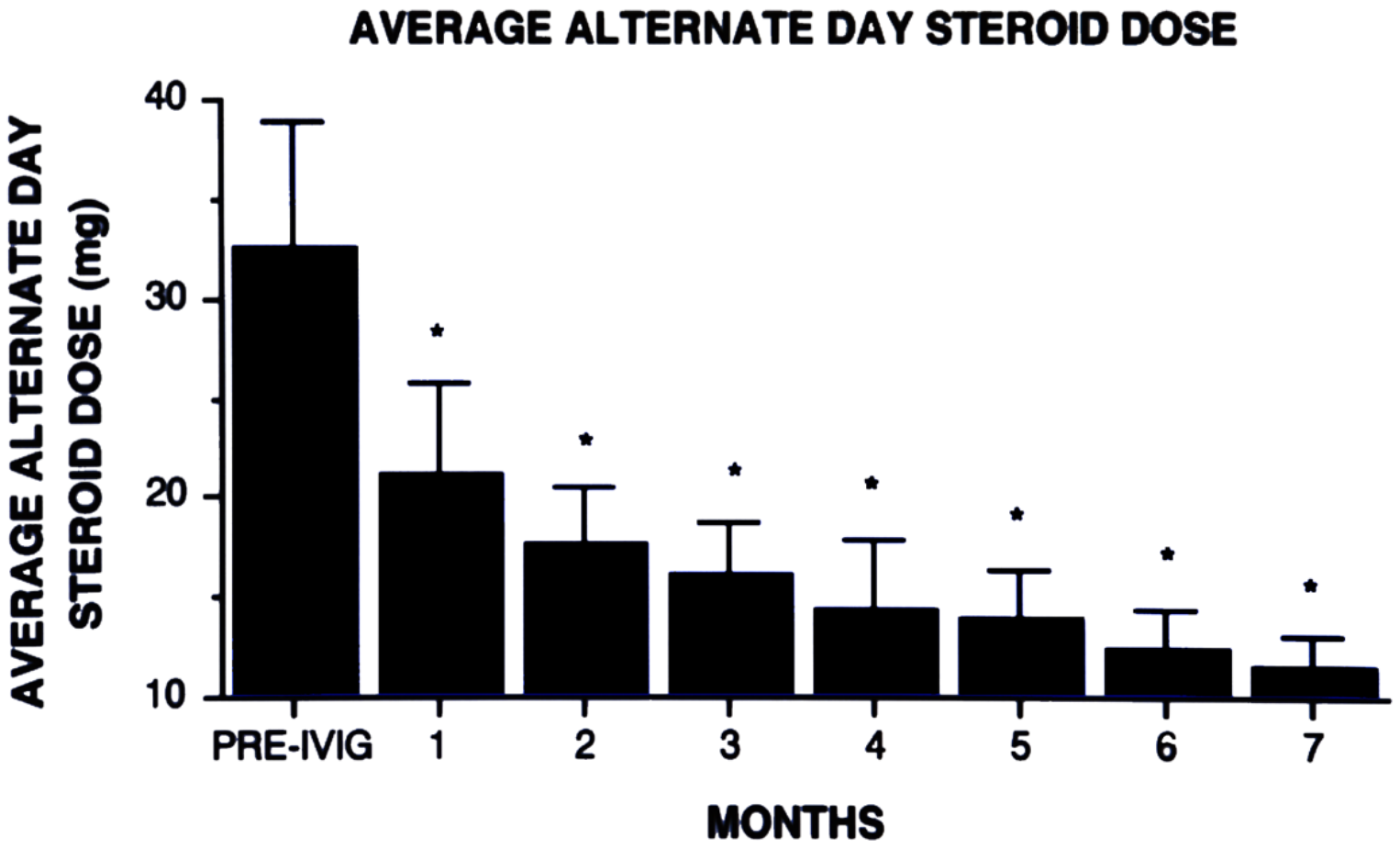
Immunomodulatory properties of Tregitopes in a mouse model of asthma (allergic airway disease - AAD)

Dr. Bruce Mazer
McGill University
Epivax Update 20180227

EpiVax

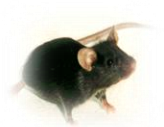
B. Mazer: IVIg reduces steroid dependency in asthma: Do Tregitopes have the same effect?

