The Road to Using Engineered Regulatory T and Cytotoxic T-cells with Chimeric Antigen Receptors
Mechanisms of Inhibitor Development: The Role of B-cells and T-cells and a Novel Therapeutical Approach

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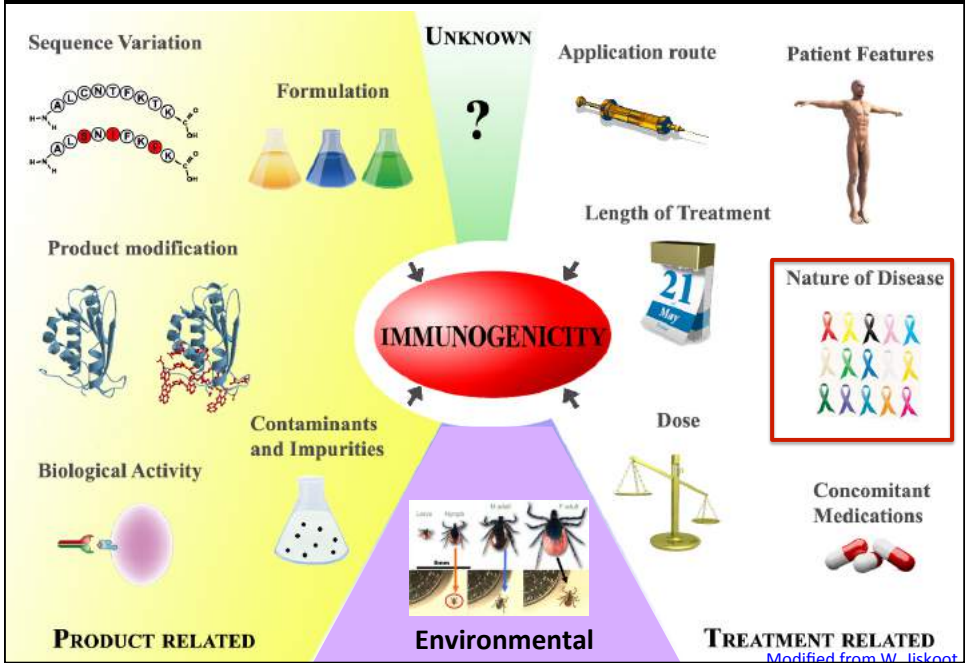
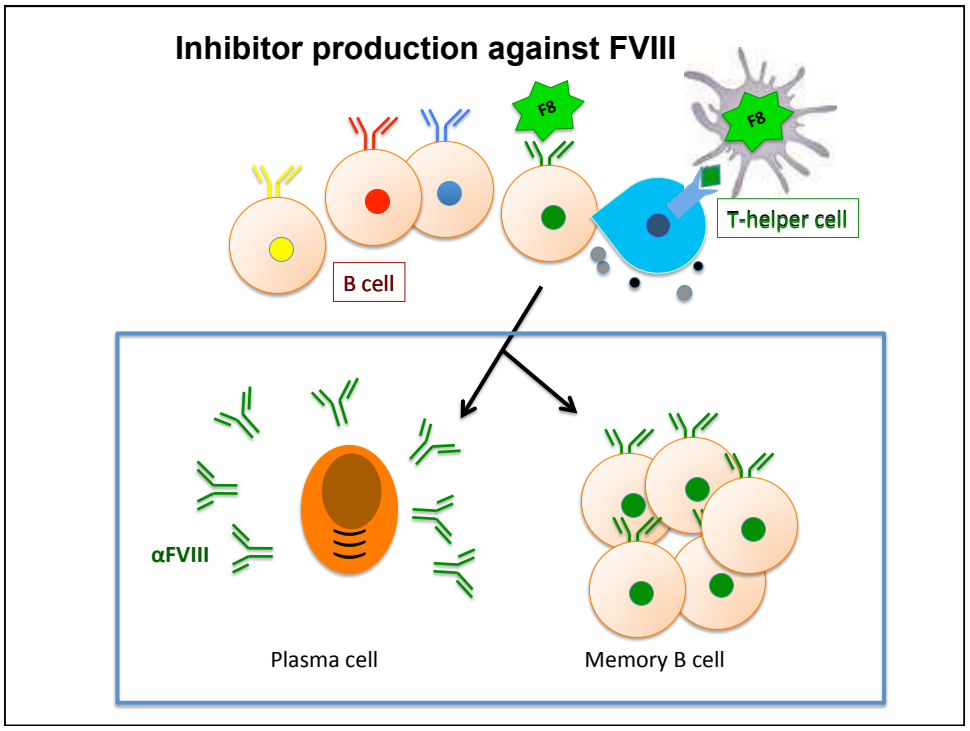
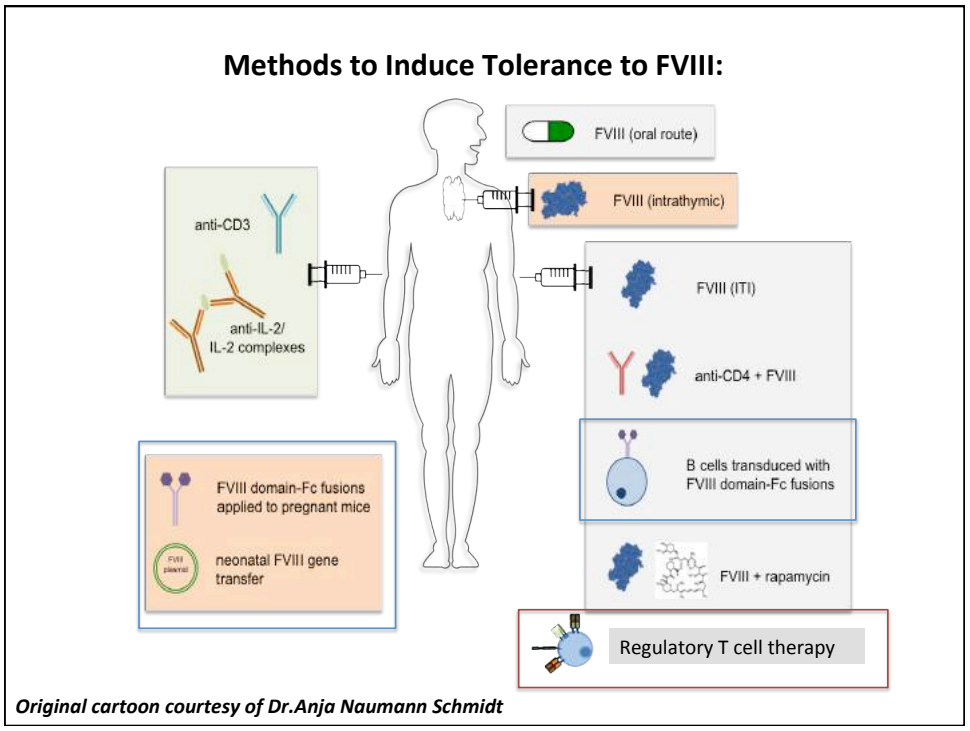
"USU is America's Medical School"

Factors Affecting the Immune Response to FVIII



Modified from W. Jiskoot


Inhibitor production against FVIII
FB
FB
T-helper cell
B cell
αFVIII
Plasma cell
Memory B cell

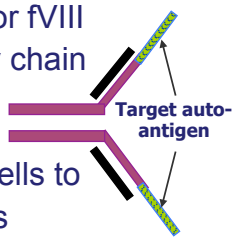

Methods to Induce Tolerance to FVIII:
FVIII (oral route)
FVIII (intrathymic)
anti-CD3
anti-IL-2/
IL-2 complexes
FVIII (ITI)
anti-CD4 + FVIII
B cells transduced with
FVIII domain-Fc fusions
FVIII domain-Fc fusions
applied to pregnant mice
neonatal FVIII gene
transfer
FVIII + rapamycin
Regulatory T cell therapy
Original cartoon courtesy of Dr.Anja Naumann Schmidt

History of B-cell Delivered Gene Therapy for Tolerance

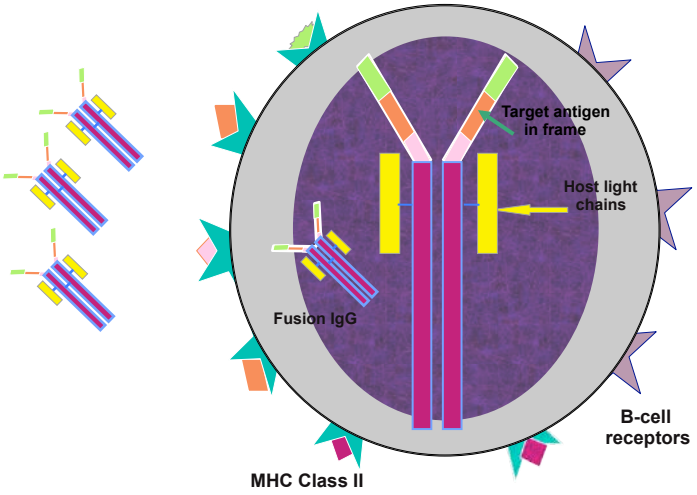
- We developed a platform technique for tolerance induction to multiple epitopes expressed at the N-terminus of an IgG scaffold, and transduced B cells to express this fusion IgG.
- "Pre-clinical efficacy" has been achieved in rodent models for multiple diseases, including EAE (MS), EAU (uveitis), type 1 diabetes and hemophilia.
- Tolerance, so induced, is specific, long-lasting, affects both Th1 and Th2 CD4 T cells and is more effective and long-lasting on the IgG carrier.
- **CD25+ (FoxP3+) regulatory T cells are involved in both the induction and the maintenance of tolerance, and B-cell recruitment of Tregs may be critically dependent on the presence of unique epitopes in the IgG and Fc receptors.**

Key strategies in our studies

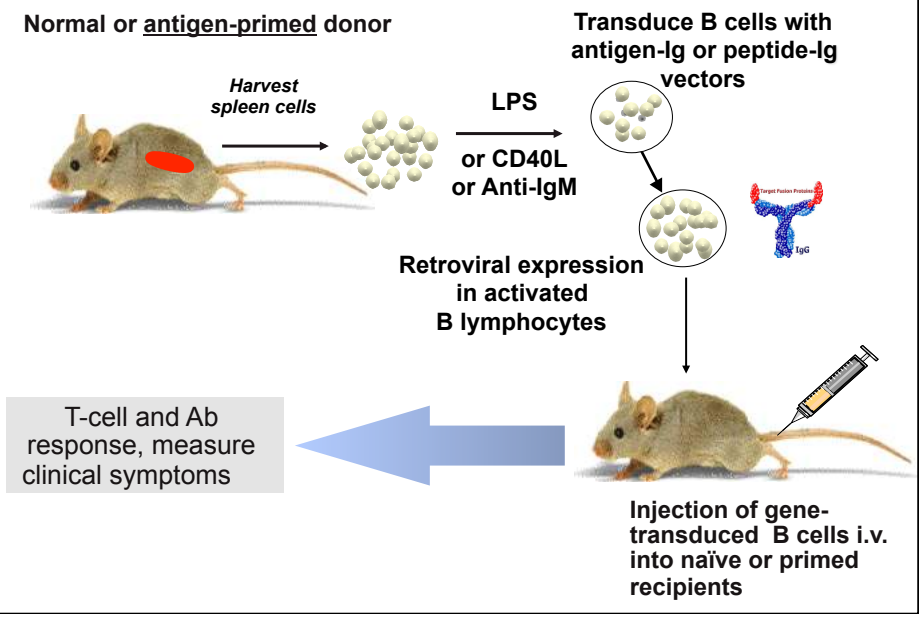
- Use isologous immunoglobulins as carriers based on the tolerogenicity of IgG (Borel, Weigle)
- Engineer target protein, *e.g.*, autoantigens or fVIII domains, at the N-terminus of an IgG heavy chain scaffold
- Transduce myeloma cells or "activated" B cells to produce or present fusion protein and act as tolerogenic APC, respectively.

