Modulation of immunogenicity by engineered antigen-specific regulatory T cells: Fighting fire with fireman or police CARs

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Dealing with immunogenicity and adverse responses

Rationale and issues

Clinical studies with expanded human regulatory T-cell therapy are already in progress. However, these are polyclonal T cells that include a diverse repertoire of relativities.

Caveats:

- The frequency of relevant regulatory T cells (Tregs) may be quite low.
- Expanded polyclonal Tregs (multiple specificities) may be non-specifically immunosuppressive.

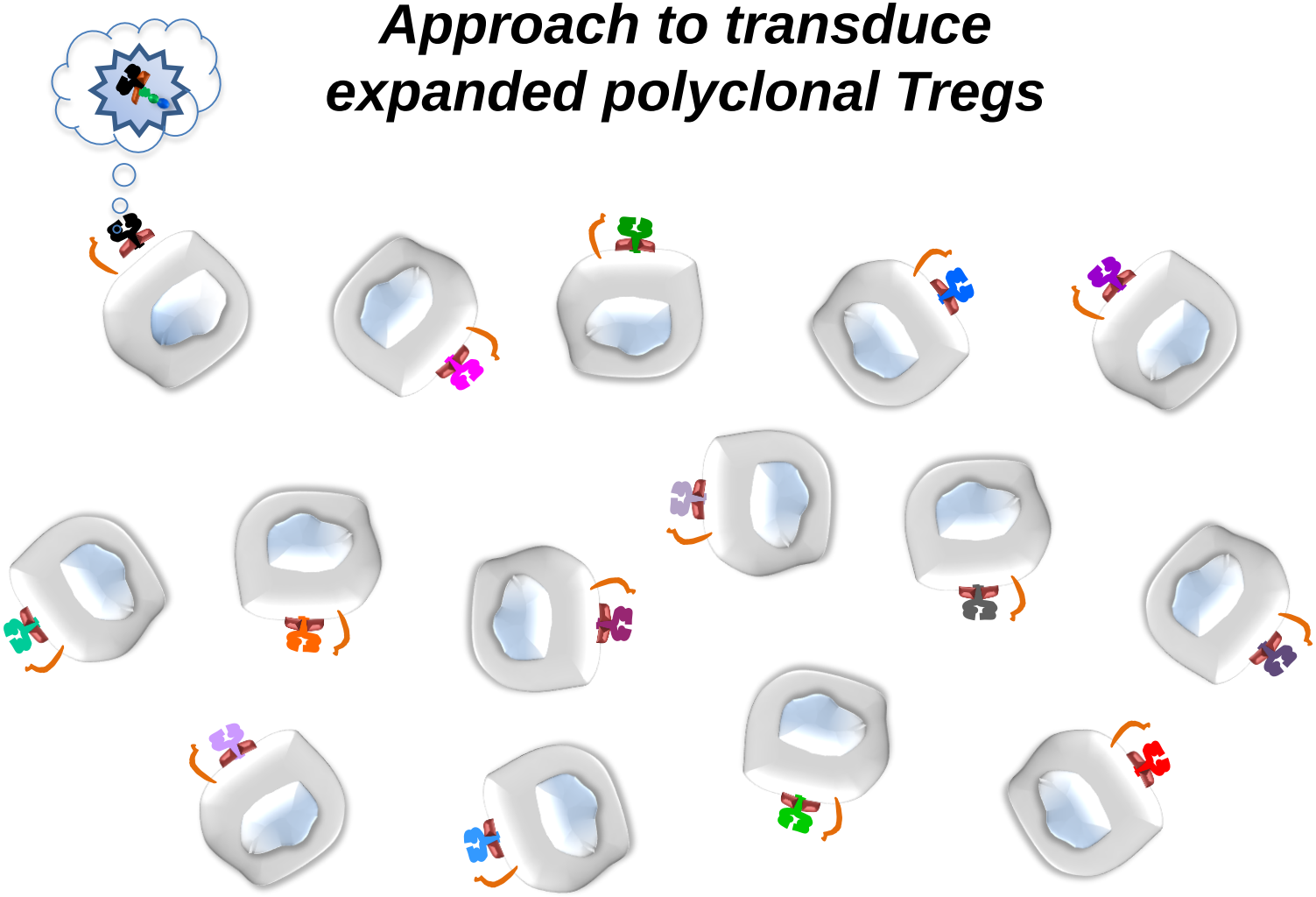
Approaches

- Enrich and expand Tregs with antigen/tetramer, etc.

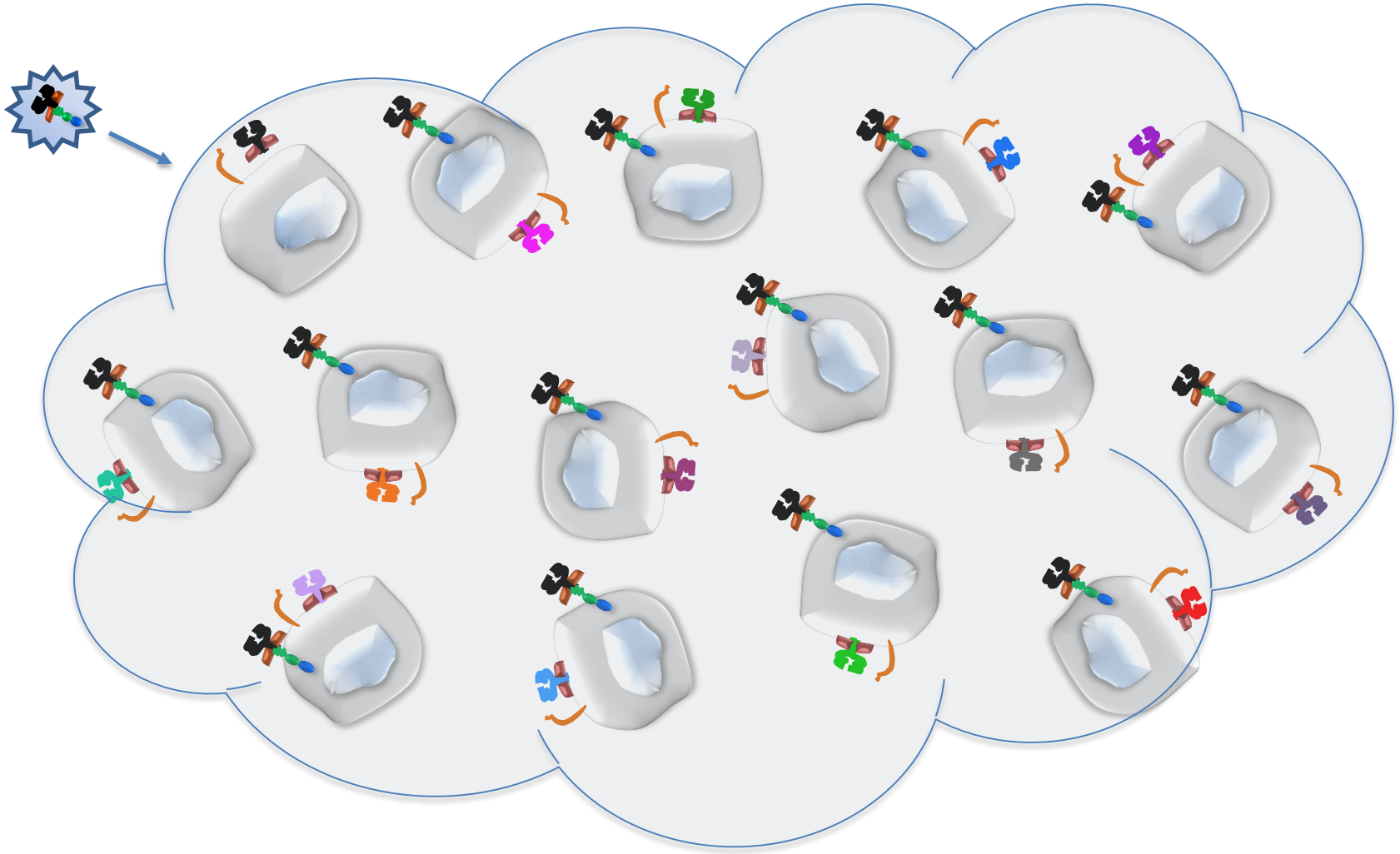
Possible solution based on chimeric antigen receptor (CAR) therapy for cancer:

- Engineer specificity into polyclonal Tregs via retroviral transduction of specific "receptors", e.g.:
  - T-cell receptor (TCR)
  - CAR (scFv) or
  - Antigen (B-cell antibody receptor=BAR)

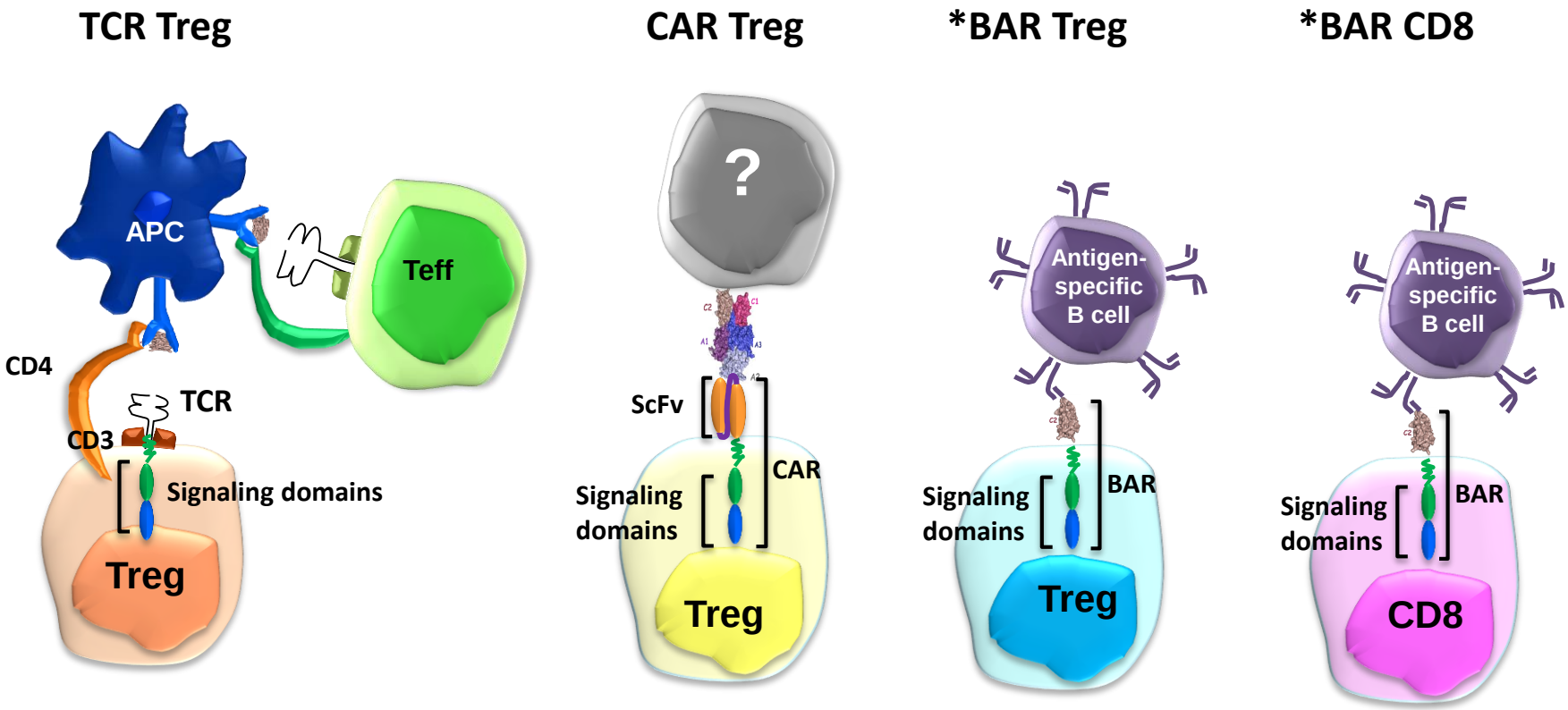
Approach to transduce expanded polyclonal Tregs