EpiVax

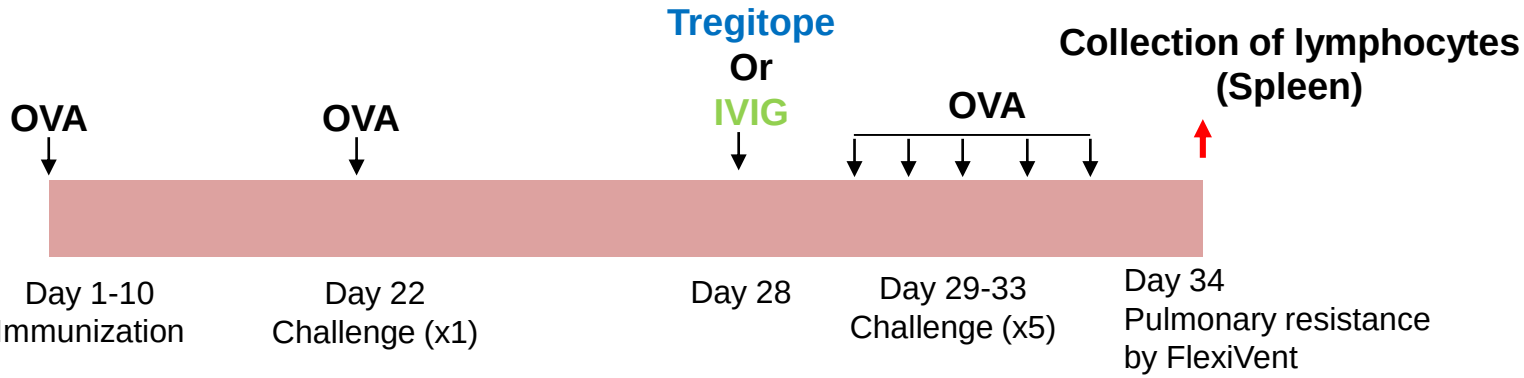


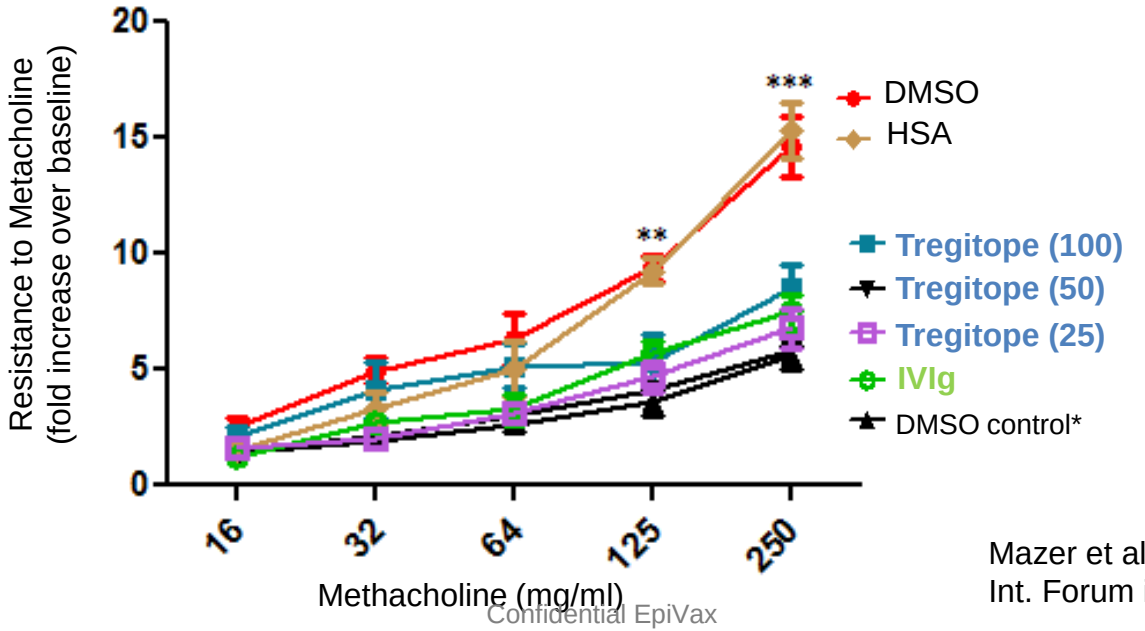
Mazer BD and Gelfand EW, J Allergy Clin Immunol 1991; 87:976-83.

Tregitopes in a Mouse Model of Asthma



C57BL/6



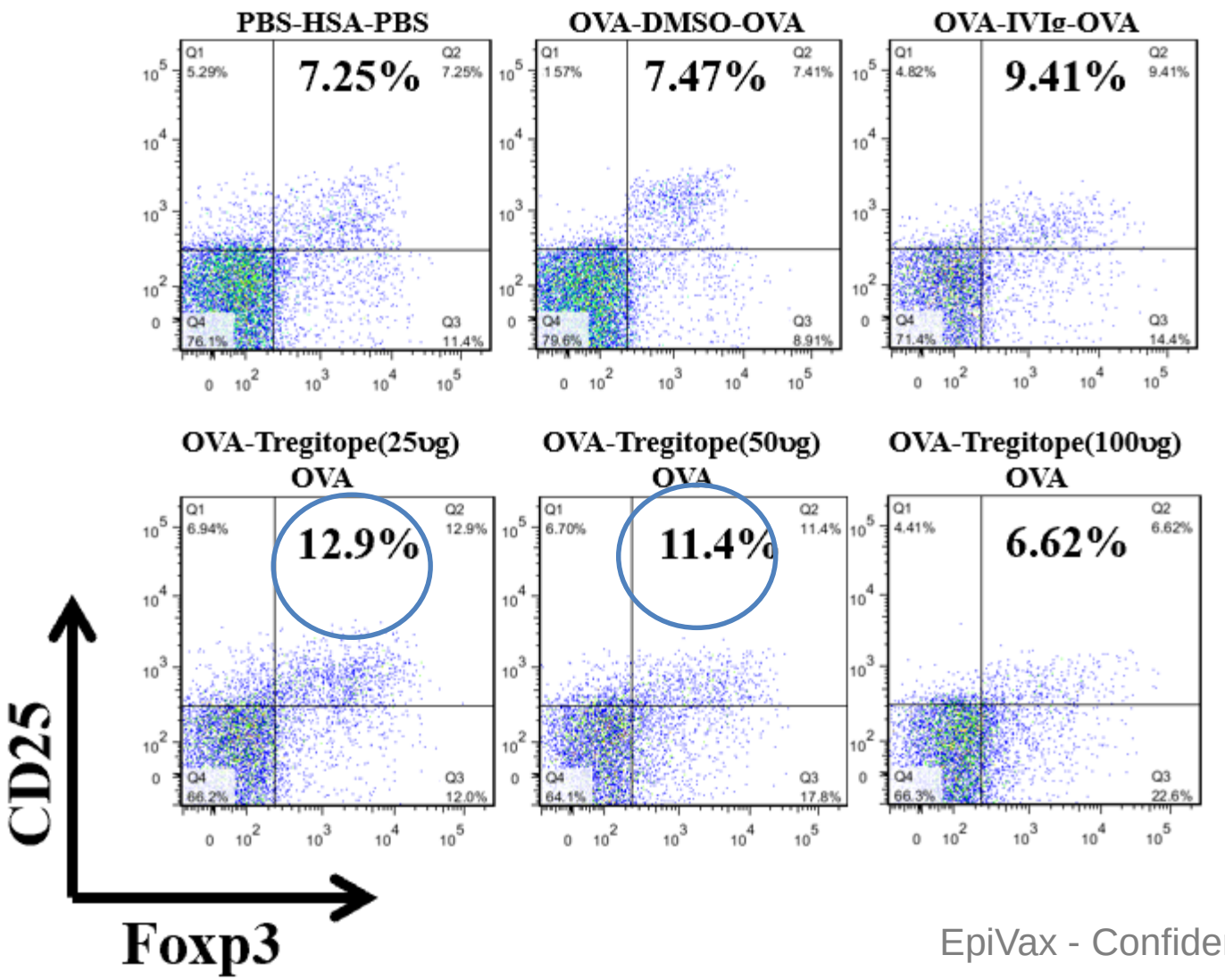


** p<0.01
*** p<0.001

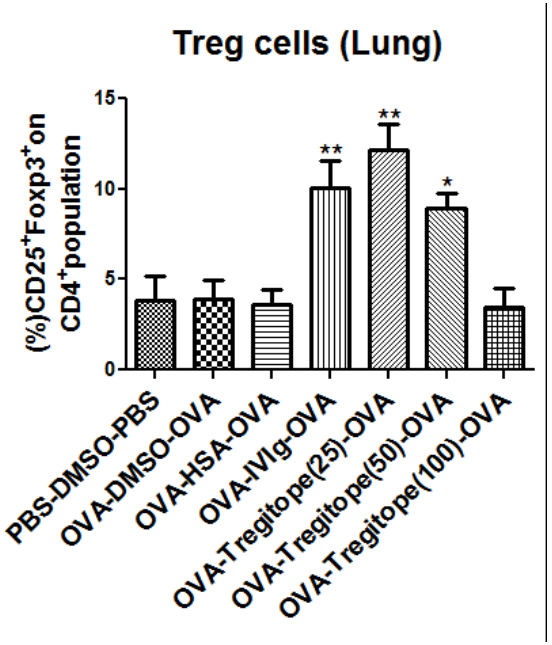
*immunization and challenge by PBS

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.