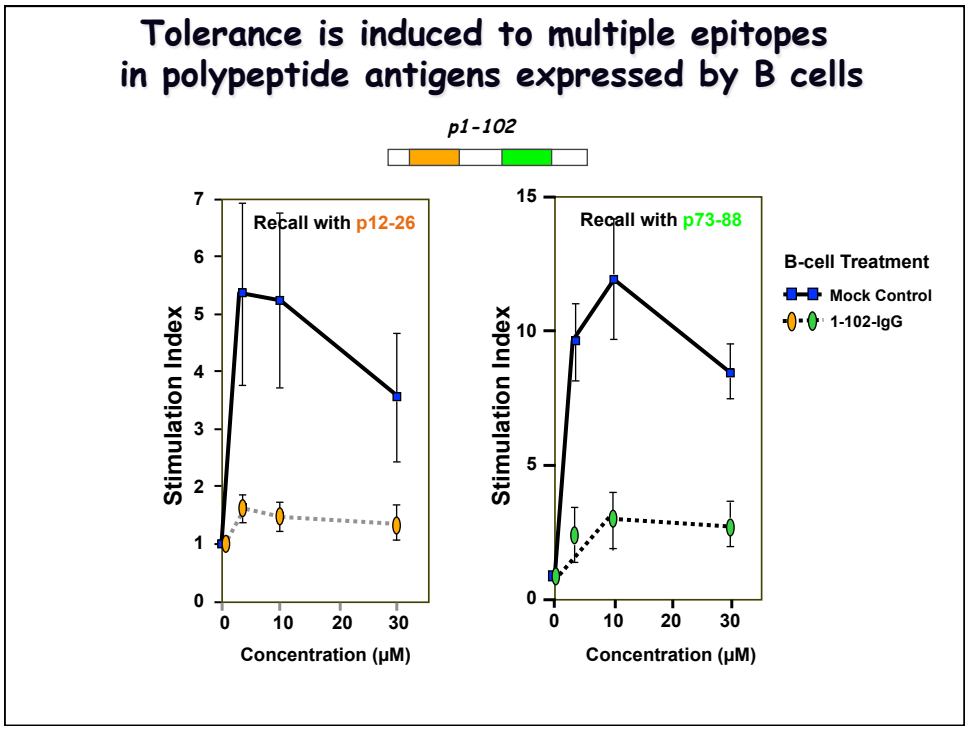


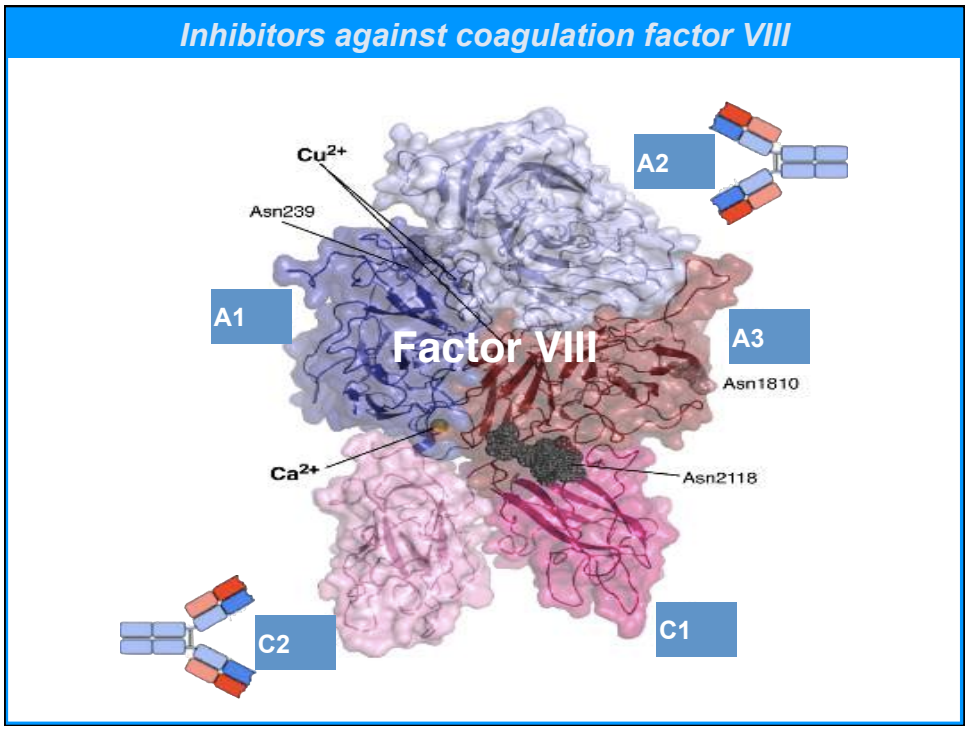
Production and Presentation of IgG Fusion Protein in B Cells



Gene therapy protocol for B-cell delivered tolerance




Tolerance is induced to multiple epitopes
in polypeptide antigens expressed by B cells
p1-102
Recall with p12-26
Recall with p73-88
Stimulation Index
Concentration (μM)
B-cell Treatment
Mock Control
1-102-IgG


Inhibitors against coagulation factor VIII
Cu2+
Asn239
A1
A2
A3
Factor VIII
Asn1810
Ca2+
Asn2118
C1
C2

Plenary Paper

CLINICAL TRIALS AND OBSERVATIONS

Phase 3 study of recombinant factor VIII Fc fusion protein in severe hemophilia A

Johnny Mahlangu,[1] Jerry S. Powell,[2] Margaret V. Ragni,[3] Pratima Chowdary,[4] Neil C. Josephson,[5] Ingrid Pabinger,[6] Hideji Hanabusa,[7] Naresh Gupta,[8] Roshni Kulkarni,[9] Patrick Fogarty,[10] David Perry,[11] Amy Shapiro,[12] K. John Pasi,[13] Shashikant Apte,[14] Ivan Nestorov,[15] Haiyan Jiang,[15] Shuanglian Li,[15] Srividya Neelakantan,[15] Lynda M. Cristiano,[15] Jaya Goyal,[15] Jurg M. Sommer,[15] Jennifer A. Dumont,[15] Nigel Dodd,[15] Karen Nugent,[15] Gloria Vigliani,[15] Alvin Luk,[15] Aoife Brennan,[15] and Glenn F. Pierce,[15] for the A-LONG Investigators

[1]Haemophilia Comprehensive Care Centre, Faculty of Health Sciences, University of the Witwatersrand and National Health Laboratory Service,

Krishnamoorthy et al. Recombinant Factor VIII Fc (rFVIIIFc) Fusion Protein Reduces Immunogenicity and Induces Tolerance in Hemophilia A Mice. Cellular Immunology *in press (2016)*

Hospital, Department of Hematology, Mahara, India, and Biogen Idec Hemophilia, Cambridge, MA

Key Points

- A novel recombinant factor VIII with prolonged half-life, rFVIIIFc, was developed to reduce prophylactic injection frequency.
- rFVIIIFc was well-tolerated in patients with severe hemophilia A, and resulted in low bleeding rates when dosed 1 to 2 times per week.

This phase 3 pivotal study evaluated the safety, efficacy, and pharmacokinetics of a recombinant FVIII Fc fusion protein (rFVIIIFc) for prophylaxis, treatment of acute bleeding, and perioperative hemostatic control in 165 previously treated males aged ‡12 years with severe hemophilia A. The study had 3 treatment arms: arm 1, individualized prophylaxis (25-65 IU/kg every 3-5 days, n ≤ 118); arm 2, weekly prophylaxis (65 IU/kg, n ≤ 24); and arm 3, episodic treatment (10-50 IU/kg, n ≤ 23). A subgroup compared recombinant FVIII (rFVIII) and rFVIIIFc pharmacokinetics. End points included annualized bleeding rate (ABR), inhibitor development, and adverse events. The terminal half-life of rFVIIIFc (19.0 hours) was extended 1.5-fold vs rFVIII (12.4 hours; $P < .001$). Median ABRs observed in arms 1, 2, and 3 were 1.6, 3.6, and 33.6, respectively. In arm 1, the median weekly dose was 77.9 IU/kg; approximately 30% of subjects achieved a 5-day dosing interval (last 3 months on study). Across arms, 87.3% of bleeding episodes resolved with 1 injection. Adverse events were consistent with those expected in this population; no subjects developed inhibitors. rFVIIIFc was well-tolerated, had a prolonged half-life compared with rFVIII, and resulted in low ABRs when dosed prophylactically 1 to 2 times per week. This trial was registered at www.clinicaltrials.gov as #NCT01181128. (Blood. 2014;123(3):317-325)

Reduction of Factor VIII Inhibitor Titers During Immune Tolerance Induction With Recombinant Factor VIII-Fc Fusion Protein

C. Groomes et al. (Pediatrics, USUHS) *Pediatric Blood and Cancer,* in press.

Pediatr Blood Cancer

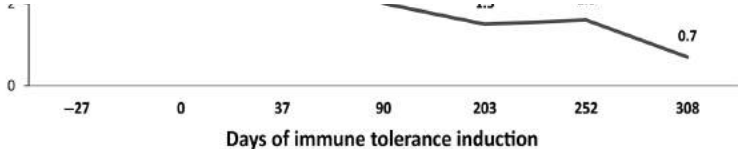
BRIEF REPORT

Reduction of Factor VIII Inhibitor Titers During Immune Tolerance Induction With Recombinant Factor VIII-Fc Fusion Protein

Charles L. Groomes, MD,[1,2] David M. Gianferante, MD,[2] Gary D. Crouch, MD, MSHA,[1,2] Dina S. Parekh, MD,[1,2] David W. Scott, PhD,[1] and Kenneth Lieuw, MD, PhD[1,2]*