Transduced antigen-specific polyclonal Tregs

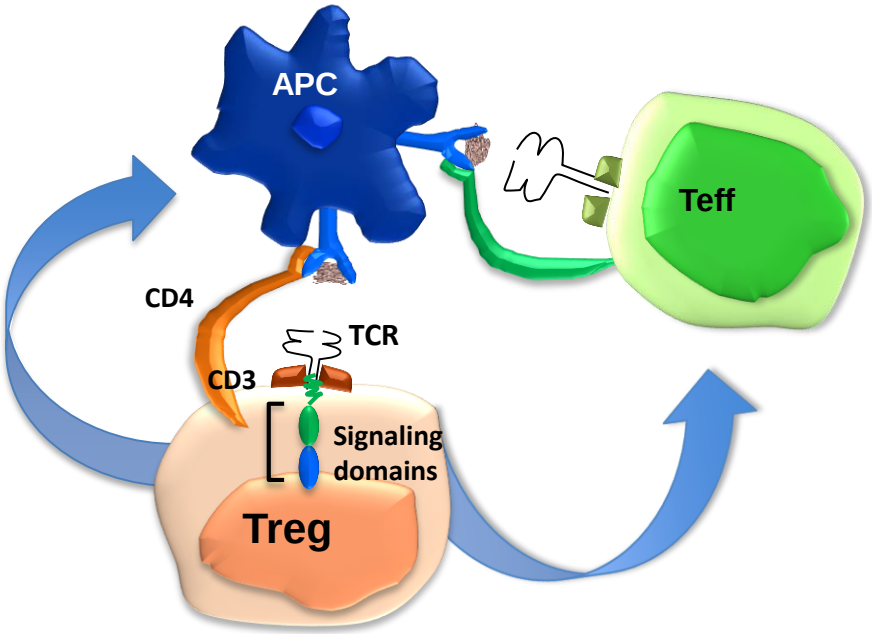


Engineering antigen-specificity into polyclonal T cells: Four flavors



**B-cell antibody receptor=BAR*

Engineering antigen-specificity into polyclonal T cells: TCR V-regions



TCR Treg effects

Aihong (Allan) Zhang

Yong Chan Kim

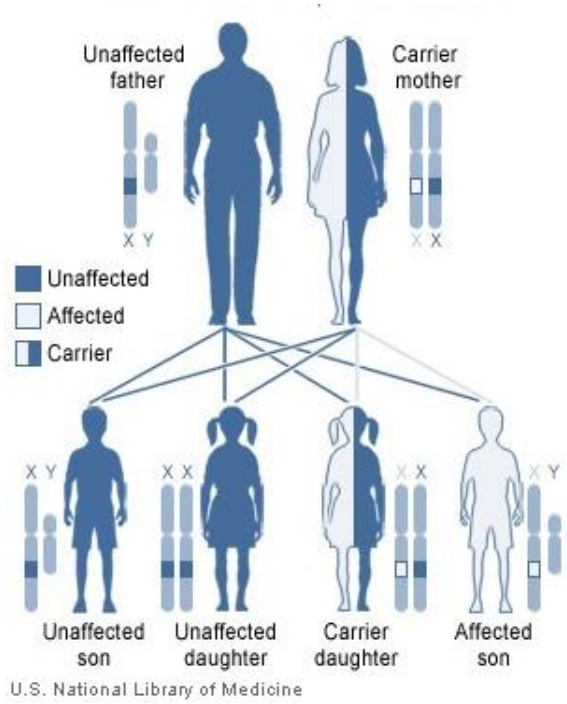
FVIII in hemophilia A

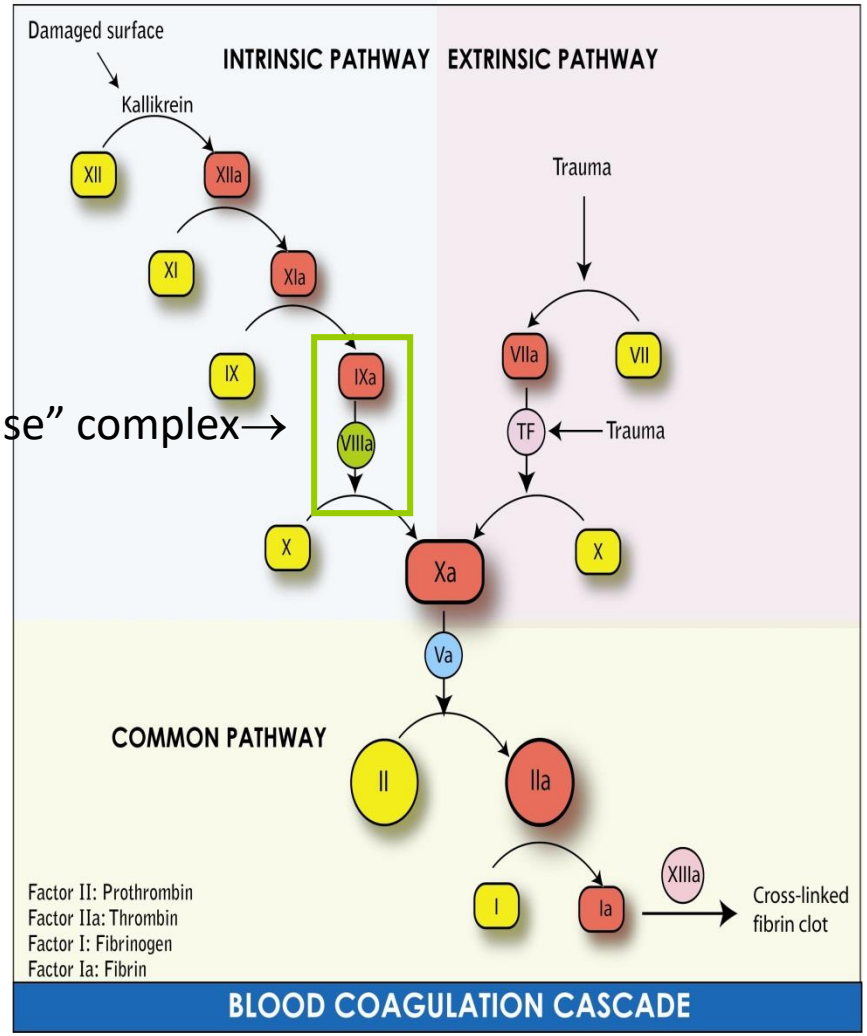
MBP in multiple sclerosis

Systems and targets

- Multiple systems:
  - ✓ **Hemophilia inhibitors (FVIII)**
  - ✓ Autoimmunity, e.g., MS (MBP) or Type 1 diabetes
  - ✓ Allergy (OVA)
  - ✓ Future targets (ADA's)

Hemophilia





"Tenase" complex→

X-linked blood clotting disorder

FVIII mutations cause Hemophilia A*

FIX mutations cause Hemophilia B

***Deletions, inversions, missense, stop codons**

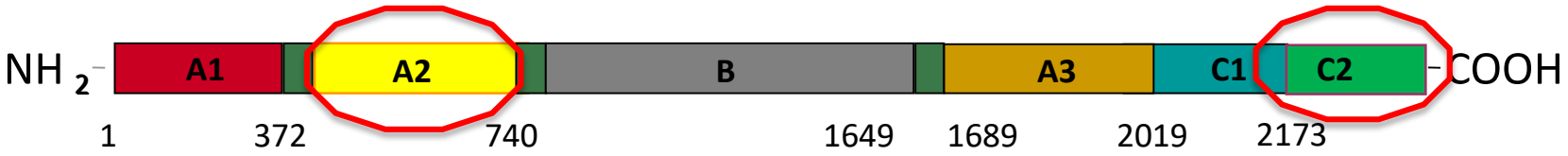
Hemophilia

What is standard treatment for bleeds?