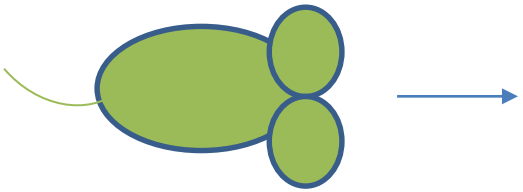
Tregitope treatment with Allergen
Do Tregitopes abrogate allergy responses?

Marieme Dembele PhD
Bruce Mazer MD
McGill University

Ragweed-induced model of asthma

EpiVax

Foxp3 GFP mice
C57/BL6



Ragweed model

Broncho-alveolar lavage (BAL)
Th2, Th1 and Th17 cytokines

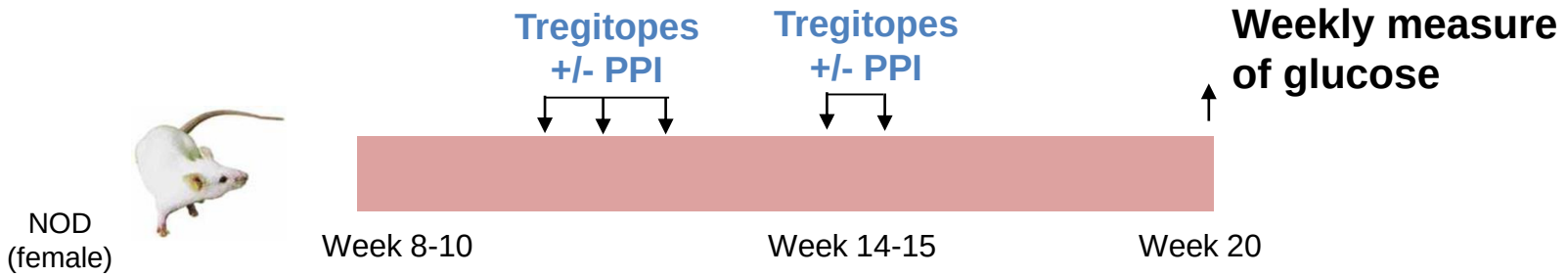
Lung homogenate:
Th2, Th1 and Th17 cytokines

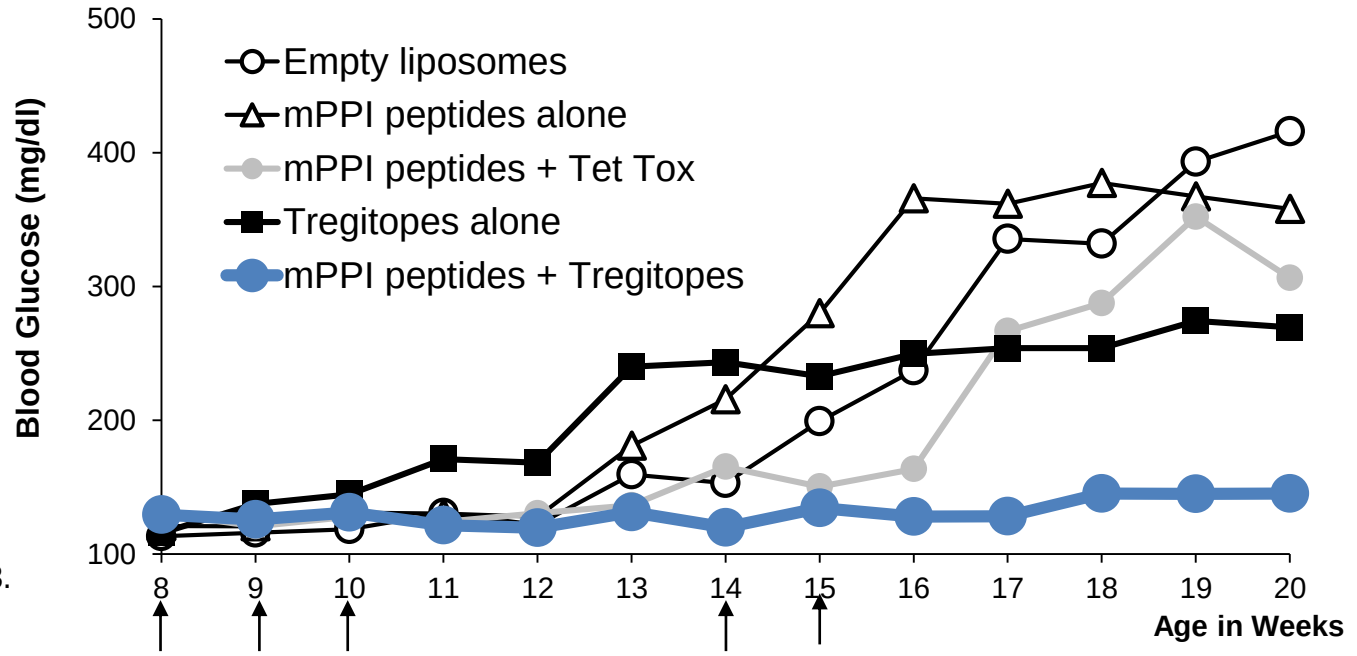
Phenotyping using flow cytometry
Granulocytes
T regulatory cells
Intracellular Cytokines production by CD4+ T cells

Histology

Blood:
IgE
Antigen-specific IgE

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



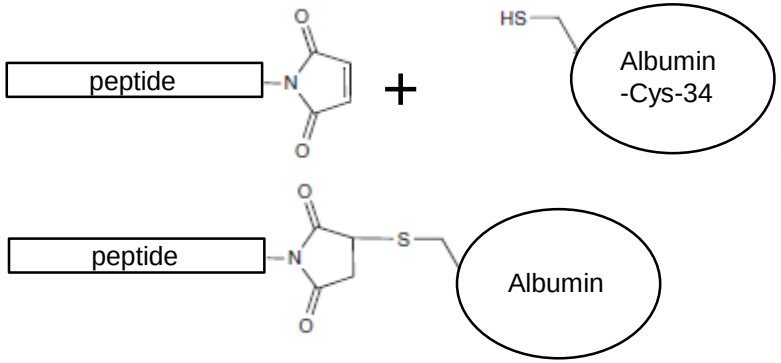


Cousens LP et al
J Diabetes Res.
2013;2013:621693.

T1D ASATI with Albumin-Tregitope Fusions

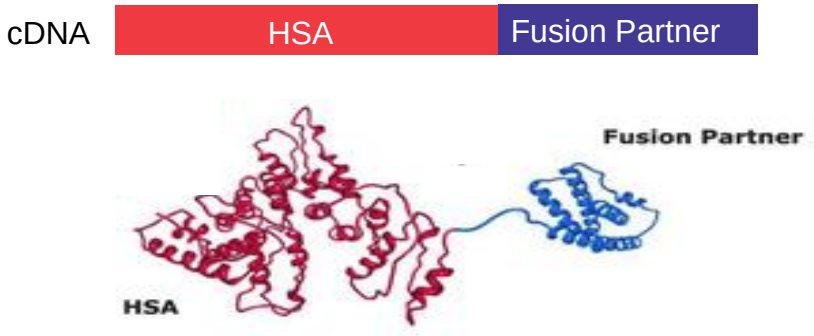
Bioconjugation

- 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



Human Serum Albumin causes Death in NOD mice (Yu et al.)