The development of inhibitors toward factor VIII (FVIII) is a common and serious complication of hemophilia A (HA) therapy. Patients with hemophilia who develop inhibitors often undergo time- and resource-intensive immune tolerance induction (ITI) protocols. We report a 15-month-old male with severe HA and a high-titer inhibitor that occurred while receiving prophylactic treatment with recombinant FVIII (rFVIII), in whom significant inhibitor titer reduction was achieved with thrice weekly infusions of a new, prolonged half-life rFVIII-Fc fusion protein product (trade name *Eloctate*). Further studies are warranted to explore the potential of *Eloctate* in ITI protocols. Pediatr Blood Cancer

Key words: eloctate; factor VIII inhibitors; hemophilia A; immune tolerance induction; recombinant factor VIII-Fc fusion protein



RESEARCH ARTICLE

IMMUNOLOGY

Regulation of immune responses to protein therapeutics by transplacental induction of T cell tolerance

Nimesh Gupta,[1,2,3] Slobodan Culina,[4,5,6] Yann Meslier,[1,2,3] Jordan Dimitrov,[1,2,3] Christophe Arnoult,[7] Sandrine Delignat,[1,2,3] Bagirath Gangadharan,[1,2,3] Maxime Lecerf,[1,2,3] Sune Justesen,[8] Valérie Gouilleux-Gruart,[7,9] Benoit L. Salomon,[3,10] David W. Scott,[11] Srinivas V. Kaveri,[1,2,3,12] Roberto Mallone,[4,5,6,13] Sébastien Lacroix-Desmazes[1,2,3,12]*

Central tolerance plays a key role in modulating immune responses to self and exogenous antigens. The absence of self-antigen expression, as in patients with genetic deficiencies, prevents the development of antigen-specific immune tolerance. Hence, a substantial number of patients develop neutralizing antibodies to the corresponding protein therapeutics after replacement treatment. In this context, the administration of missing antigens during fetal development, a key period for self-tolerance establishment, should confer early and long-lasting antigen-specific tolerance. To this end, we exploited the physiological pathway of the neonatal Fc receptor (FcRn) through which maternal immunoglobulins are transplacentally transferred to fetuses. We demonstrate that Fc-fused antigens administered to pregnant mice reach fetal lymphoid organs in an FcRn-dependent manner, accumulate in antigen-presenting cells of myeloid origin, and promote the generation of both thymic and peripheral antigen-specific regulatory T cells. This strategy was successfully pursued in a mouse model of hemophilia A, where maternofetal transfer of the Fc-fused immunodominant domains of coagulation factor VIII conferred antigen-specific tolerance. Transplacental tolerance induction with Fc-fused proteins may thus prove valuable to prevent alloimmunization after replacement protein therapy for congenital deficiencies.

Gupta et alia. ScienceTranslationalMedicine.org 2015 Vol 7 Issue 275: 275

B-cell Delivered Gene Therapy for Tolerance

- We developed a platform technique for tolerance induction to multiple epitopes expressed at the N-terminus of an IgG scaffold, and transduced B cells to express this fusion IgG.
- "Pre-clinical efficacy" has been achieved in rodent models for multiple diseases, including EAE (MS), EAU (uveitis), type 1 diabetes and hemophilia.
- Tolerance, so induced, is specific, long-lasting, affects both Th1 and Th2 CD4 T cells and is more effective and long-lasting on the IgG carrier.
- CD25+ (FoxP3+) regulatory T cells are involved in both the induction and the maintenance of tolerance, and B-cell recruitment of Tregs may be critically dependent on the presence of unique epitopes in the IgG and Fc receptors.

Rationale and approach:

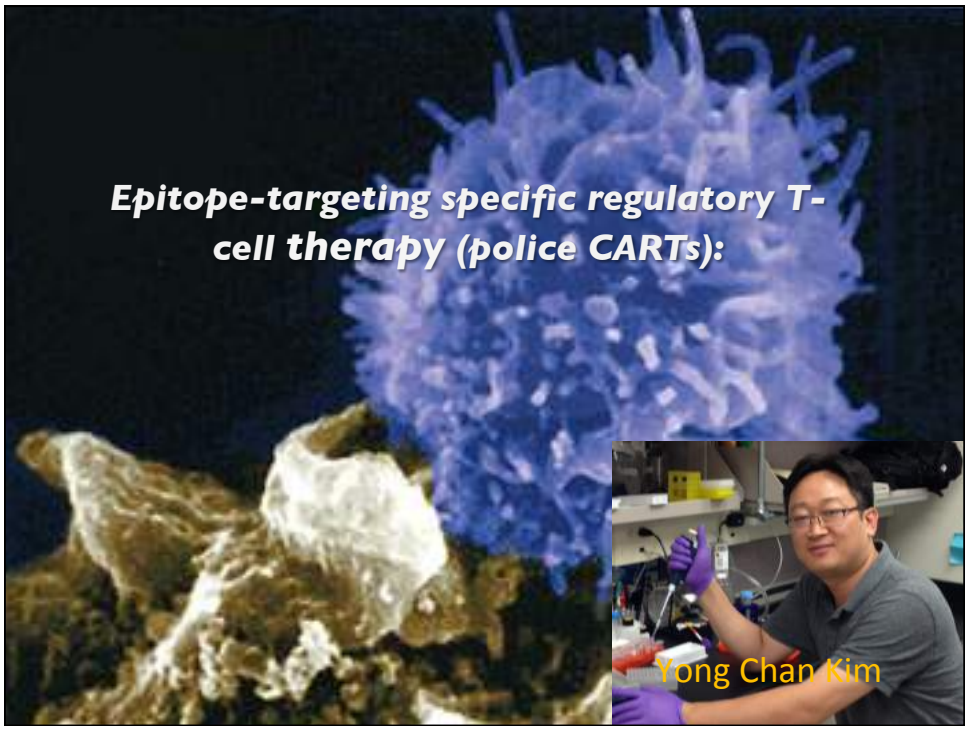
Regulatory T cell therapy has been proposed to treat autoimmune diseases as well as to suppress undesirable immune responses against bio-therapeutics, but polyclonal Tregs may be non-specifically immunosuppressive

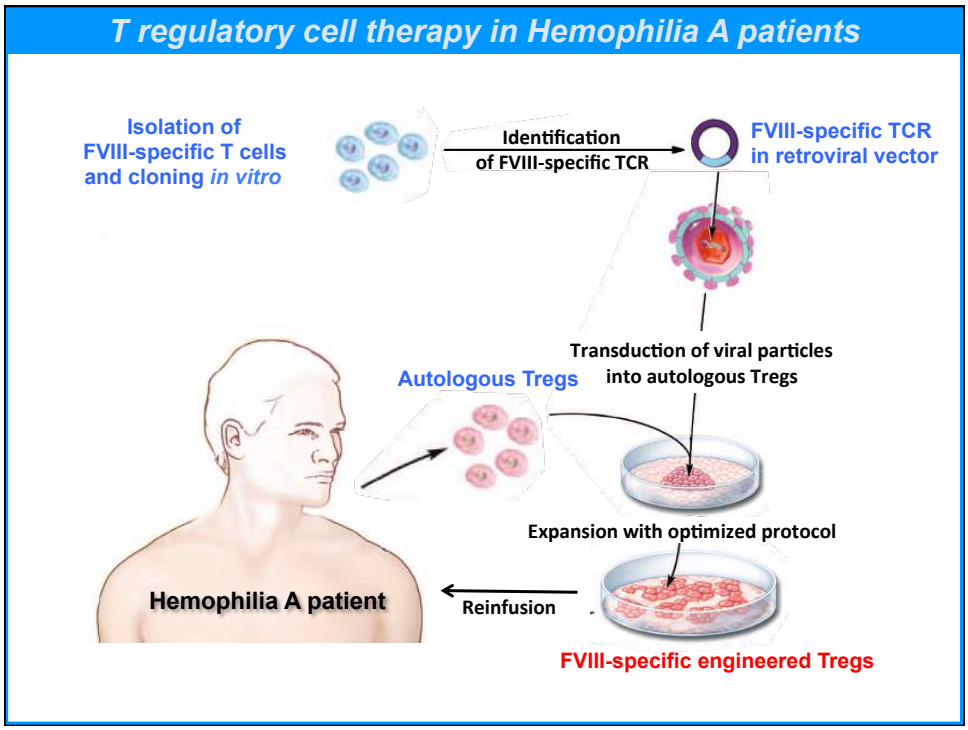
Our approach:

To create *specific* regulatory T cells recognizing target peptides using T cell receptors derived from patient clones (CARTs), as well as single chain chimeric antigen receptors (scFv, CARs)

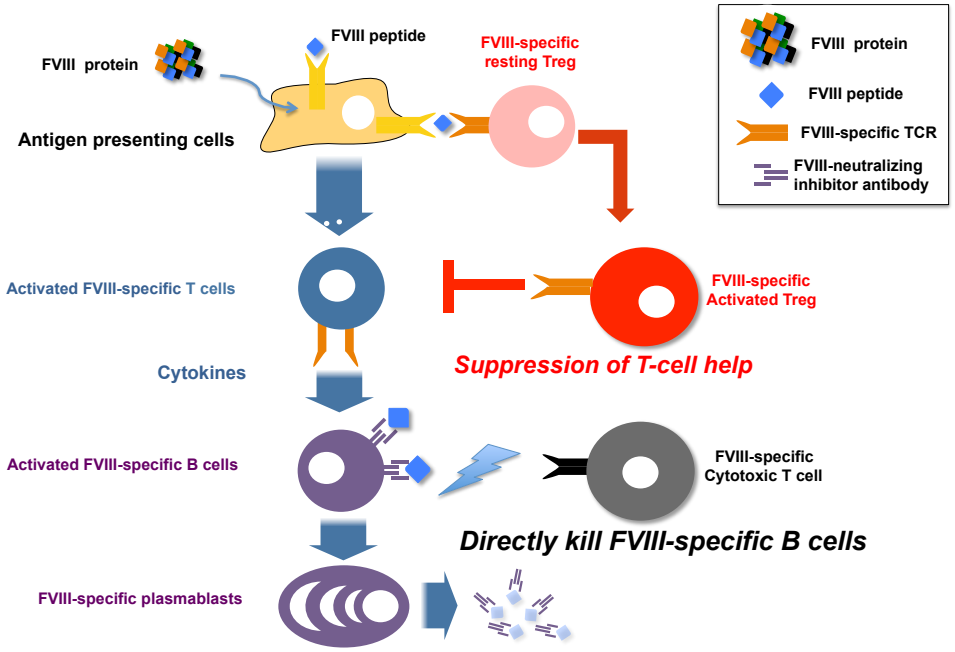
Two targets: MBP in multiple sclerosis

FVIII in hemophilia A

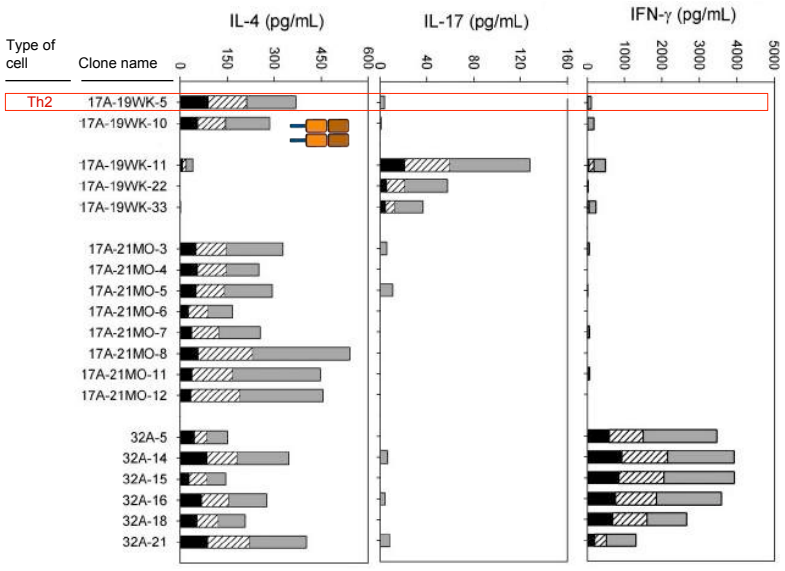

Epitope-targeting specific regulatory T-cell therapy (police CARTs):
Yong Chan Kim