The unwelcome response to a human protein, FVIII

Hemophilia A patients can mount an immune response to FVIII depending, in part, on the nature of their mutation

Because they lack FVIII, they did not develop immune tolerance to therapeutic FVIII



Specific antibodies against FVIII *inhibit* clotting by binding to domains required its bio-activity (“inhibitors”)

Application in Hemophilia A

IMMUNOBIOLOGY

Engineered antigen-specific human regulatory T cells: immunosuppression of FVIII-specific T- and B-cell responses

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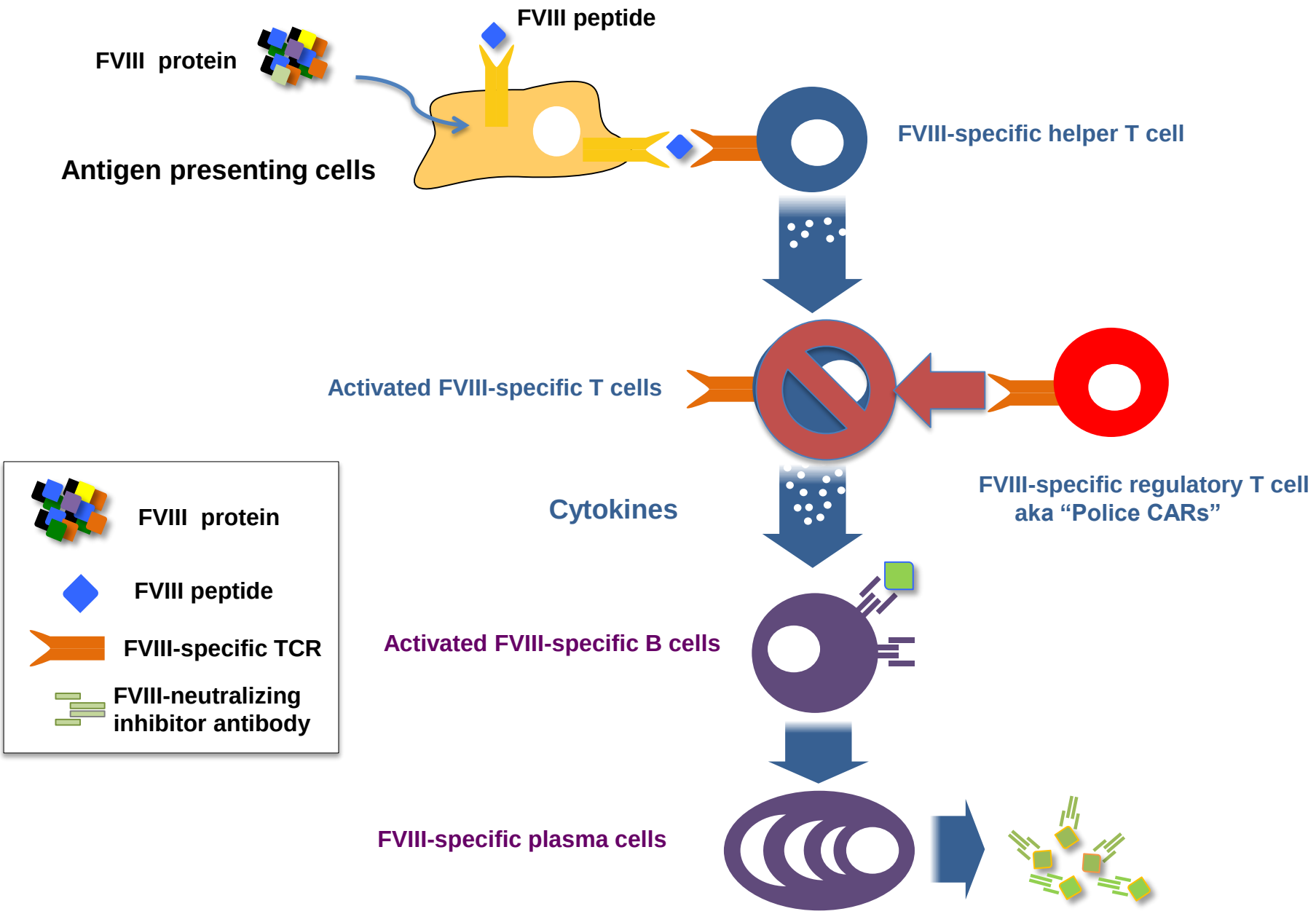
Key Points

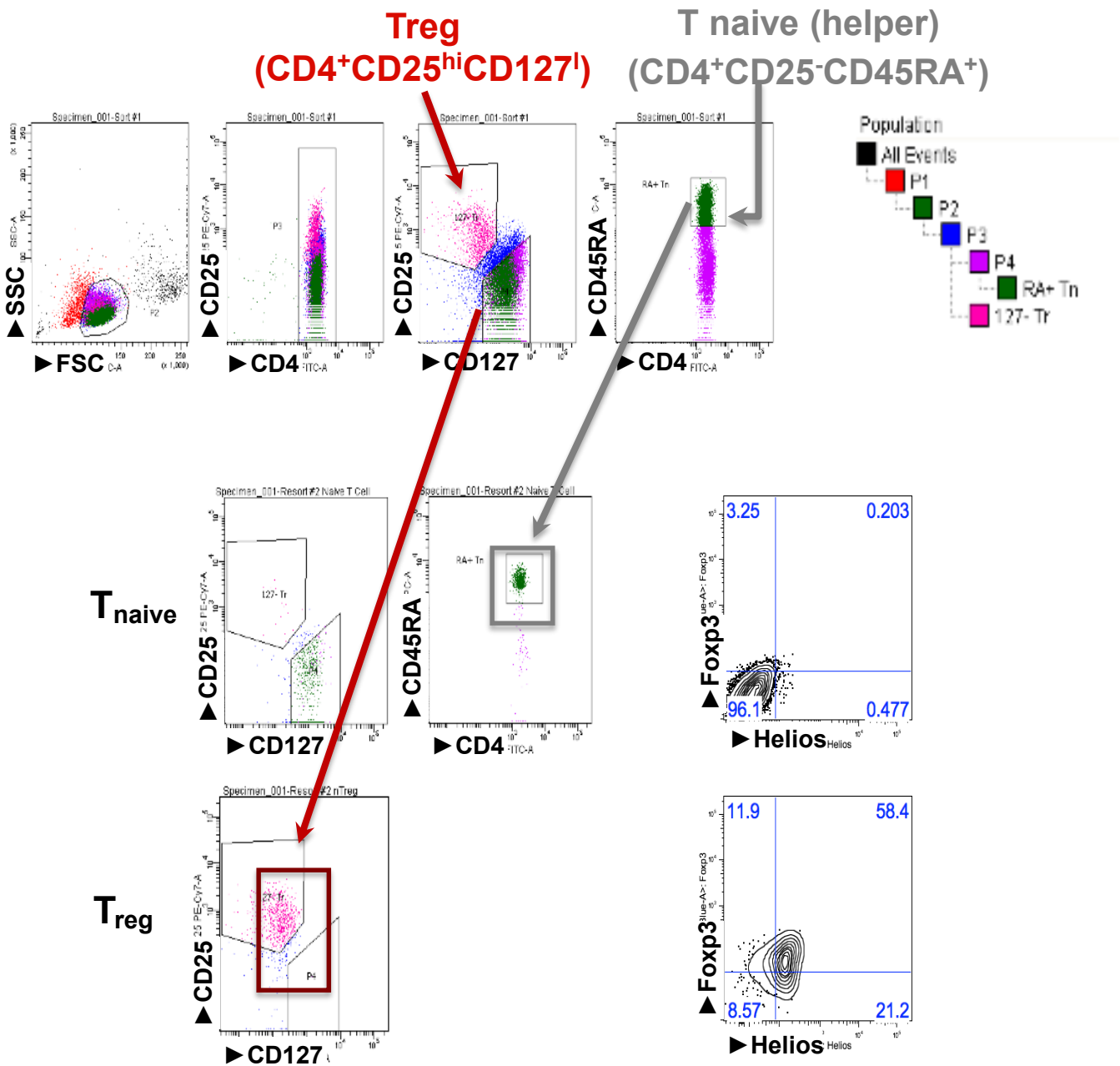
- Generation and function of specific human Tregs.
- Specific regulation of FVIII responses by engineered human Tregs.

Expansion of human regulatory T cells (Tregs) for clinical applications offers great promise for the treatment of undesirable immune responses in autoimmunity, transplantation, allergy, and antidrug antibody responses, including inhibitor responses in hemophilia A patients. However, polyclonal Tregs are nonspecific and therefore could potentially cause global immunosuppression. To avoid this undesirable outcome, the generation of antigen-specific Tregs would be advantageous. Herein, we report the production and properties of engineered antigen-specific Tregs, created by transduction of a recombinant T-cell receptor obtained from a hemophilia A subject's T-cell clone, into expanded human FoxP3^{+} Tregs. Such engineered factor VIII (FVIII)-specific Tregs efficiently suppressed the proliferation and cytokine production of FVIII-specific T-effector cells. Moreover, studies with an HLA-transgenic, FVIII-deficient mouse model demonstrated that antibody production from FVIII-primed spleen cells in vitro were profoundly inhibited in the presence of these FVIII-specific Tregs, suggesting potential utility to treat anti-FVIII inhibitory antibody formation in hemophilia A patients. (*Blood*. 2015;125(7):1107-1115)