- NOD mice are intolerant of Human HSA.
- Of methods tested to overcome this issues, split dosing was the preferred.
- Split dosing was not required for Tregitope-HSA; the mice did not anaphylax.

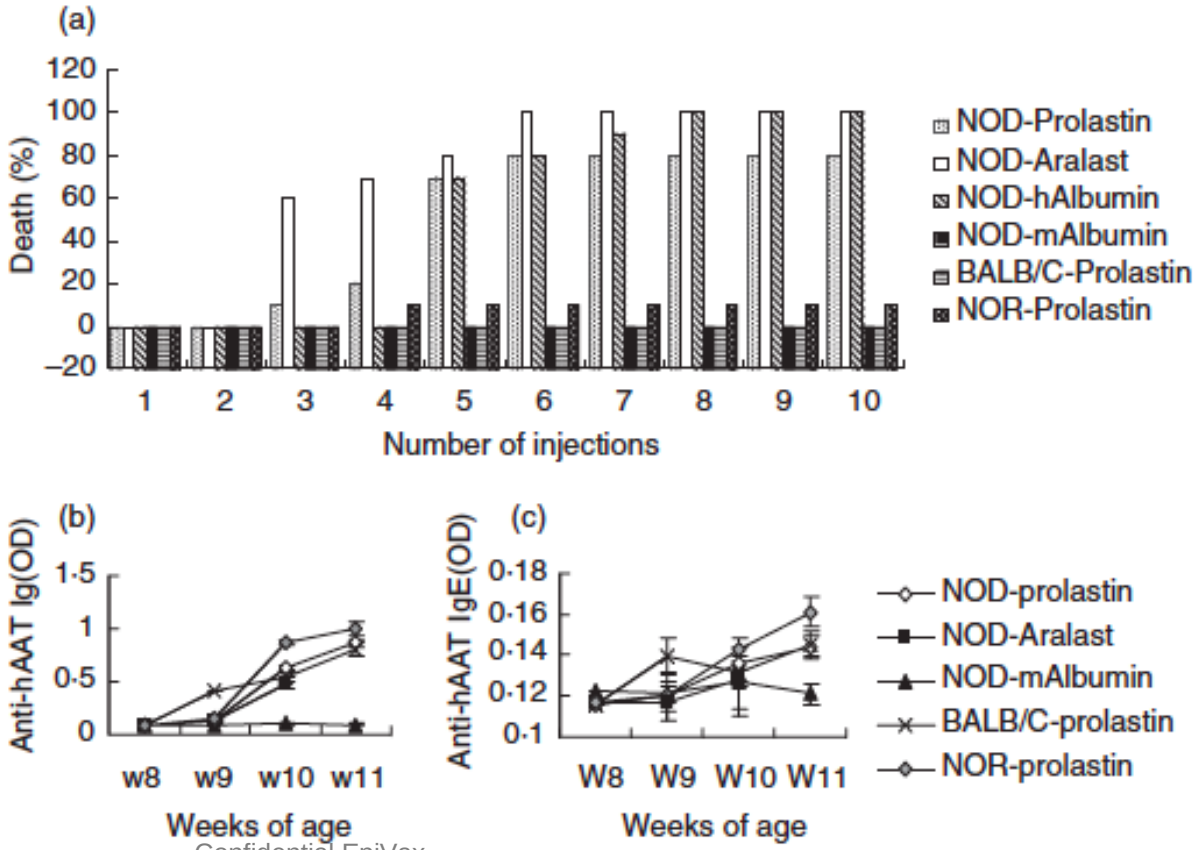
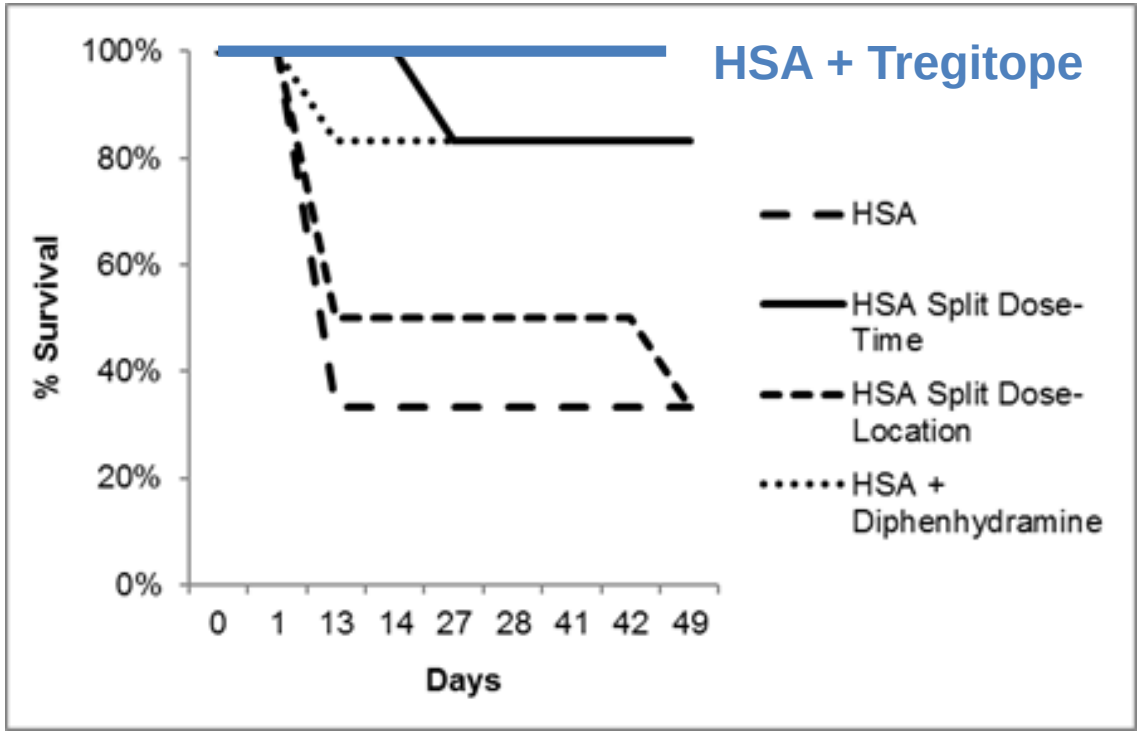


Fig. 2. Anaphylaxis is non-obese diabetic (NOD)-specific, but not human alpha-1 antitrypsin (hAAT)-specific. (a) Mortality rates in NOD, non-obese resistant and Balb/c mice (8 weeks of age) injected with hAAT (Prolastin® or Aralast®), human albumin (Albuminar®) or mouse albumin as indicated ($n = 10$, 2 mg/mouse, two injections per week); (b) anti-hAAT immunoglobulin G (IgG) levels. Each line represents the average optical density (OD) at 100× dilution; (c) anti-hAAT IgE levels. Each line represents the average OD at 50× dilution.