T regulatory cell therapy in Hemophilia A patients
Isolation of FVIII-specific T cells and cloning in vitro
Identification of FVIII-specific TCR
FVIII-specific TCR in retroviral vector
Transduction of viral particles into autologous Tregs
Autologous Tregs
Expansion with optimized protocol
Hemophilia A patient
Reinfusion
FVIII-specific engineered Tregs

How can we prevent or reverse FVIII-specific 'inhibitor " formation?



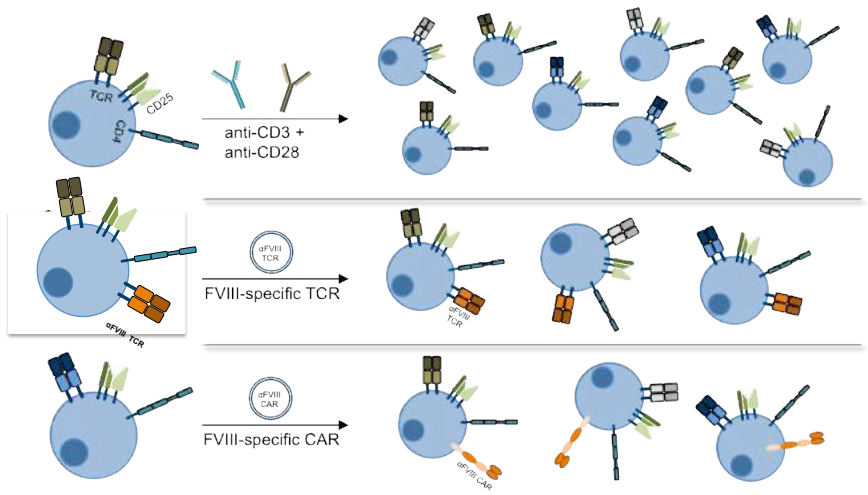
FVIII-specific T cell clones as a source of TCR V regions



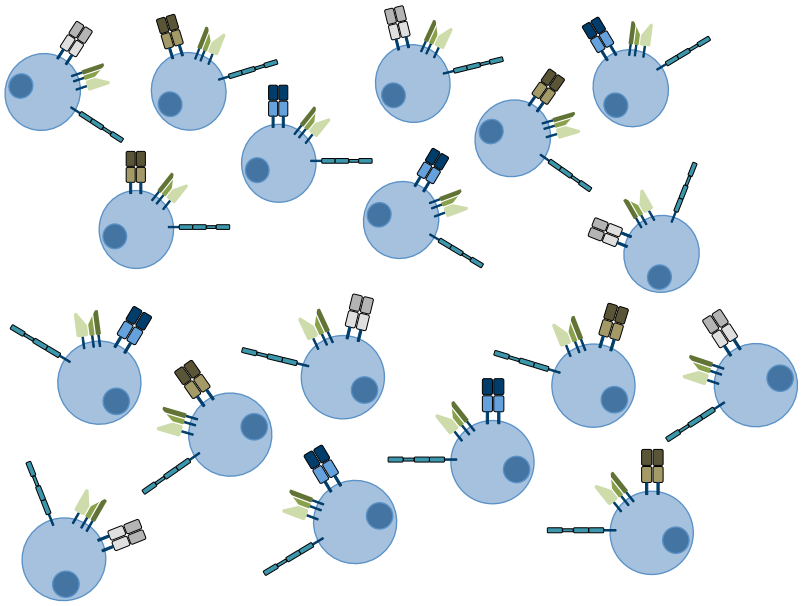
Clone 17195 recognizes a DR1-restricted peptide in the C2 domain of FVIII

Ettinger *et al*. Blood. 2009 Aug 13;114(7):1423-1428.

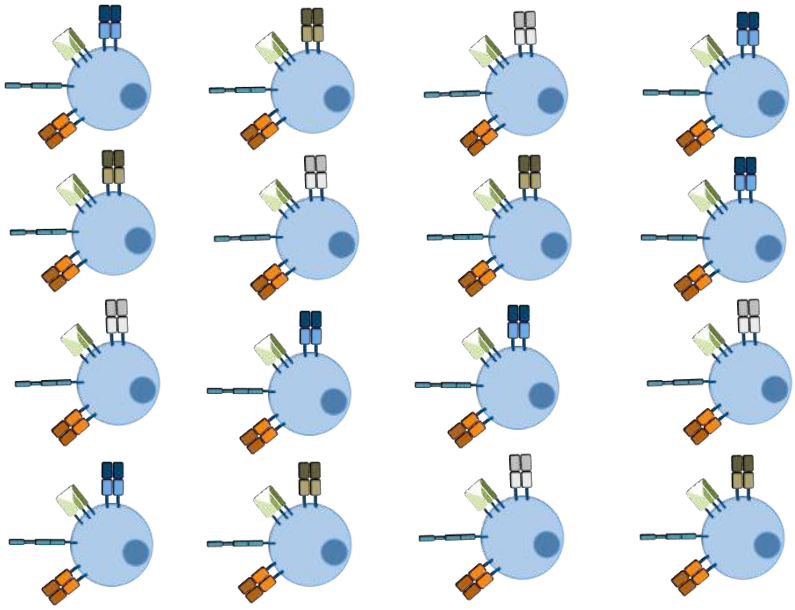
Engineered T-cell receptors or chimeric antigen receptors versus polyclonal Tregs



Normal polyclonal CD25⁺ T regulatory cells



Expanded FVIII-specific TCR-transduced polyclonal Tregs



FVIII-specific TCR (17195TCR)

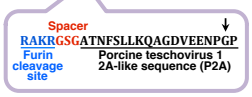
Recombinant 17195 TCR for retroviral expression

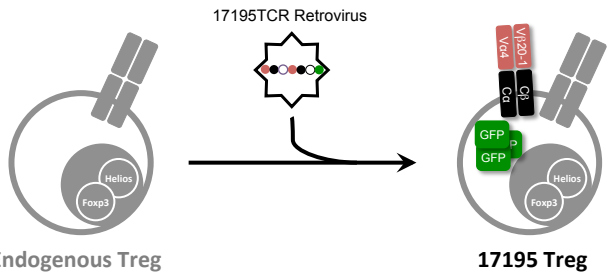
TCR gene usage:

TRAV	TRAJ	Junction	TRBV	TRBD	TRBJ	Junction
4*01	29*01	CLVGDAPNSGNTPLVS	20-1*05	1*01	1-2*01	CSAHTRANYGYTF

Construct structure:

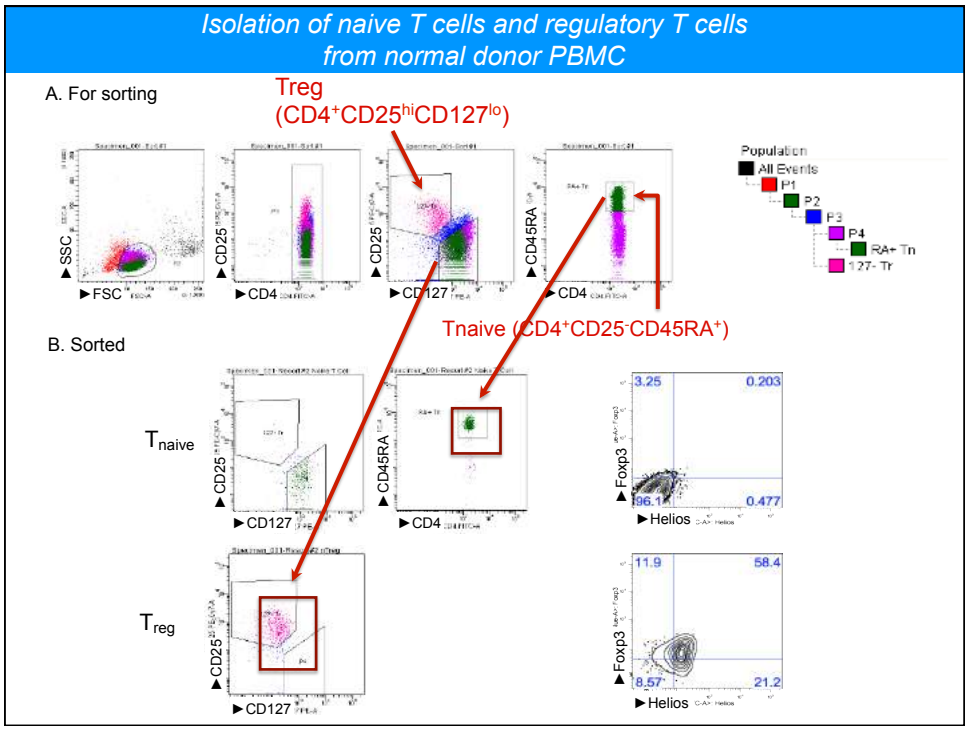
P2A structure:

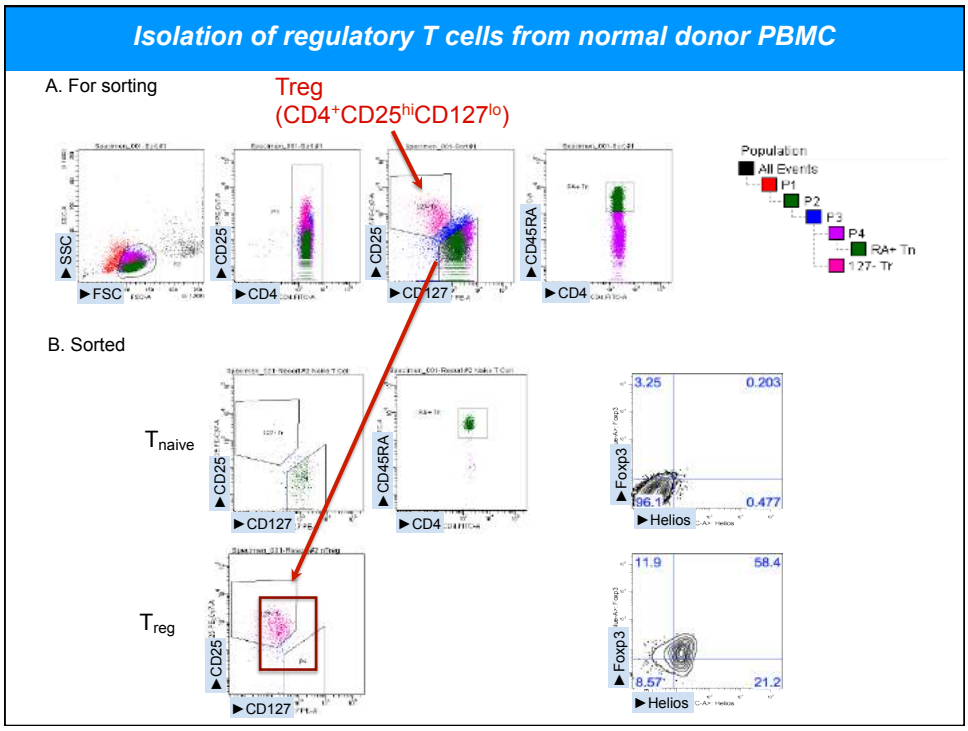




Endogenous Treg

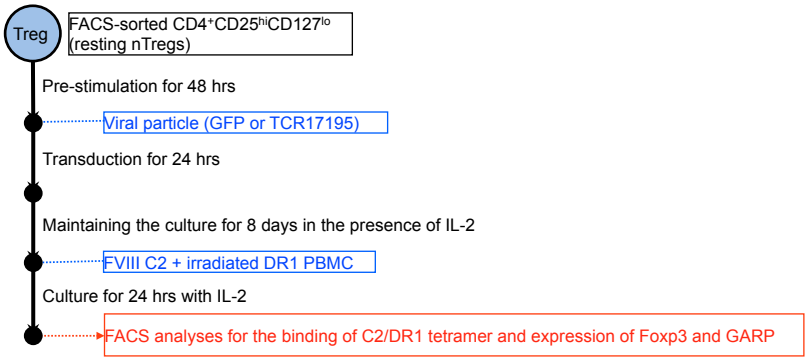
17195 Treg


Isolation of naive T cells and regulatory T cells from normal donor PBMC
A. For sorting
Treg (CD4+CD25hiCD127lo)
Tnaive (CD4+CD25-CD45RA+)
B. Sorted
Tnaive
Treg
Population
All Events
P1
P2
P3
P4
RA+ Tn
127- Tr
SSC
FSC
CD25
CD4
CD127
CD45RA
Foxp3
Helios
3.25
0.203
96.1
0.477
11.9
58.4
8.57
21.2


Isolation of regulatory T cells from normal donor PBMC
A. For sorting
Treg (CD4+CD25hiCD127lo)
B. Sorted
Tnaive
Treg
Population
All Events
P1
P2
P3
P4
RA+ Tn
127- Tr
SSC
FSC
CD25
CD4
CD127
CD45RA
Foxp3
Helios
3.25
0.203
96.1
0.477
11.9
58.4
8.57
21.2

TCR17195 is functional to activate primary Treg

- Measurement of Foxp3 and Treg markers in TCR17195-transduced Treg

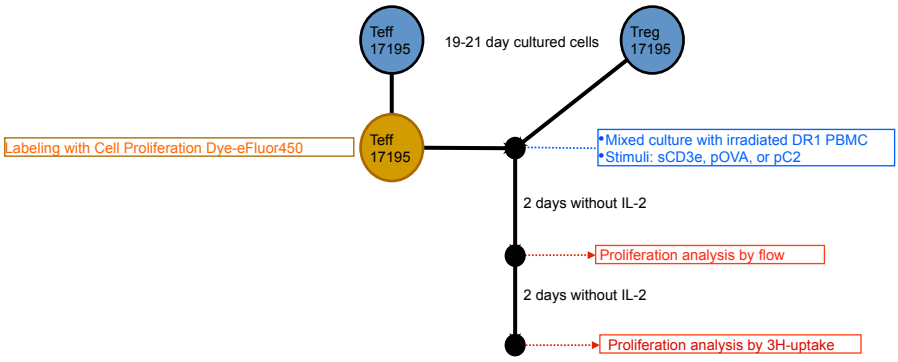


So these cells bear specific receptors, they look like Tregs, smell like Tregs…..

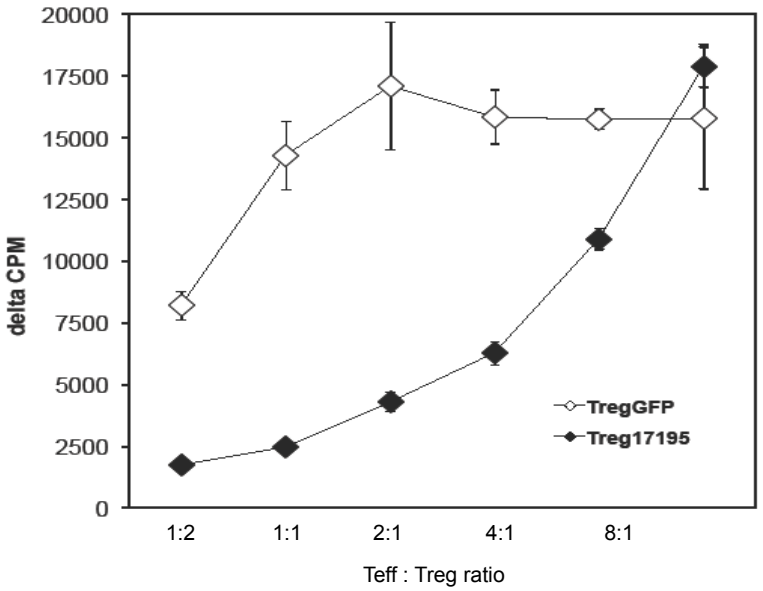
But do they *function* like Tregs?

Immunosuppression by FVIII-specific Tregs

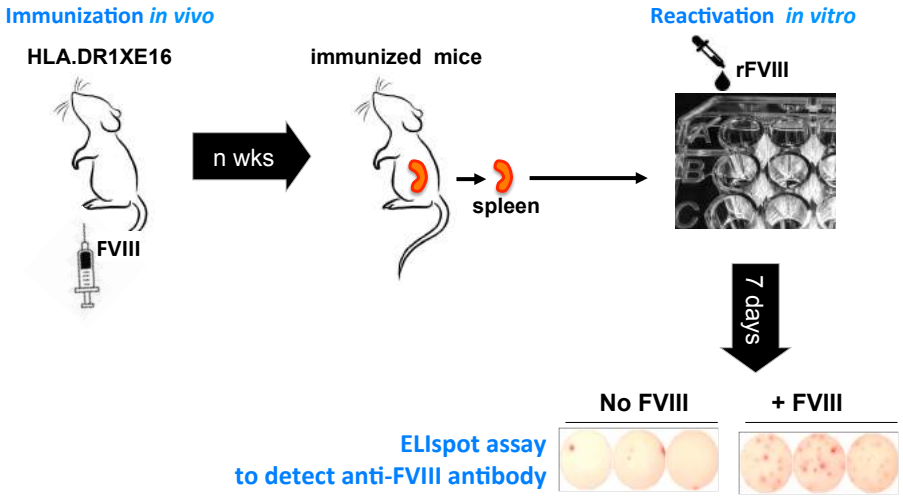
pC2-specific proliferation assay of TCR17195-transduced conventional T cells in the presence of Treg17195



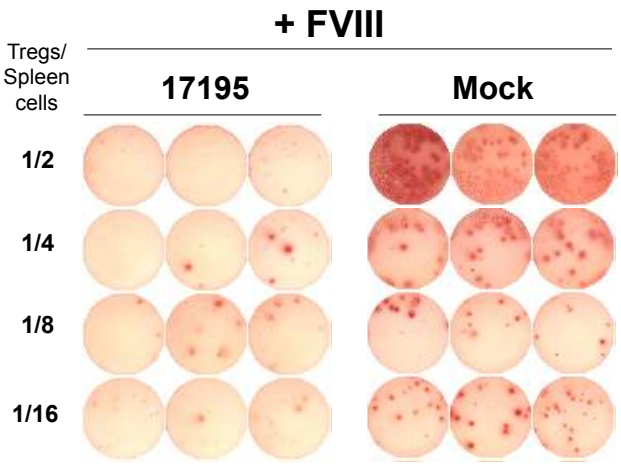
Immunosuppression by FVIII-specific Tregs



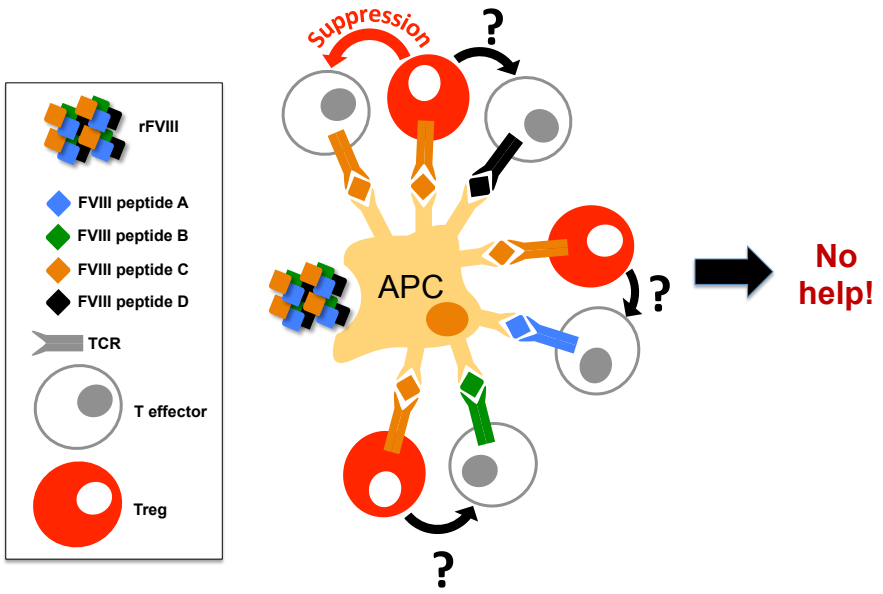
FVIII-specific suppression of secondary antibody formation by 17195 Tregs: Protocol