Immunology 101: The immune Response to FVIII

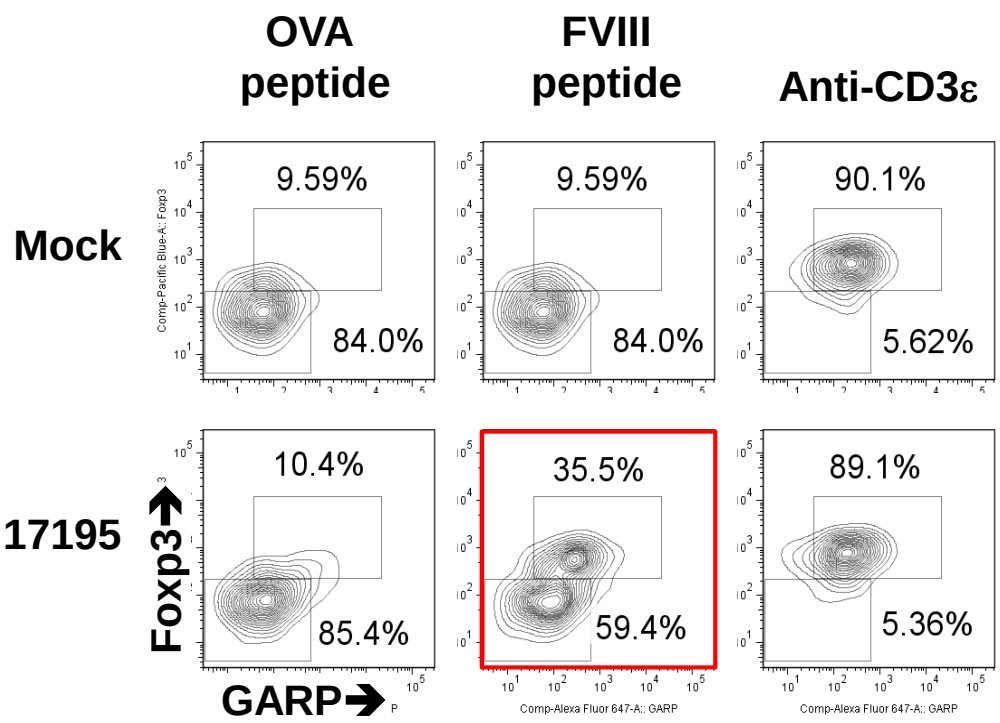


Isolation of naive T cells and regulatory T cells from normal donor PBMC

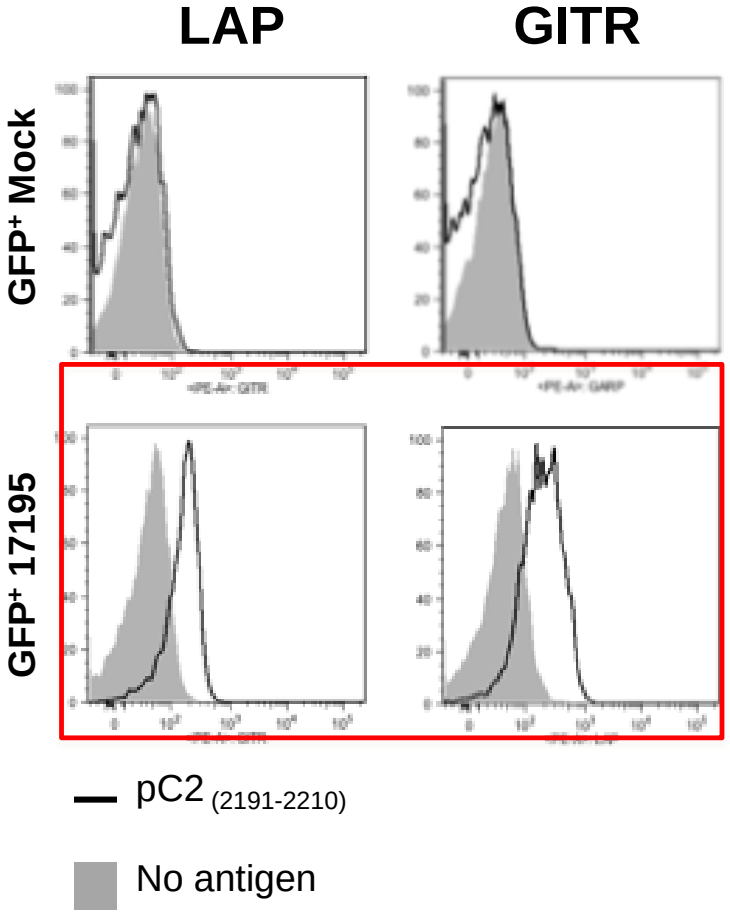


Antigen-specific upregulation of Treg markers in 17195 Tregs

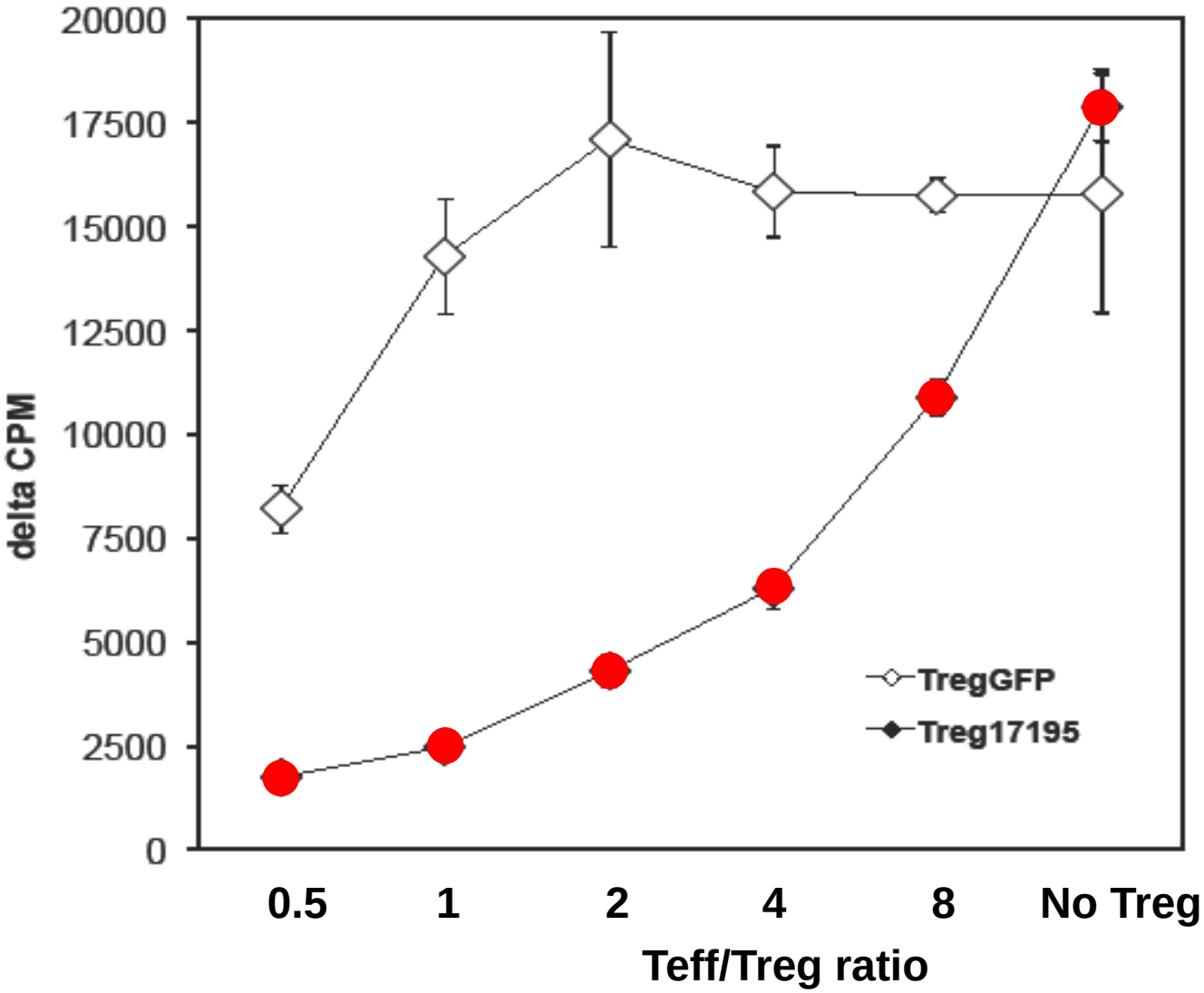
Induction of Foxp3 and GARP



Induction of LAP and GITR



FVIII-C2-specific immunosuppression by Treg17195



YC Kim et al. Blood **125**: 1107, 2015.

Can this approach work to prevent or reverse inhibitor responses in hemophilia A mice *?*

Protocol of FVIII-specific suppression of secondary antibody formation by engineered FVIII-specific human Tregs