Kaplan-Meier survival curve for HSA Toxicity Pilot Study in NOD mice

NOD mice were injected s.c. with 800ug total dose of HSA.



- – – HSA single dose mice were injected (800ug/100uL) once at each time point (Day 0, 13, 27 ,41)
- ——— HSA split dose-time injections of 400ug/100uL (Day 0/1, 13/14, 27/28, 41/42)
- – – – HSA split dose-location of 400 ug/100uL (2 flanks; Day 0, 13, 27, 41)
- ····· HSA + diphenhydramine, where diphenhydramine was administered 15 minutes before each HSA single dose on Day 13, 27, 41.

T1D-ASATI: Tregitope-HSA fusion

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

EpiVax

HSA-Fusion E + PPI peptides

"Drug"

Immunizations:

Mice Enrolled	-1	0	3	7	10	14	17	21	24	28	31	35	38	42	45	49
	208	331	505	532	513	600	523	**600**	600	600	600	600	600	600	600	600
	278	243	184	182	131	171	168	157	129	129	129	129	129	129	129	129
	324	337	334	363	356	413	580	489	536	600	600	562	600	**572**	600	600
	337	294	295	461	511	463	374	390	213	245	198	203	130	196	228	239
	235	291	255	263	124	179	139	179	185	210	147	165	138	154	183	125
	230	286	125	155	176	192	177	136	143	114	140	130	139	126	148	157
	274	202	270	207	169	193	211	172	180	164	208	169	179	205	154	139
	246	273	294	146	261	365	209	222	126	211	171	119	187	160	203	123
	285	223	546	600	504	600	549	593	560	491	**416**	600	600	600	600	600
	355	348	266	310	286	418	264	173	232	257	217	150	167	146	204	131
	218	292	395	257	354	313	462	457	600	600	514	429	344	379	324	428
	336	292	340	324	365	494	445	576	600	600	376	**437**	600	600	600	600

HSA only

Immunizations:

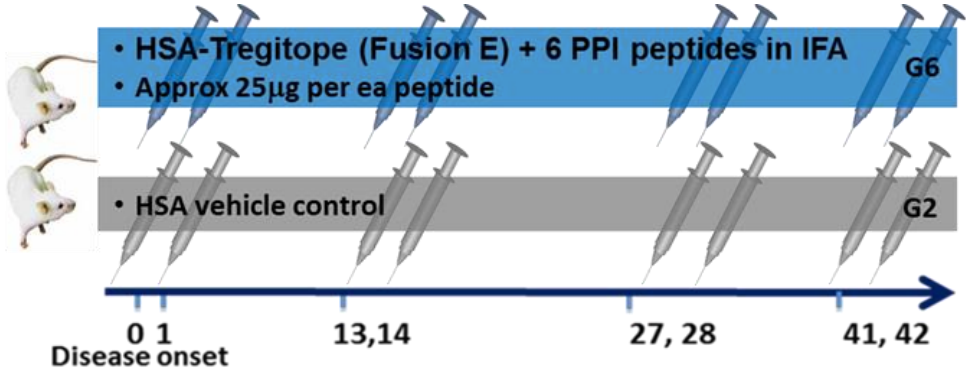
Mice Enrolled	-1	0	3	7	10	14	17	21	24	28	31	35	38	42	45	49
	208	275	115	132	98	105	103	157	104	118	105	116	97	93	94	106
	316	327	448	432	600	558	555	600	600	**419**	600	600	600	600	600	600
	314	342	558	600	600	600	600	600	549	**480**	600	600	600	600	600	600
	344	329	401	367	453	507	**600**	600	600	600	600	600	600	600	600	600
	342	324	600	600	600	593	**525**	600	600	600	600	600	600	600	600	600
	205	296	343	413	443	384	320	521	510	**137**	600	600	600	600	600	600
	235	332	346	184	185	188	564	600	434	573	600	**474**	600	600	600	600
	265	337	211	268	246	284	233	219	**167**	600	600	600	600	600	600	600
	200	249	131	402	492	551	572	600	**481**	600	600	600	600	600	600	600
	299	231	146	276	338	223	319	266	468	438	328	278	386	223	297	226
	202	343	367	557	457	556	544	589	410	374	600	600	600	600	600	600
	324	214	271	234	355	288	465	206	162	184	159	326	184	283	282	241

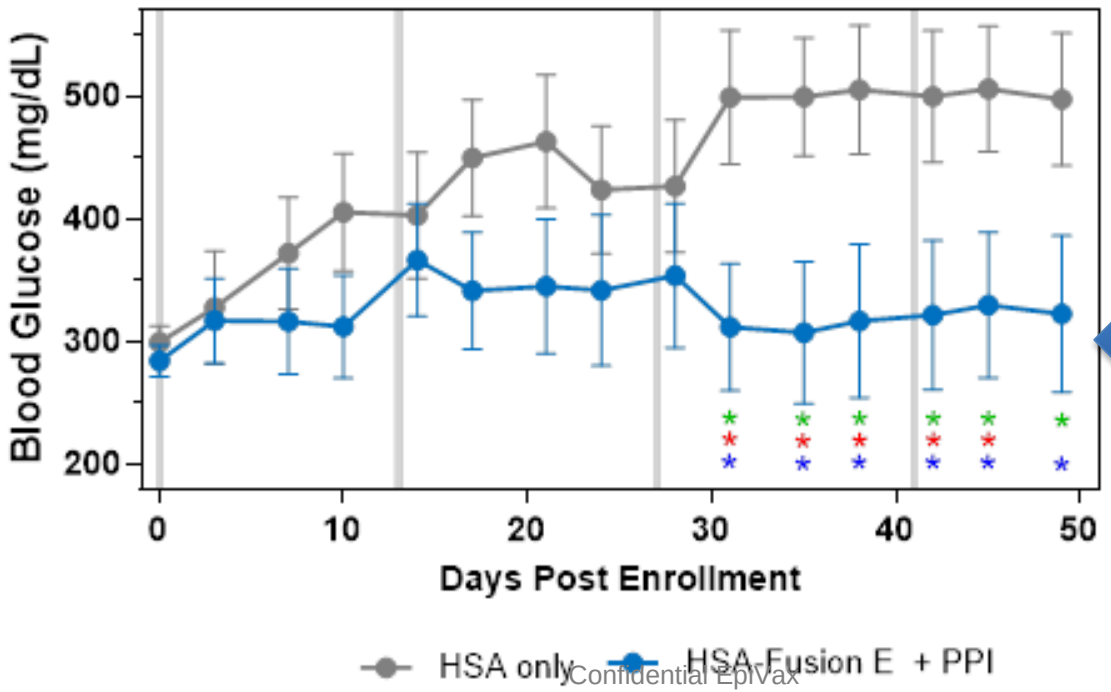
Blood glucose (mg/dL)

- <200
- 200-300
- 300-400
- 400-500
- 500-599
- 600 or greater

T1D-ASATI: Tregitope-HSA fusion

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





Mice alive at end of study

3/12

p<0.05
* nonparametric-diff
* mann-whitney
* ttest

8/12

"Drug"

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

Clinical trials and drug discovery

LUPUS SCIENCE & MEDICINE

Safety and efficacy of hCDR1 (Edratide) in patients with active systemic lupus erythematosus: results of phase II study

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ABSTRACT

Objective: To evaluate the safety and efficacy of hCDR1 (Edratide) in patients with systemic lupus erythematosus (SLE).