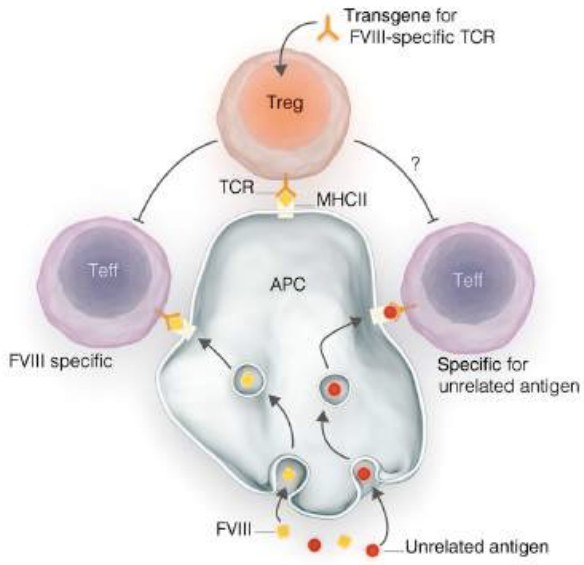
FVIII-specific suppression of secondary antibody formation by 17195 Tregs



FVIII activates C2-specific Tregs to suppress antibody to multiple epitopes



Mechanism of Treg suppression by engineered specific Tregs (police CARs/CARTs)

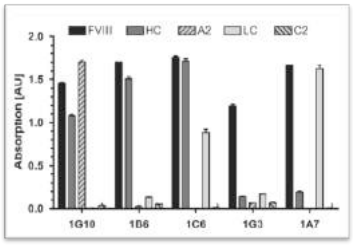


scFv anti-FVIII-28z CAR (ANS8CAR)

Selection and characterisation of FVIII-specific single chain variable fragments

A. Naumann; A. K. Scherger; J. Neuwirth; A. Orlowski; J. Kahle; D. Schwabe; C. Königs
Molecular Haemostasis and Immunodeficiency, Department of Paediatrics, University Hospital Frankfurt, Goethe University, Frankfurt am Main, Germany

Hämostaseologie 2013; 33 (Suppl 1): S39–S45

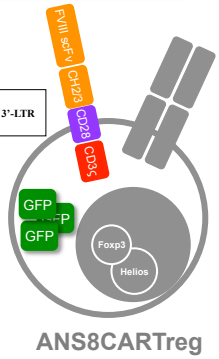


scFv anti-FVIII-28z

5'-LTR | Signal (huTCR) | FVIII-specific scFv | hinge-CH_2-CH_3 | CD28 (TM+Cyto) | CD3z (Cyto) | IRES | GFP | 3'-LTR

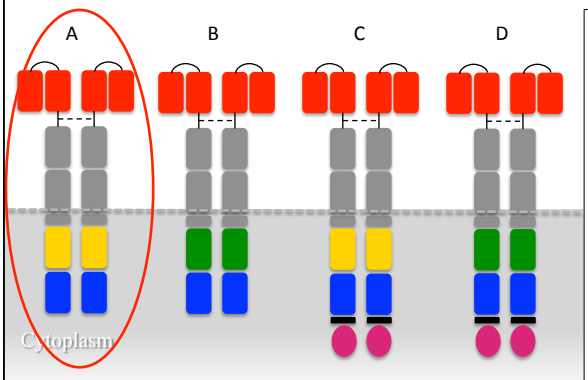
Anja N. Schmidt FVIII-specific CAR (ANS8)

Anja 'n Scott FVIII-specific CAR (ANS8)



FVIII single chain Fv (scFv) CARs

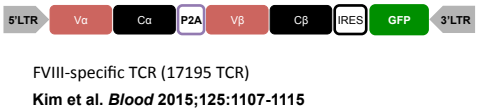
5'-LTR | Signal (msCD4) | FVIII-specific scFv | hinge-CH_2-CH_3 | CD28 (TM+Cyto) | CD3z (Cyto) | IRES | GFP | 3'-LTR



A. FVIII-specific CAR (CD28)
B. FVIII-specific CAR (41BB)
C. FVIII-specific CAR (CD28) with HSV-TK
D. FVIII-specific CAR (41BB) with HSV-TK

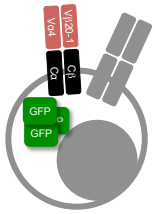
FVIII A2-specific scFv
Hinge region
CH2 and CH3 of IgG1 Fc
CD28 transmembrane domain
CD28 cytoplasmic domain
41BB cytoplasmic domain
CD3ζ cytoplasmic domain
P2A cleavage peptide
HSV-TK

Expression of FVIII-specific TCR/CAR in human Tregs

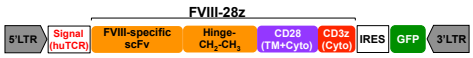


FVIII-specific TCR (17195 TCR)

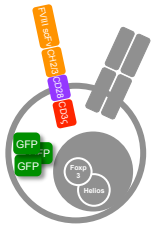
Kim et al. *Blood* 2015;125:1107-1115



17195 T cell

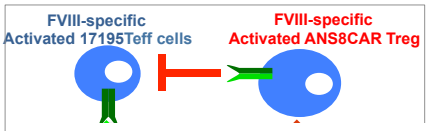


Anja N. Schmidt FVIII-specific CAR
Anja 'n Scott FVIII-specific CAR

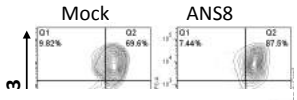


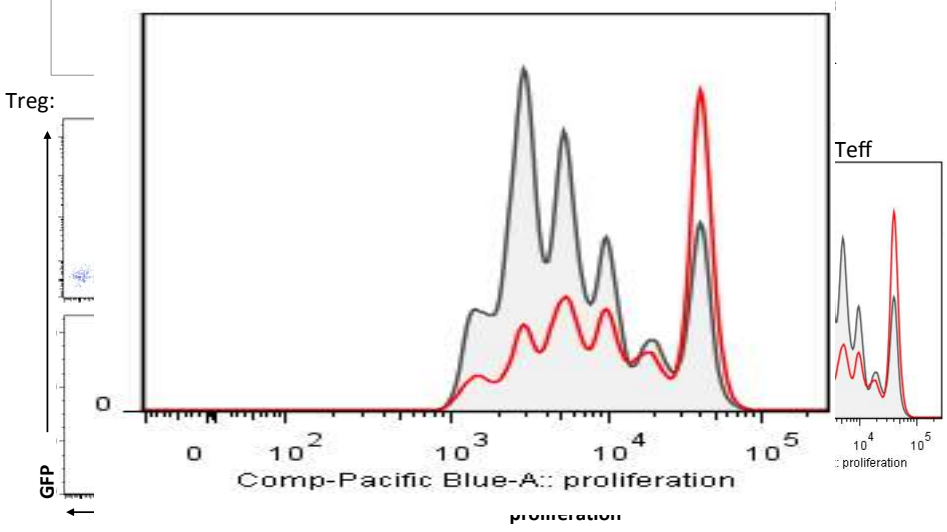
ANS8CARTreg

Immunosuppressive effect of FVIII-specific CAR (ANS8CAR) Tregs



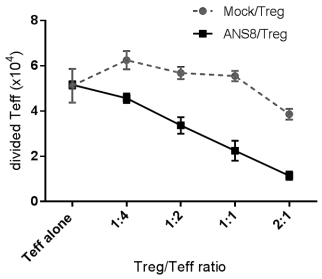
Quality test of transduced Tregs (d7)



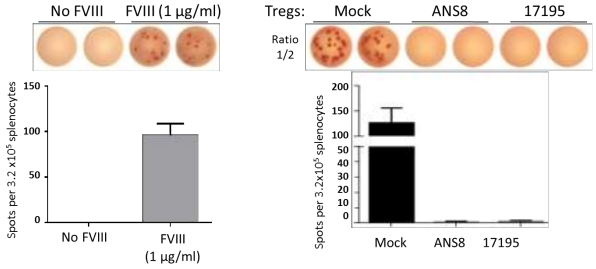


FVIII-specific immunosuppression by FVIII-CAR Tregs

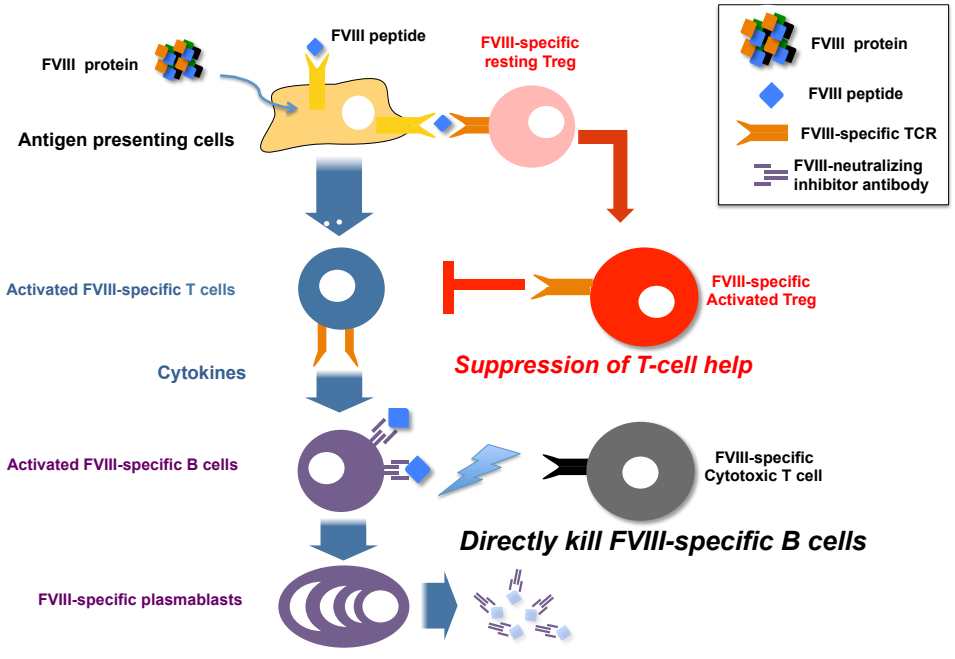
in vitro immunosuppression



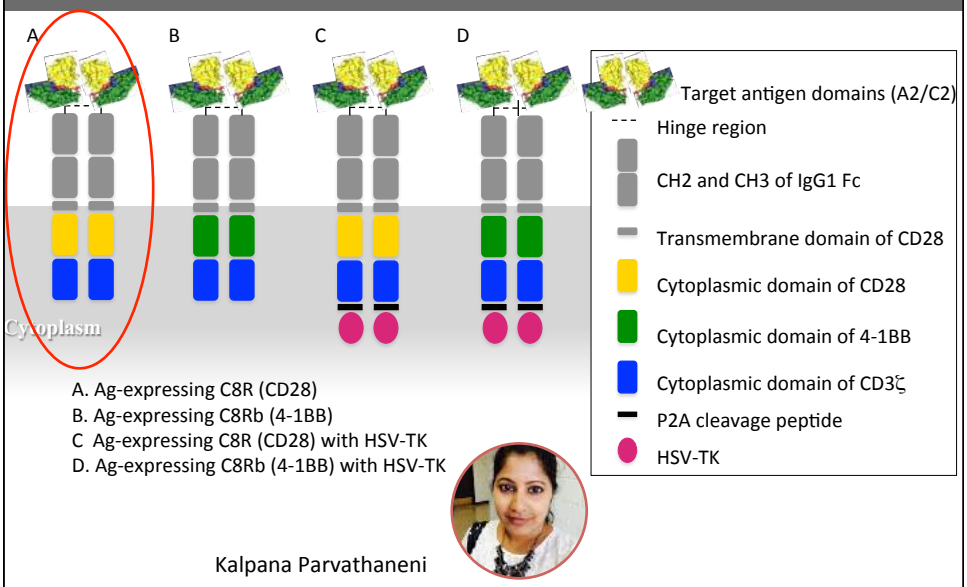
ex vivo immunosuppression



How can we prevent or reverse FVIII-specific 'inhibitor " formation?