Immunization *in vivo*

HLA.DR1XE16

FVIII

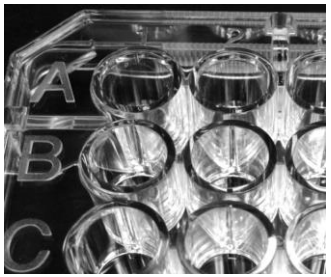
n wks

Immunized mice

spleen

Reactivation *in vitro*

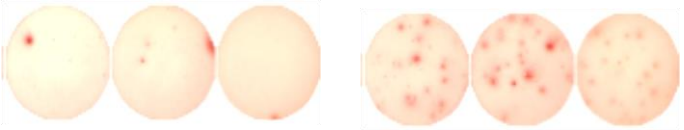
rFVIII

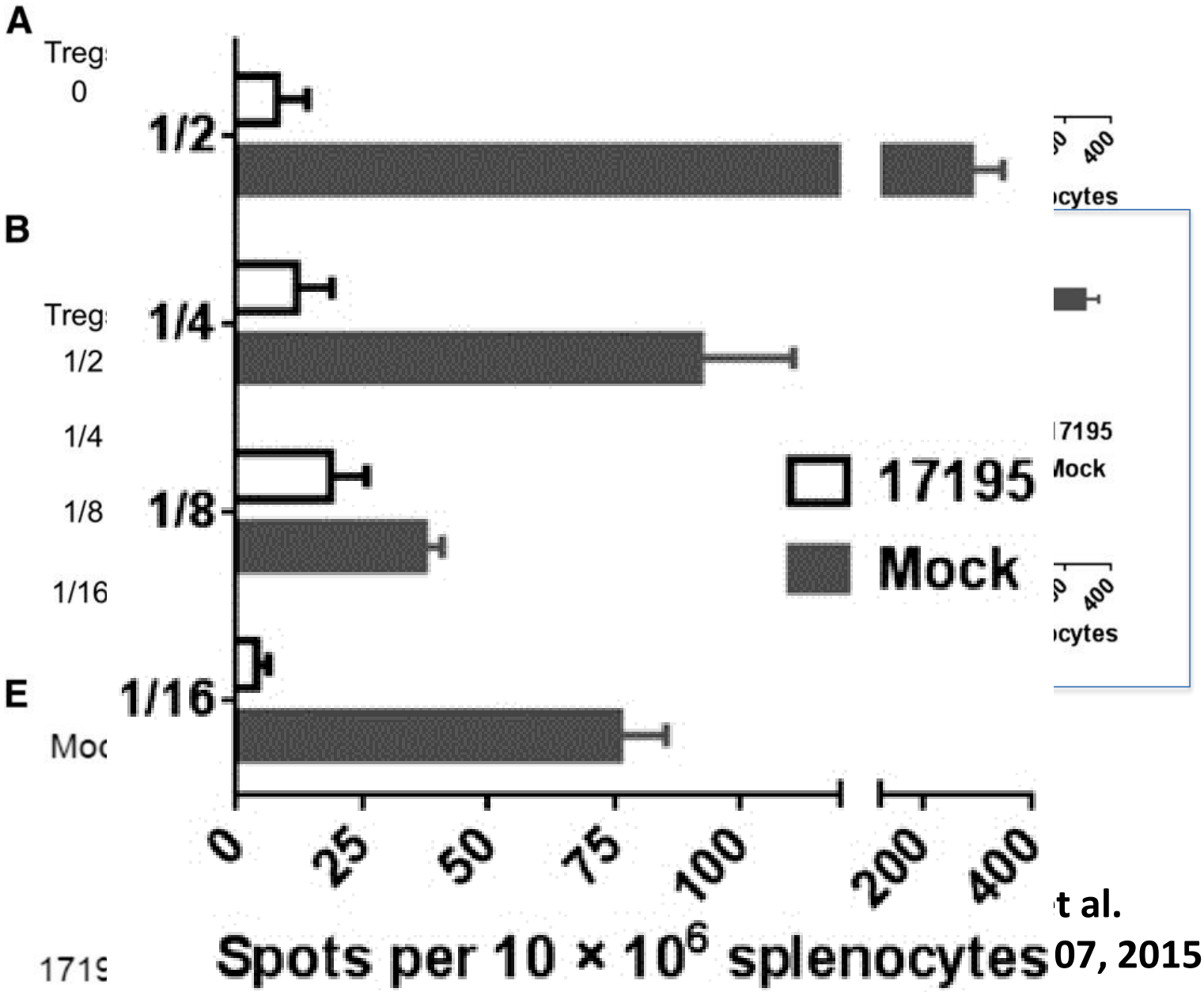


7 days

No FVIII **+ FVIII**

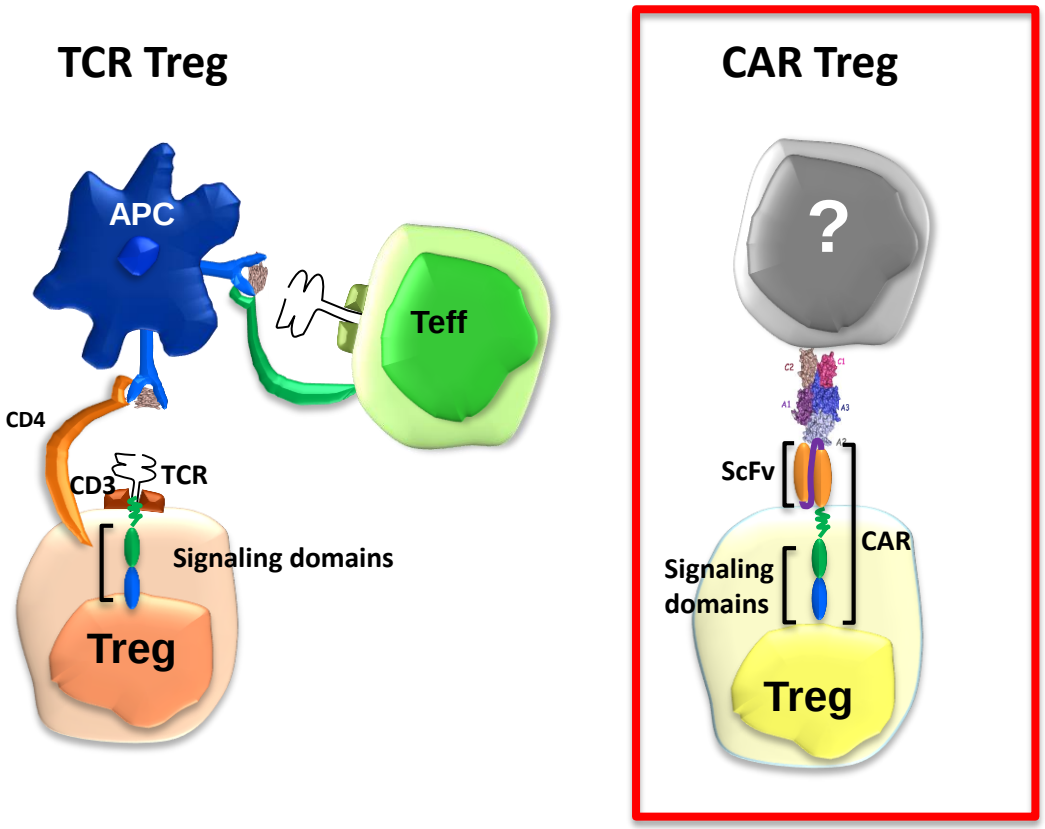
ELIspot assay to detect anti-FVIII antibody



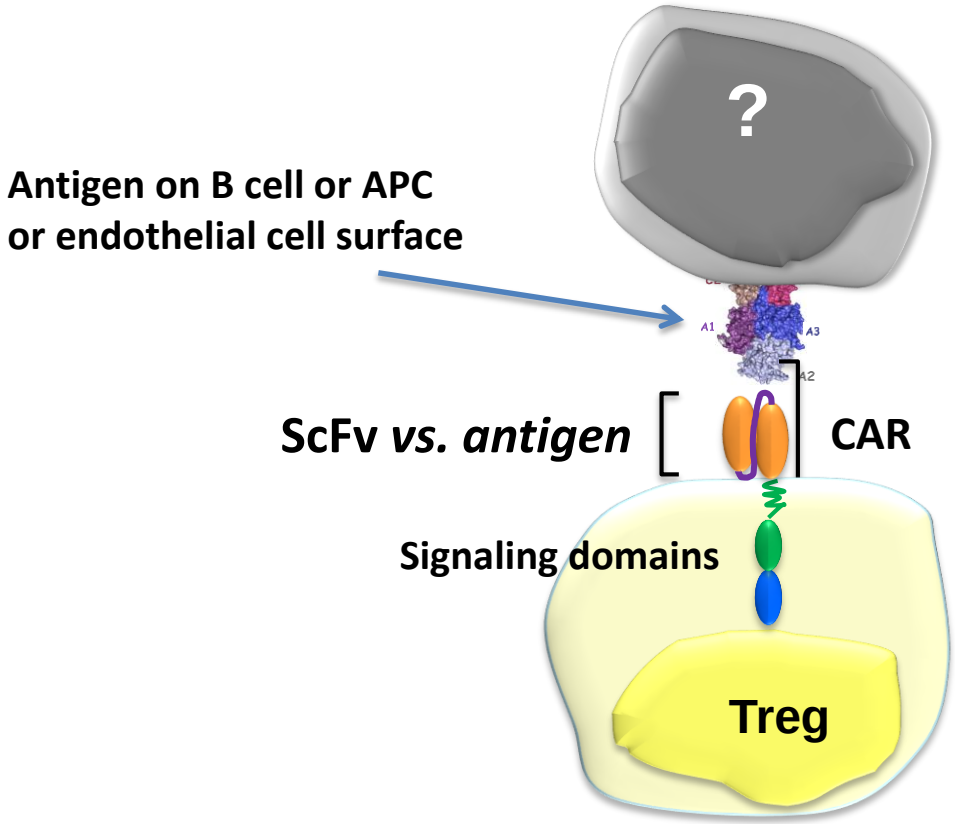

A
B
E
1/2
1/4
1/8
1/16
17195
Mock
0
25
50
75
100
200
400
Spots per 10 × 10^6 splenocytes

t al.
07, 2015.

Engineering antigen-specificity into polyclonal T cells:
<u>Single chain (scFv) CARs</u>

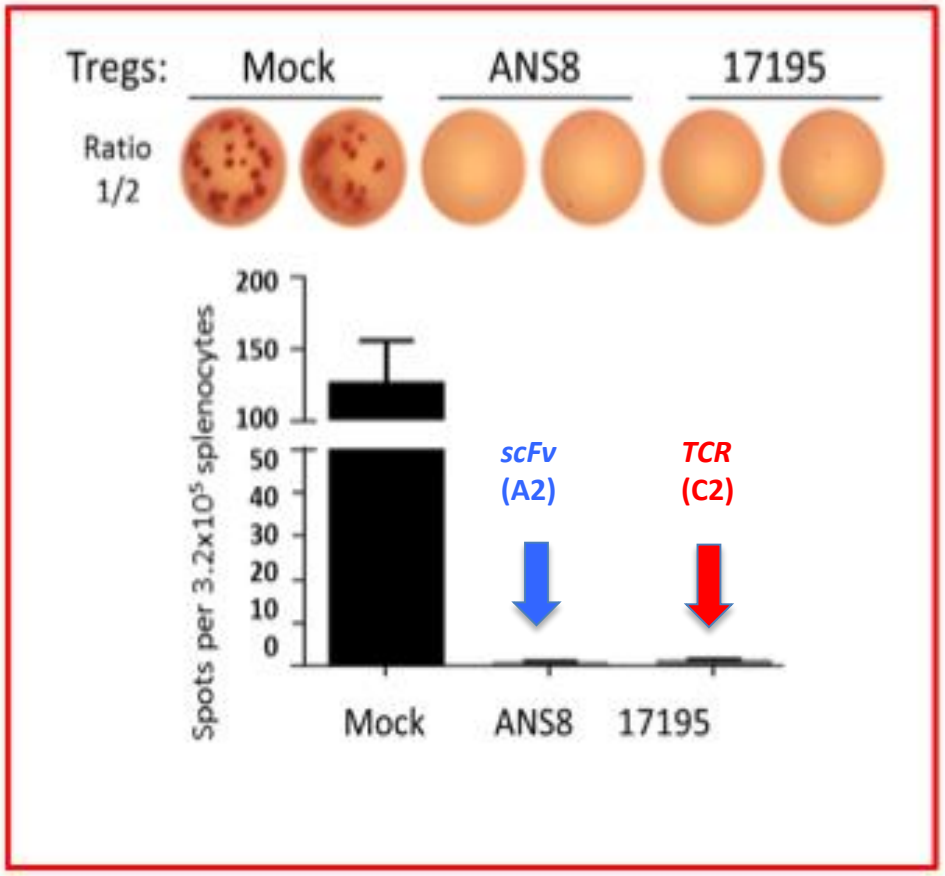


What about CAR (chimeric single chain Fv) Tregs?