Methods: Patients (n=340) with SLE ≥4 ACR criteria (4–11, mean 7) with active disease (SLEDAI-2K of 6–12). Patients were on average 7.1 years post-diagnosis and their organ involvement was mainly musculoskeletal, mucocutaneous and haematologic. Placebo or Edratide was administered subcutaneously weekly at doses of 0.5, 1.0 or 2.5 mg. The co-primary endpoints were SLEDAI-2K SLE Disease Activity and Adjusted Mean SLEDAI (AMS) reduction in patients compared with controls using a landmark analysis. Secondary outcomes were improvement in British Isles Lupus Assessment Group (BILAG) Responder Index and medicinal flare analysis.

Results: Edratide was safe and well tolerated. The primary endpoints based solely on SLEDAI-2K and AMS were not met. The secondary predefined endpoint, BILAG, was met for the 0.5 mg Edratide arm in the intention to treat (ITT) cohort (N=316) (OR=2.09, p=0.03) with trends in the 1.0 and 2.5 mg doses. There was also a positive trend in the Composite SLE Responder Index of the ITT cohort. Post hoc analysis showed that the BILAG secondary endpoint was also met for the 0.5 mg Edratide for a number of subgroup dose levels, including low or no steroids, seropositivity and patients with 2 grade BILAG improvement.

Conclusions: The favourable safety profile and encouraging clinically significant effects noted in some of the endpoints support the need for additional longer term Edratide studies that incorporate recent advances in the understanding and treatment of SLE, including steroid treatment algorithms, and using a composite primary endpoint which is likely to include BILAG.

Trial registration number: NCT00203151.

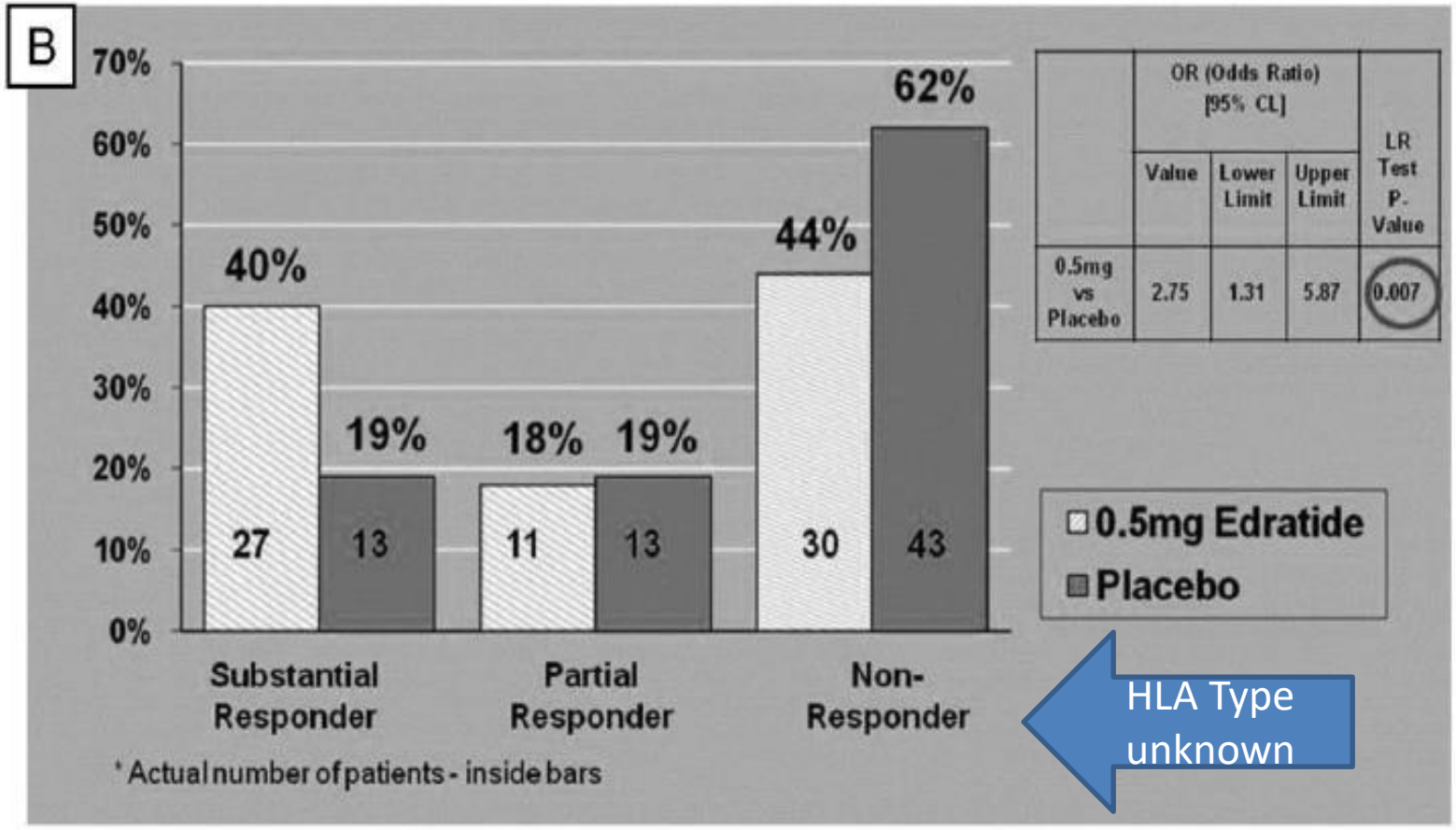
KEY MESSAGES

- Edratide demonstrated efficacy in one and possibly more clinically meaningful endpoints.
- Dose ranging studies demonstrated the 0.5 mg subcutaneous weekly was the most effective dose.
- There were no safety signals in this 26 week study.