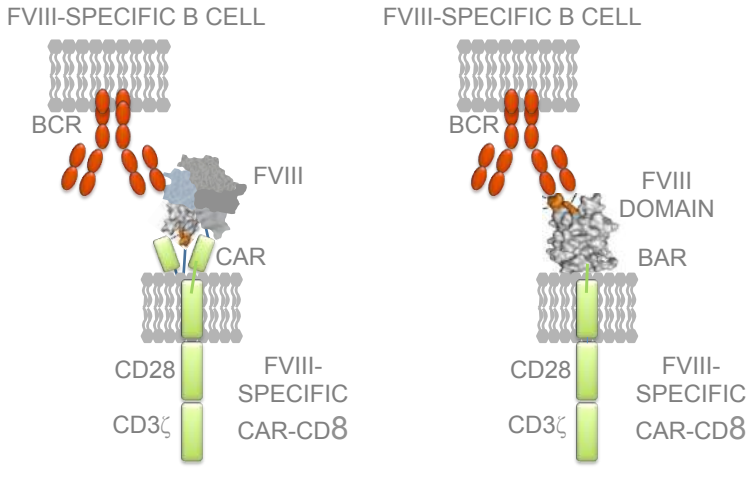
FVIII domain expression in CD8 T cells: BARs (B-cell Activating Receptors)



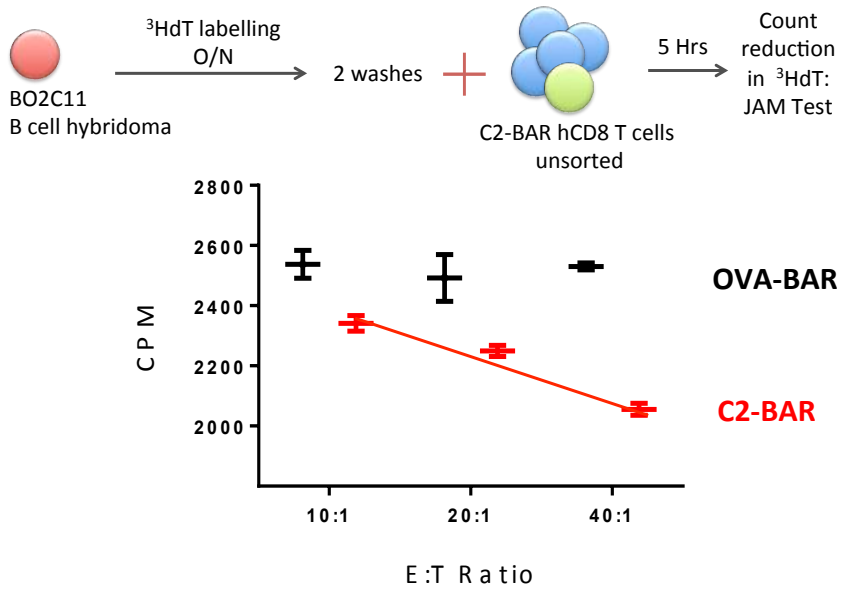
A. Ag-expressing C8R (CD28)
B. Ag-expressing C8Rb (4-1BB)
C Ag-expressing C8R (CD28) with HSV-TK
D. Ag-expressing C8Rb (4-1BB) with HSV-TK

Kalpana Parvathaneni

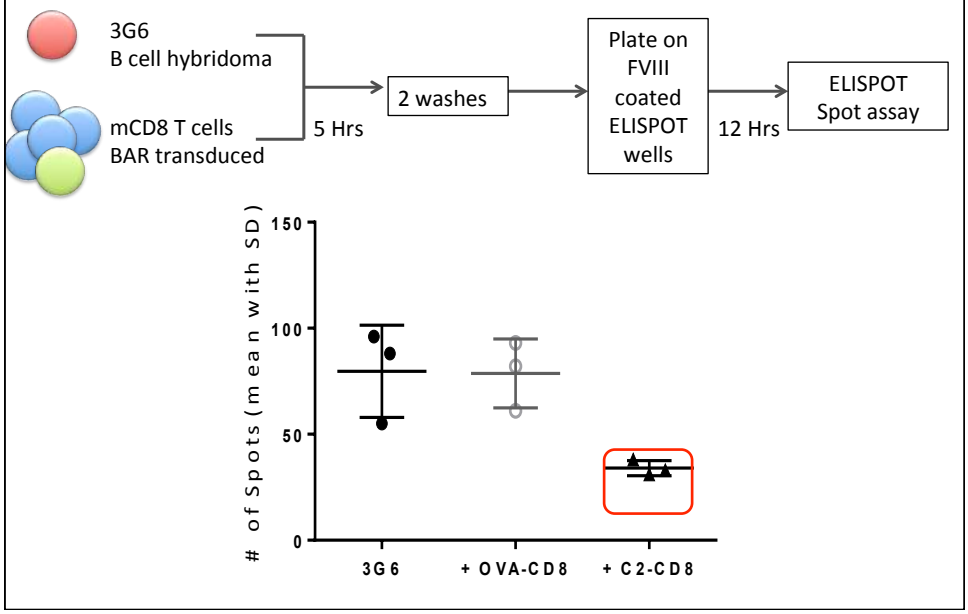
BAR=
B CELL ANTIBODY RECEPTOR

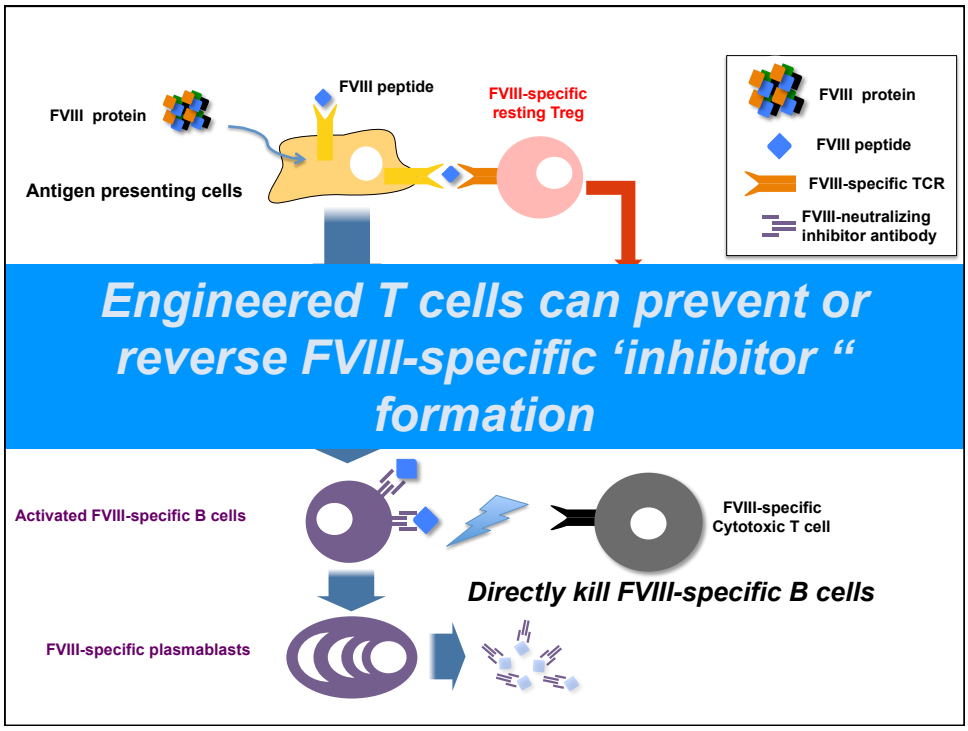


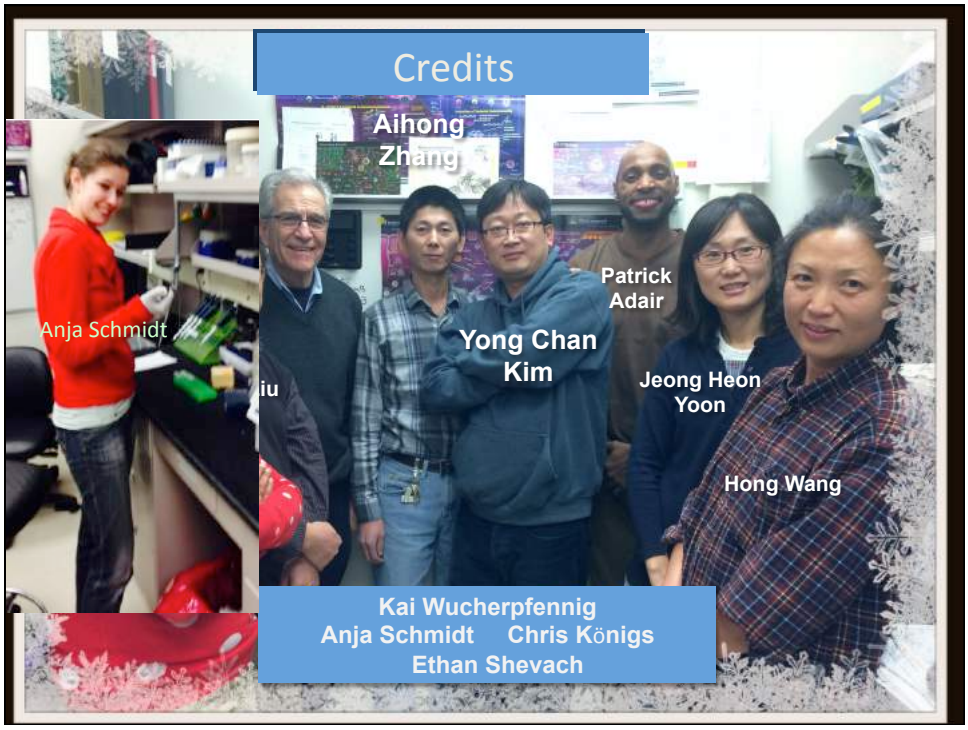
FVIII-C2 BAR - CD8 T CELLS KILL α-FVIII HYBRIDOMA: JAM TEST