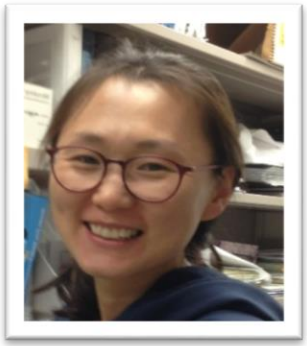
CAR Treg

Both TCR- or scFv engineered *human* Tregs suppress the <u>secondary</u> anti-FVIII response *in vitro*



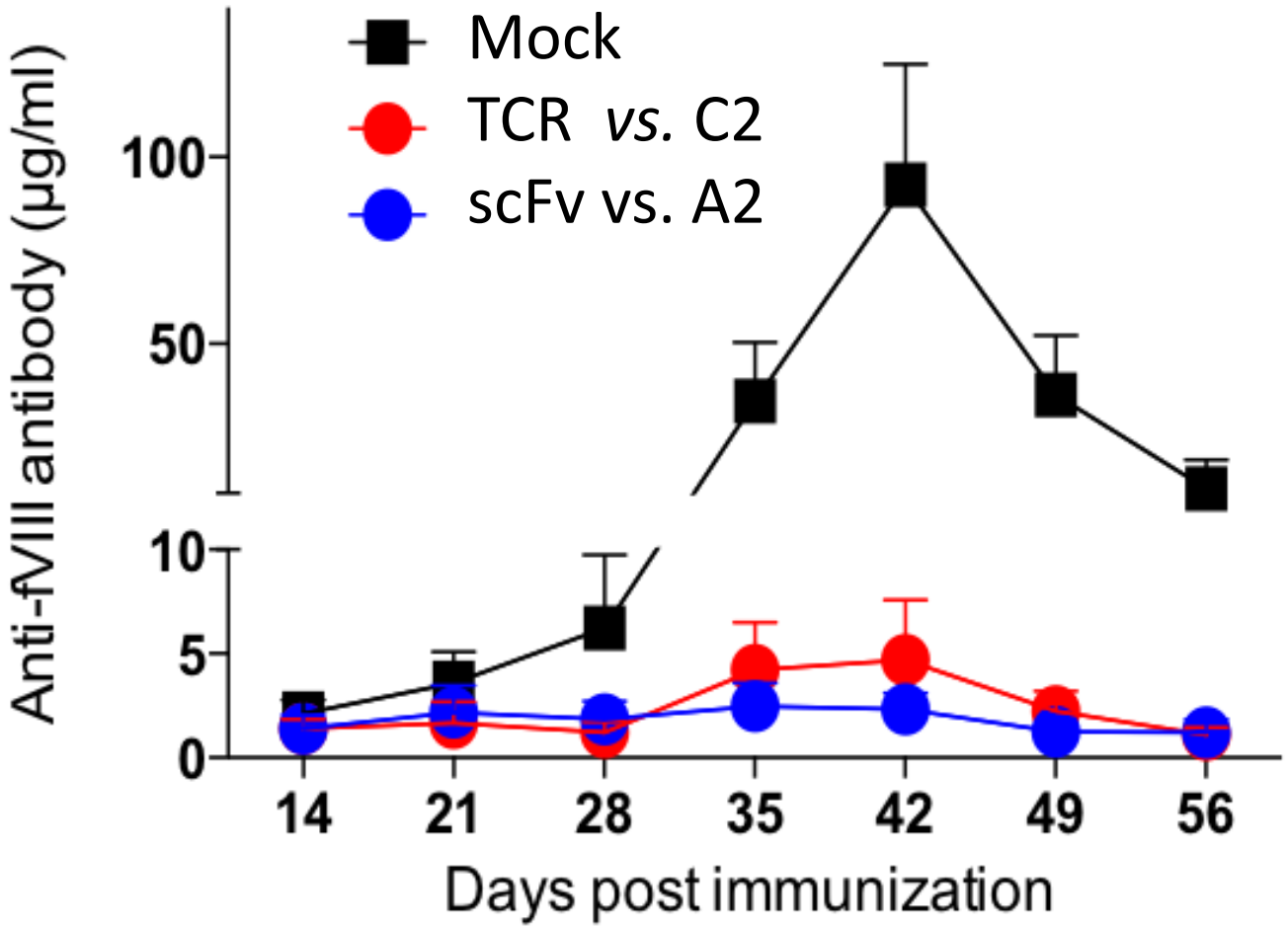
Yoon et al., Blood, **129**: 238-245, 2017.

Anja N. Schmidt

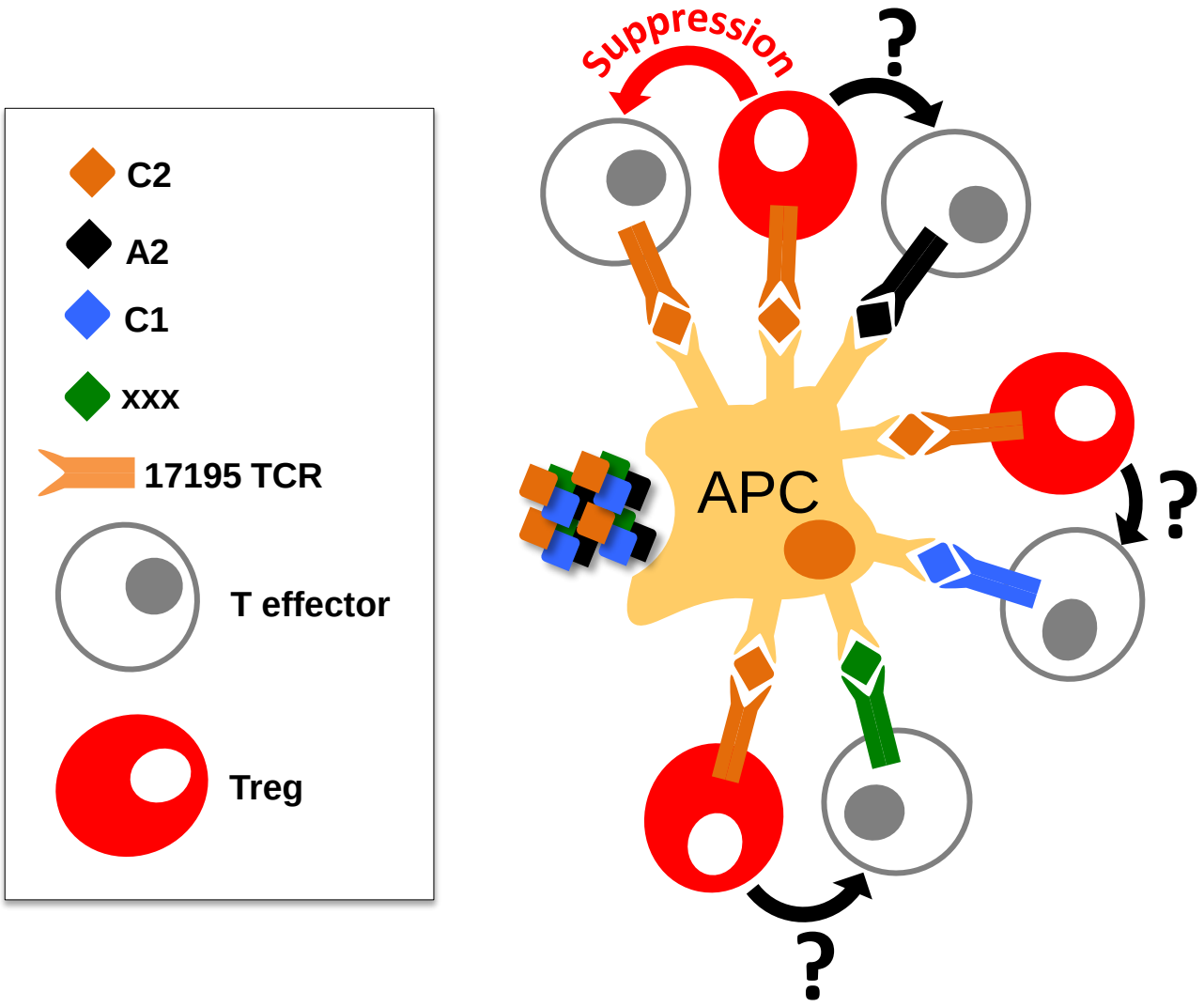


Jeongheon Yoon

TCR- or scFv engineered human Tregs suppress the anti-FVIII response *in vivo*



Scheme for bystander suppression of multiple T-cell clones by a single Treg

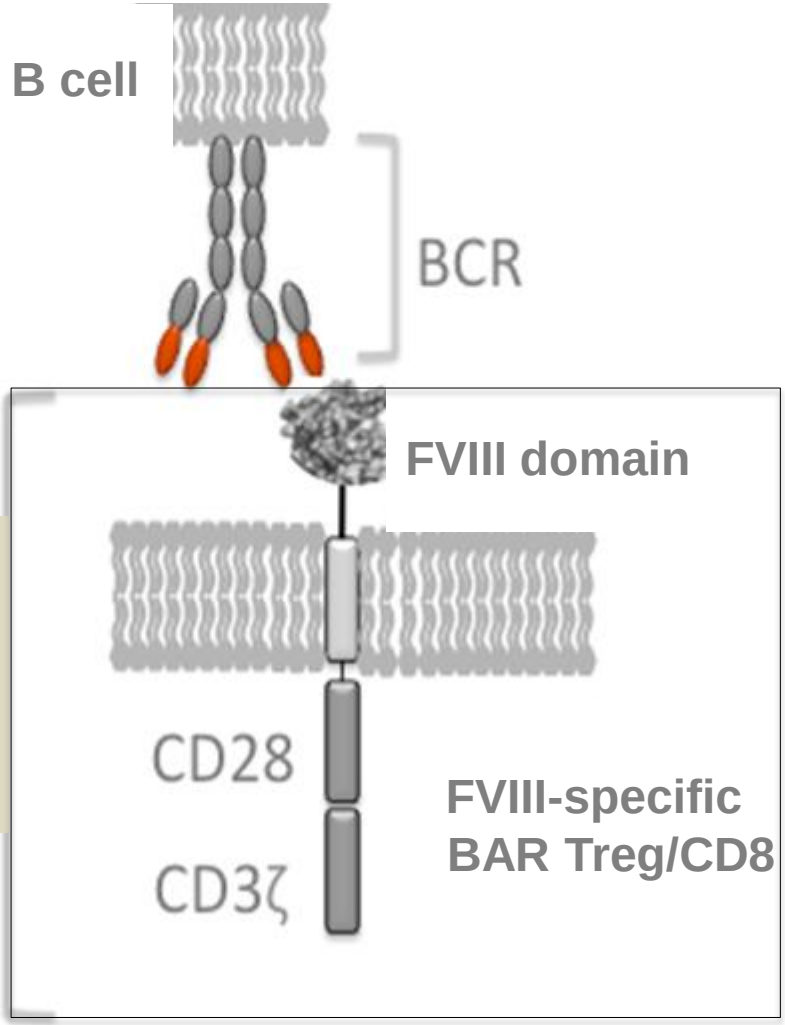


Question:

Can antigen-expressing “BAR” T-cell therapy modulate antibody responses by directly engaging antigen-specific B cells?