Due to the complex nature of the disease, progress in developing new SLE treatment modalities has been slow.[9 10] Until the approval by the Food and Drug Administration in 2011 of belimumab,[11] no new drugs had been approved for the treatment of SLE since 1955. There is therefore a clear need for new therapeutic agents.[12]

hCDR1 (Edratide) is a novel synthetic peptide of 19 amino acid residues (H-G-Y-Y-W-S-W-I-R-Q-P-P-G-K-G-E-E-W-I) based on the complementarity-determining region 1 (CDR1) of a human anti-DNA mAb that expresses a major idiotype denoted 16/6 Id.[13 14] Treatment with hCDR1 leads to a cascade of events that culminate in the down-regulation of SLE-associated autoreactive T and B cells and in the clinical amelioration of lupus. hCDR1 is therefore a candidate for treatment of patients with SLE.[15]

In mouse models of SLE, treatment with hCDR1 significantly reduced immune complex deposits in the kidney, and resulted in improvement in proteinuria and leucopenia. The treatment downregulated



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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.

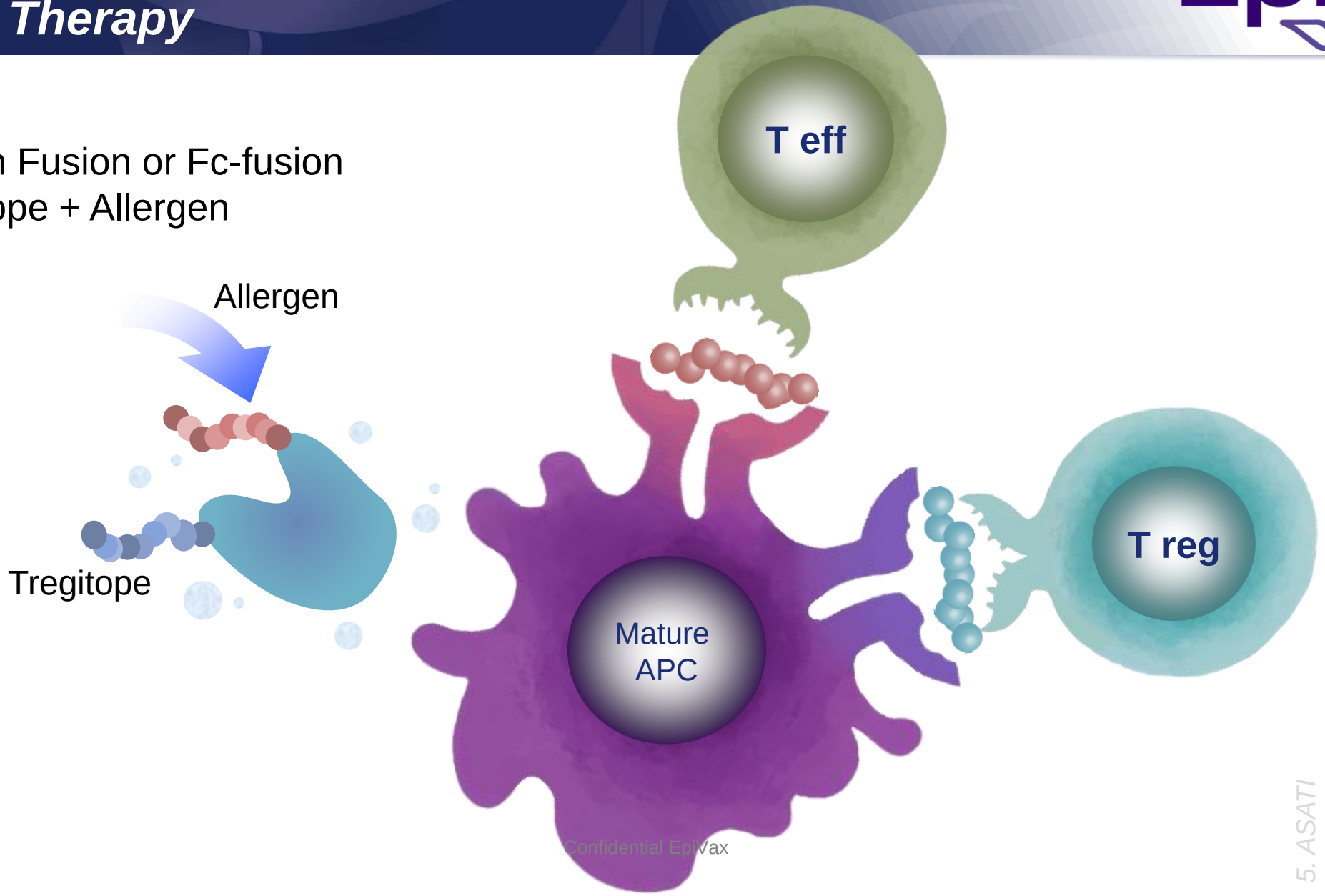
Outline

EpiVax

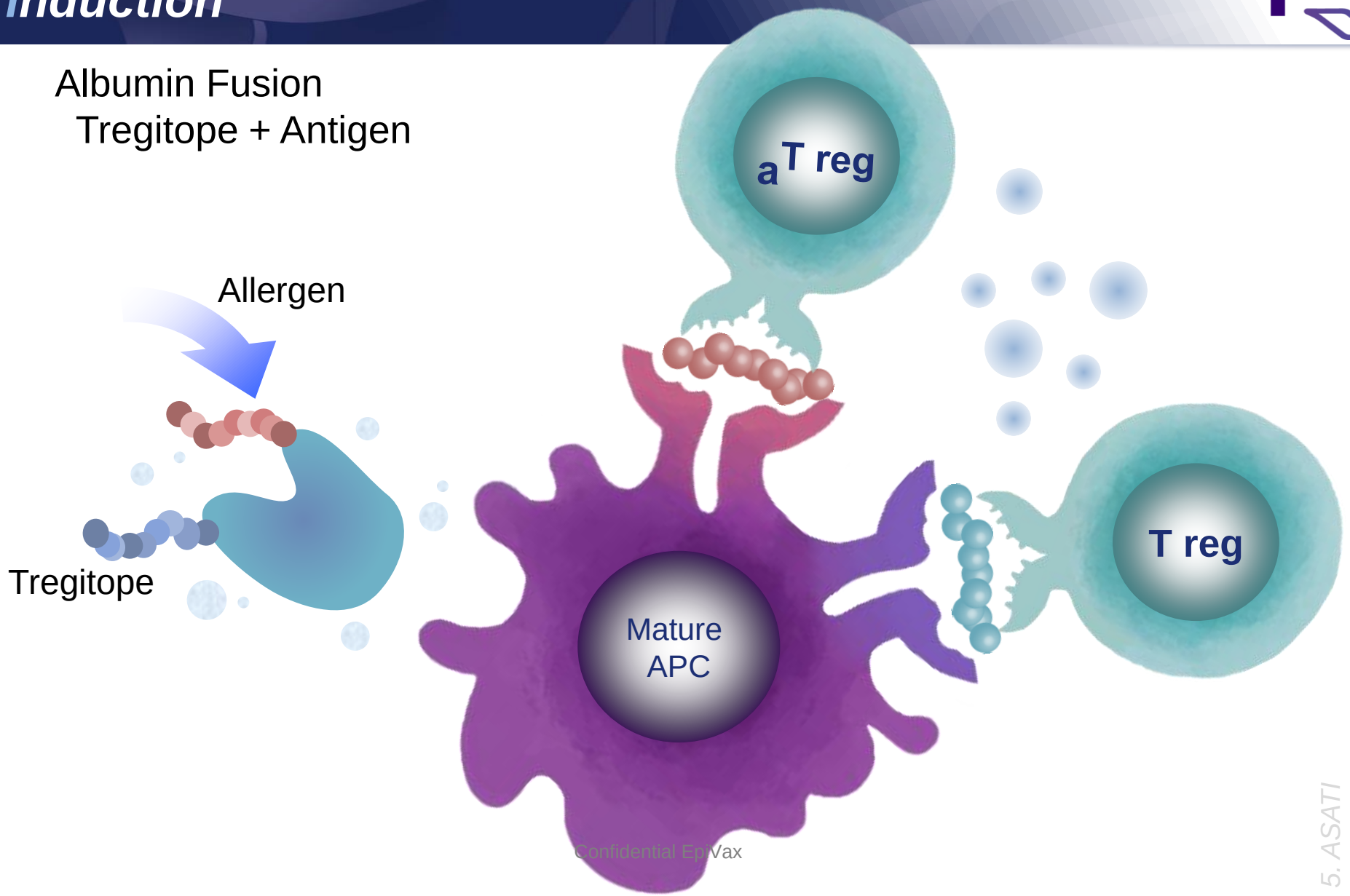
- ✓ Tregitopes – what are they?
  - ✓ Mechanism of Action
- ✓ Pre-clinical studies with Tregitopes…
- ✓ Have Tregitopes been in the clinic?
- ✓ **What's the plan for Tregitopes?**