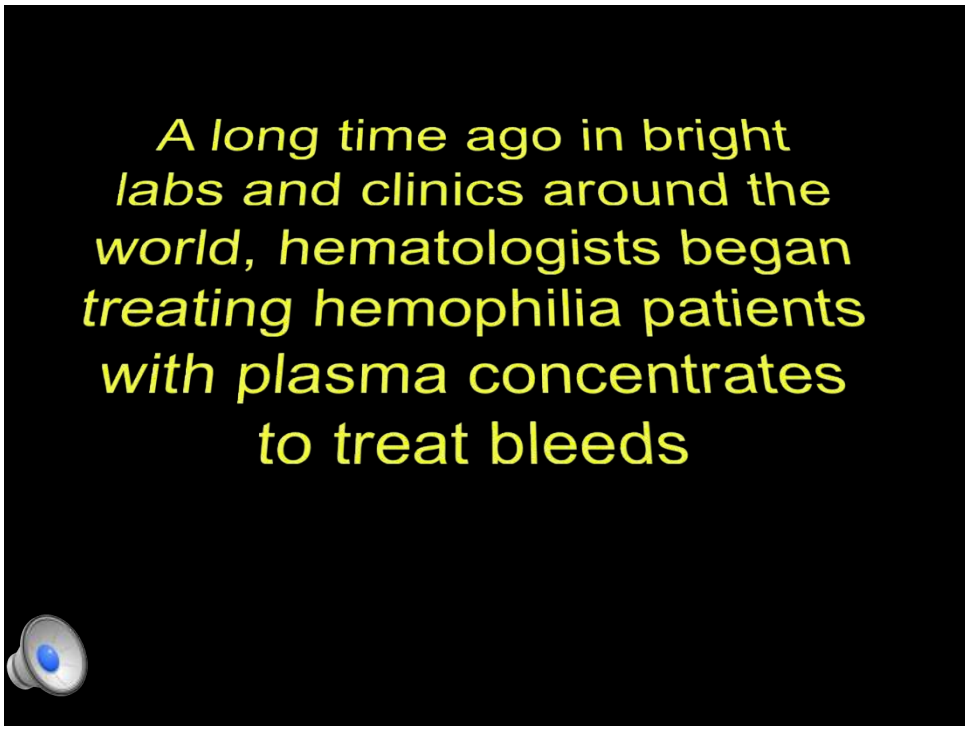
FVIII-C2 BAR-mCD8 T CELLS TO KILL α-FVIII MOUSE HYBRIDOMA

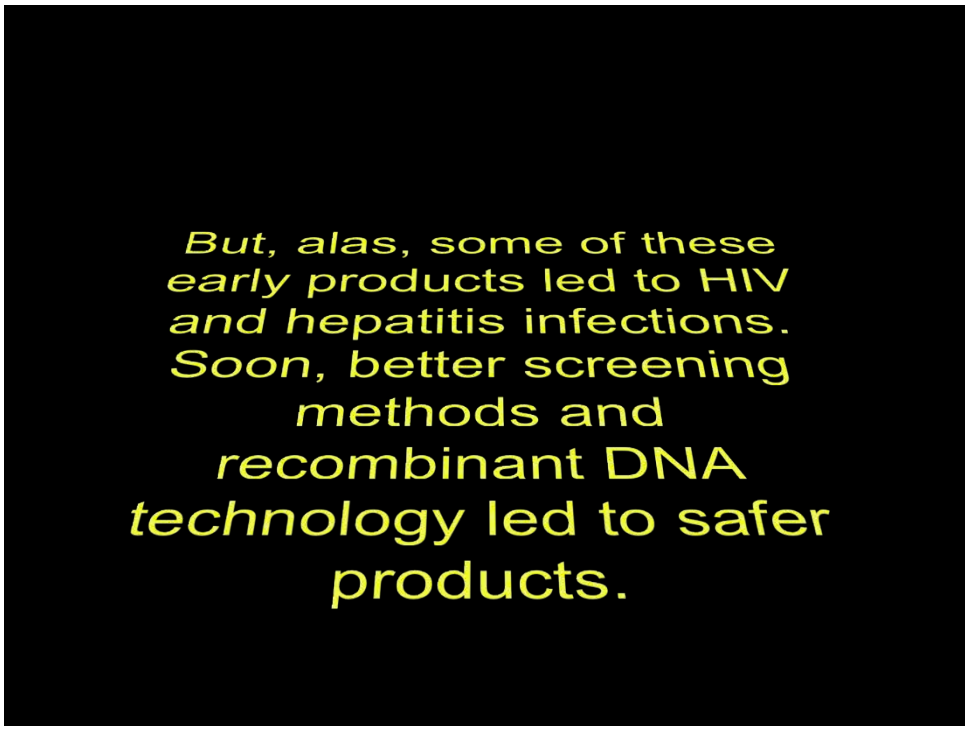


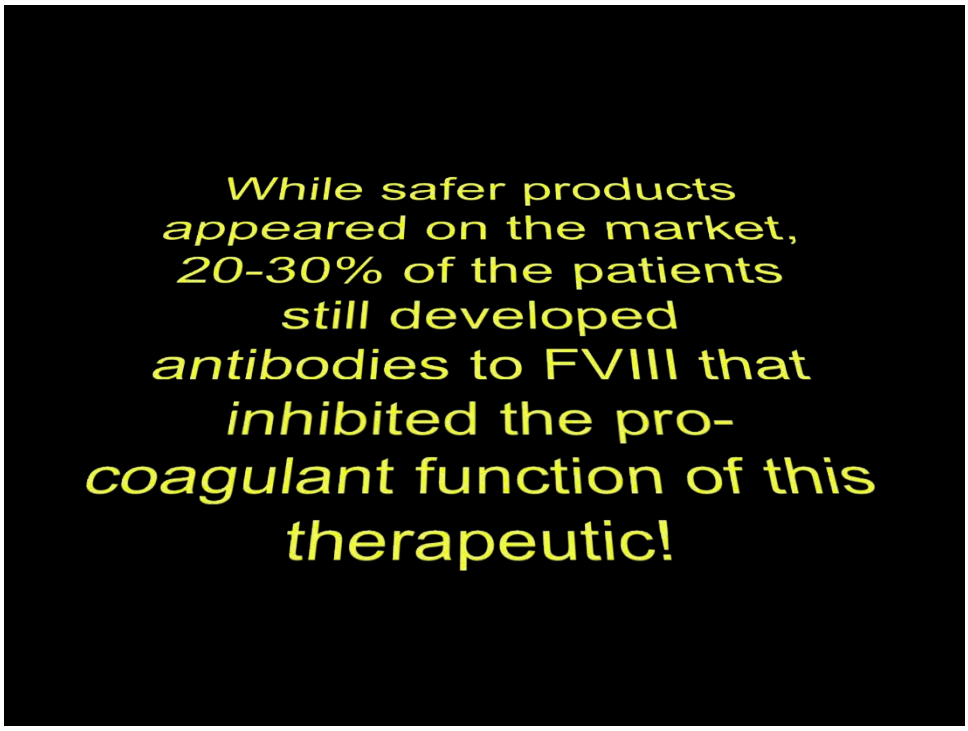

FVIII protein
FVIII peptide
FVIII-specific resting Treg
Antigen presenting cells
FVIII protein
FVIII peptide
FVIII-specific TCR
FVIII-neutralizing inhibitor antibody
Engineered T cells can prevent or reverse FVIII-specific 'inhibitor " formation
Activated FVIII-specific B cells
FVIII-specific Cytotoxic T cell
Directly kill FVIII-specific B cells
FVIII-specific plasmablasts


Credits
Aihong Zhang
Anja Schmidt
Patrick Adair
Yong Chan Kim
Jeong Heon Yoon
Hong Wang
Kai Wucherpfennig
Anja Schmidt Chris Königs
Ethan Shevach

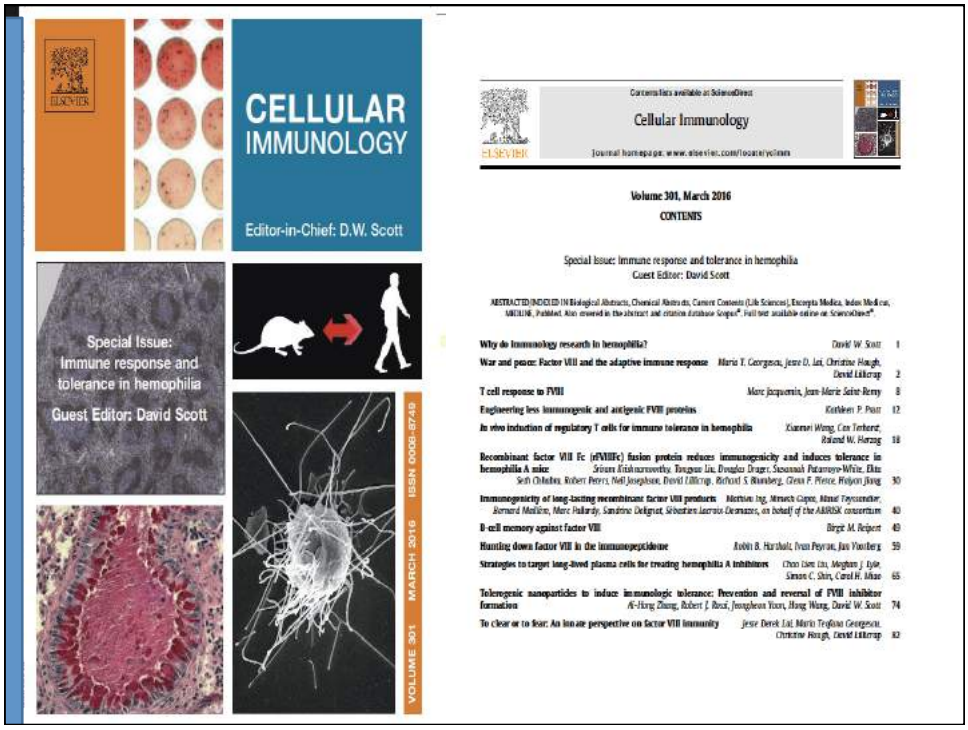

STAR WARS
IMMUNOWARS


A long time ago in bright labs and clinics around the world, hematologists began treating hemophilia patients with plasma concentrates to treat bleeds


But, alas, some of these
early products led to HIV
and hepatitis infections.
Soon, better screening
methods and
recombinant DNA
technology led to safer
products.


While safer products
appeared on the market,
20-30% of the patients
still developed
antibodies to FVIII that
inhibited the pro-
coagulant function of this
therapeutic!

Eventually, the
Immunologic Republic led
the Resistance…
developing multiple approaches to
induced tolerance to FVIII
such as oral tolerance, B-cell
delivery, Fc fusions, tolerogenic
nanoparticles,
engineered Tregs and novel
peptides




Immunology has some answers(?)