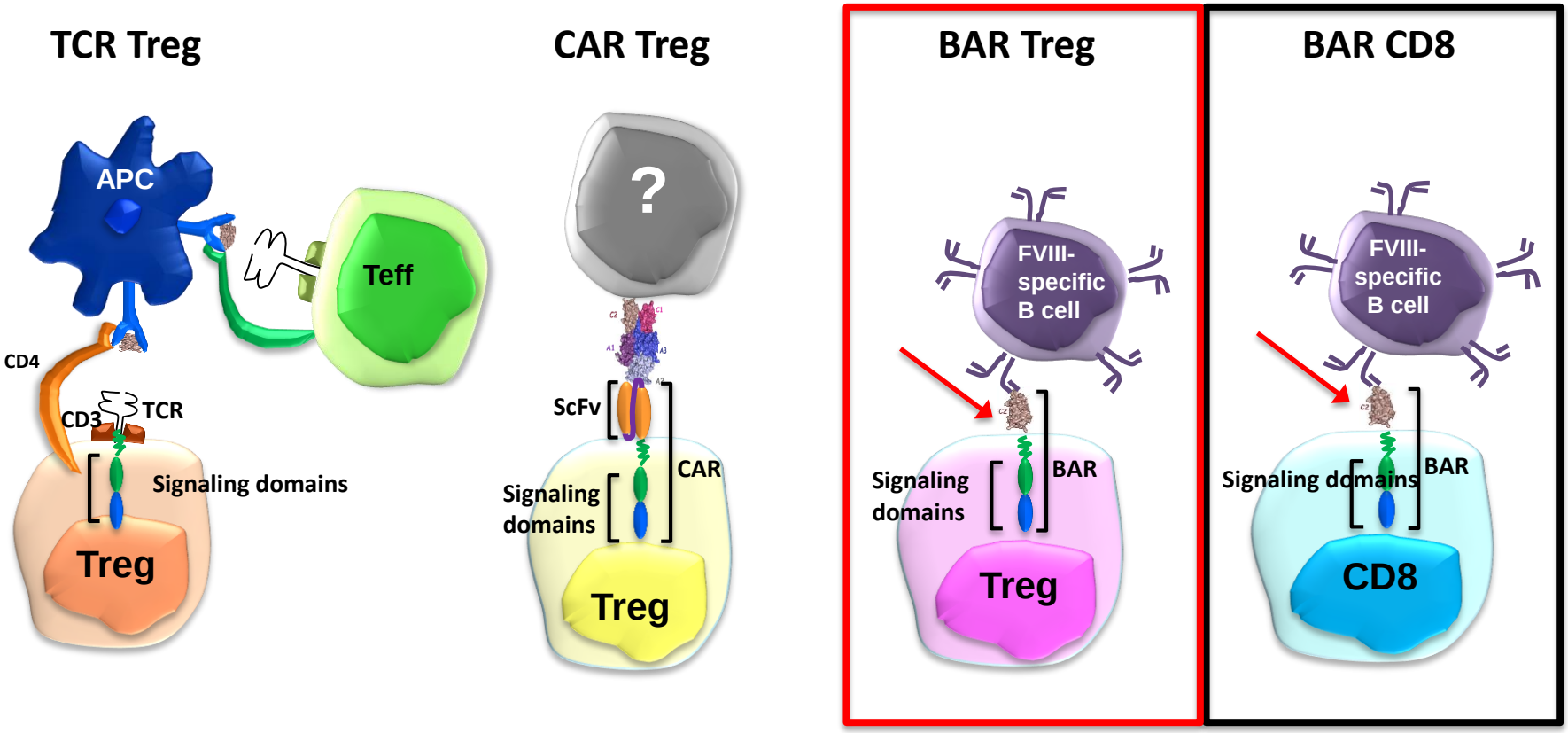
“BAR” = B-cell antibody receptor

Structure of BAR
(B-cell **A**ntibody **R**eceptor**)**
Treg or CD8 cell



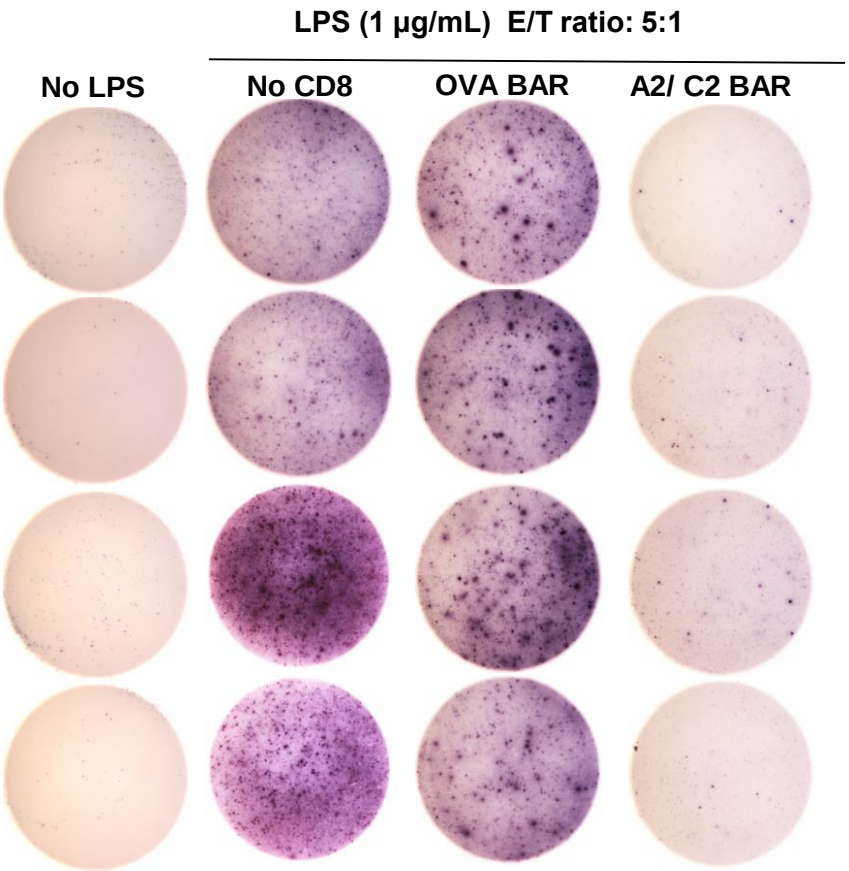
Kalpana Parvathaneni

Engineering antigen-specificity into polyclonal T cells:
<u>Targeting the B cell</u>



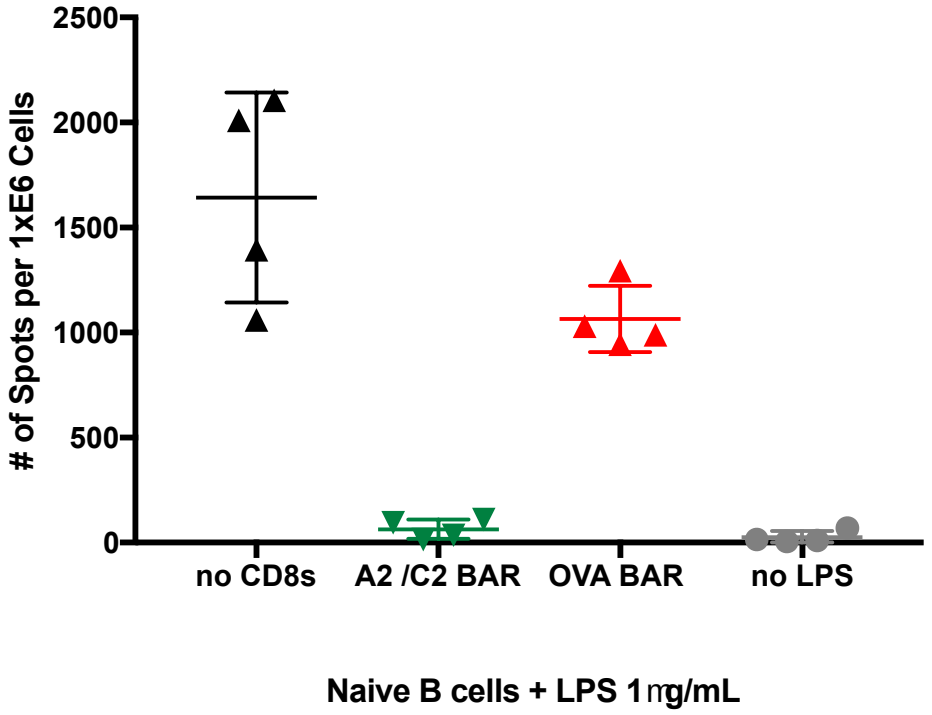
A2/C2 BAR mCD8-mediated elimination of anti-FVIII B cells from E16-mouse spleen cells stimulated with LPS