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

Albumin Fusion or Fc-fusion
Tregitope + Allergen



Tregitope ASATI : *Antigen Specific Adaptive Tolerance Induction*

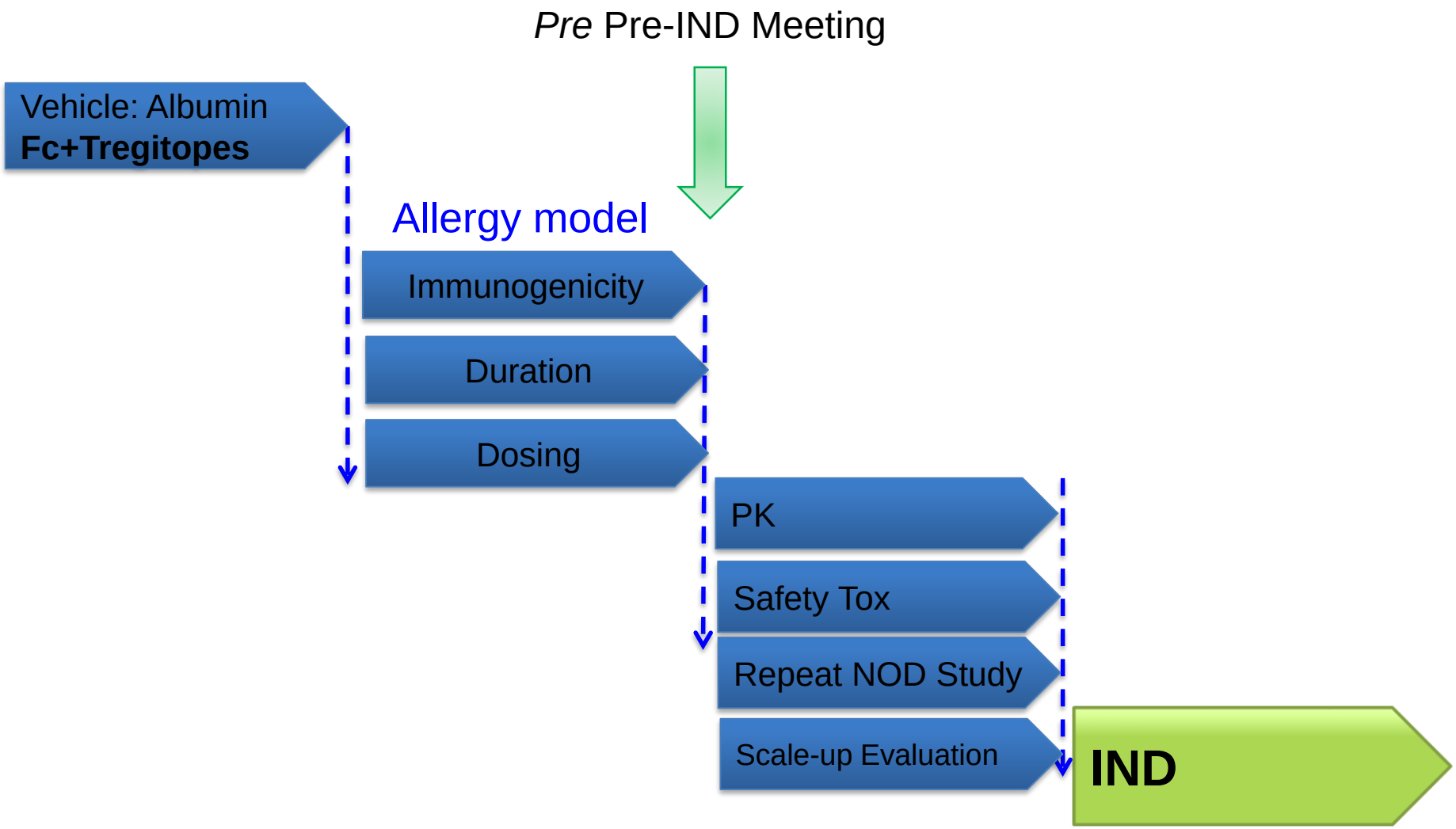


Tregitope-Fusion
Plans for Transition to Clinic

- **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**

Next Plan Tregitopes . . .
Allergy Program In Preparation



Tregitope Applications
Translating Antigen-specific tolerance to the clinic

EpiVax

Localized, specific suppression of immune response:

- **IVIg Replacement therapy**

  Tregitope Fusion Product (TFP) in development/ ready for option/license
- **Combination Therapy (ASATI) For Allergy / Spin out company ?**
- **Autoimmune diseases/Transplant** (type 1 diabetes, multiple sclerosis) --- Option/License available except for peptides/alopecia areata/derm applications, but no restrictions for the Alb or Fc Fusion.
- **Tolerization of antibodies**: introducing Tregitopes into the sequence with minimal point mutations (licenses available)
- **Tolerization of protein therapeutics**:
  co-expression or co-administration with immunogenic proteins --- Currently have Option/License for protein fusion in FVIII.

Science without fear.

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