A. αFVIII IgM+ ELISPOT assay by LPS-stimulated E16 B cells



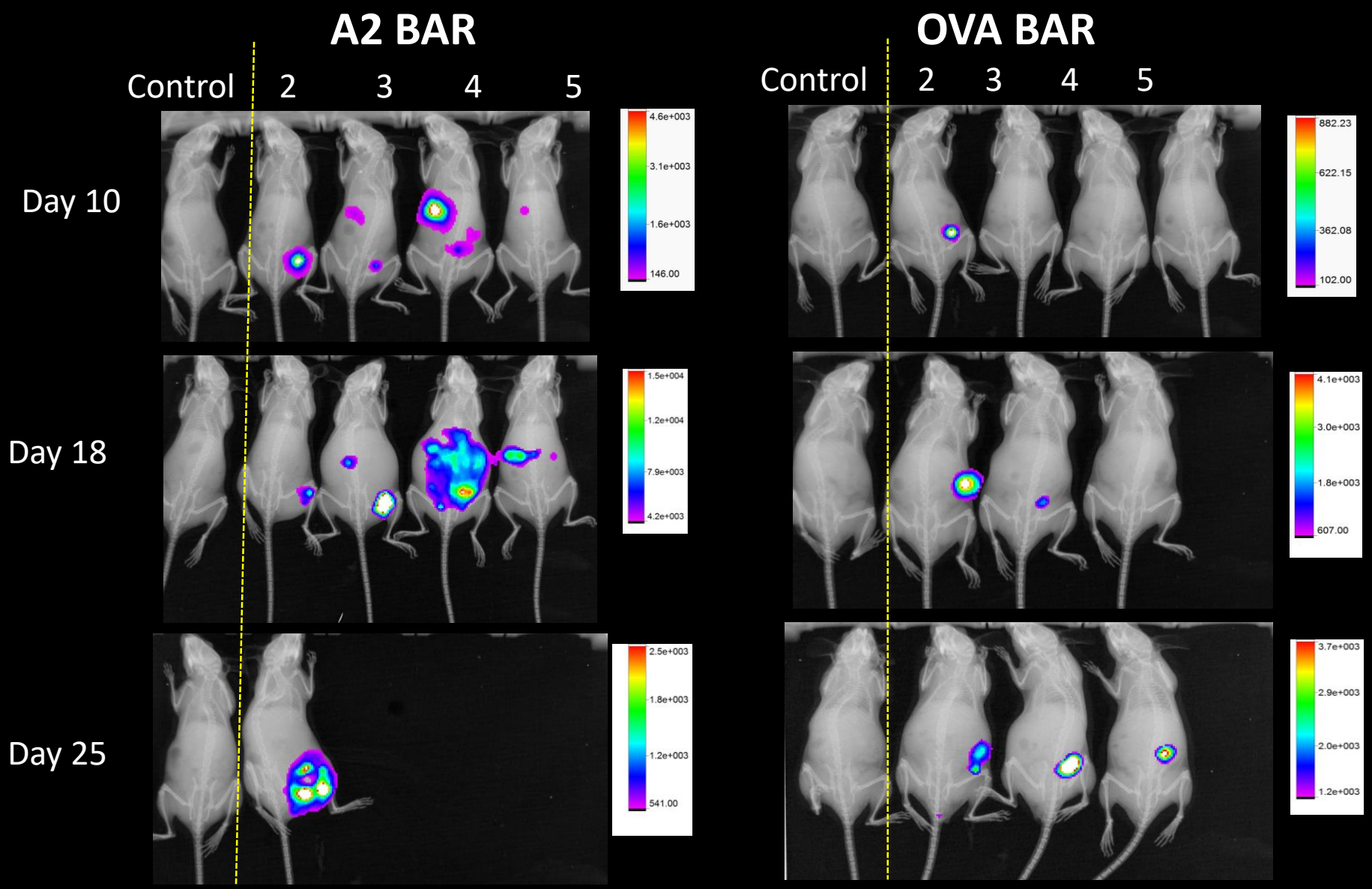
FVIII-coated wells

B. Quantification of number of spots

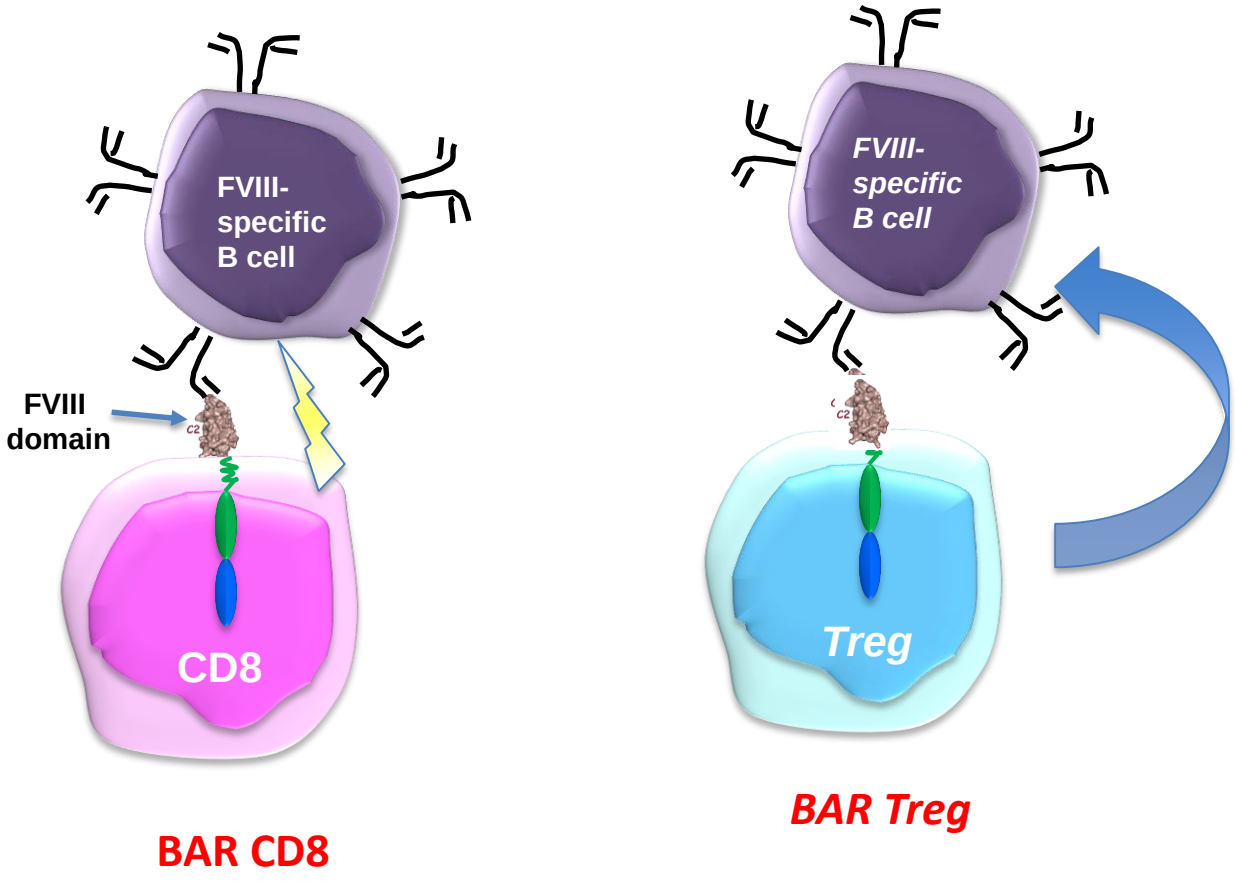


Naive B cells + LPS 1 ng/mL

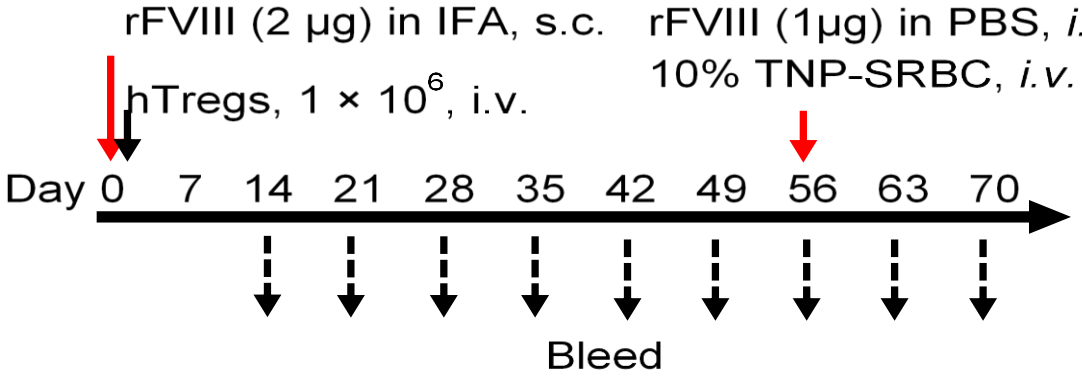
Survival of 2JLO-injected NSG mice with BAR CD8 T-cell therapy

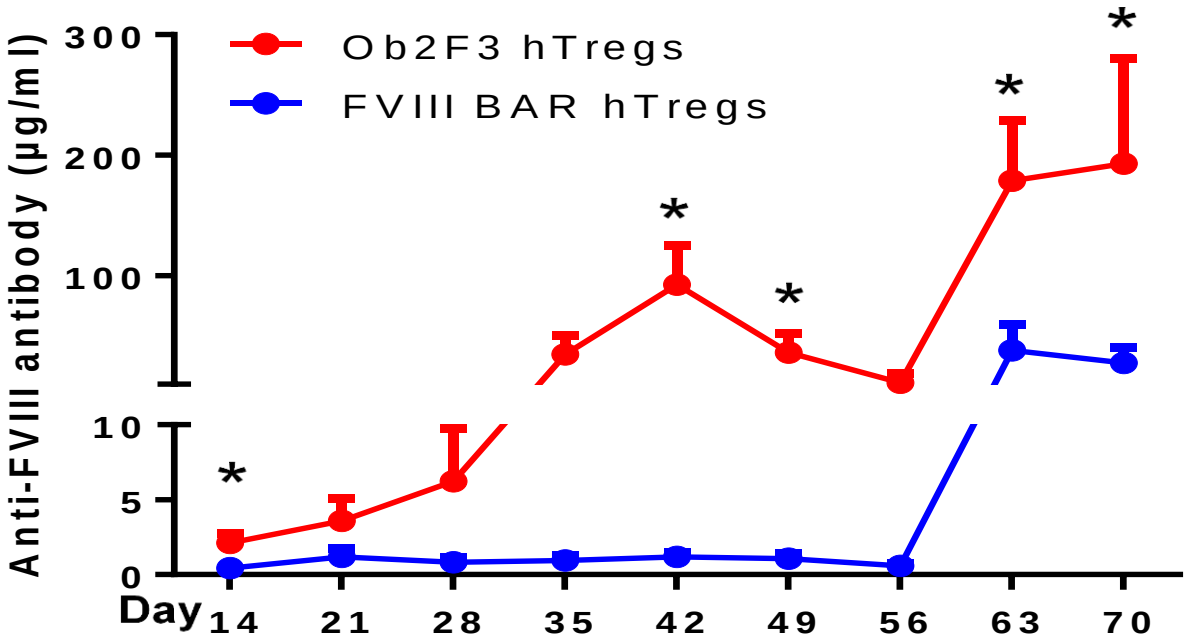


Can "BAR" engineered CD4 Tregs target and suppress FVIII-specific B cells



Prevention of anti-FVIII antibody development in naïve E16 mice by BAR human Tregs in vivo



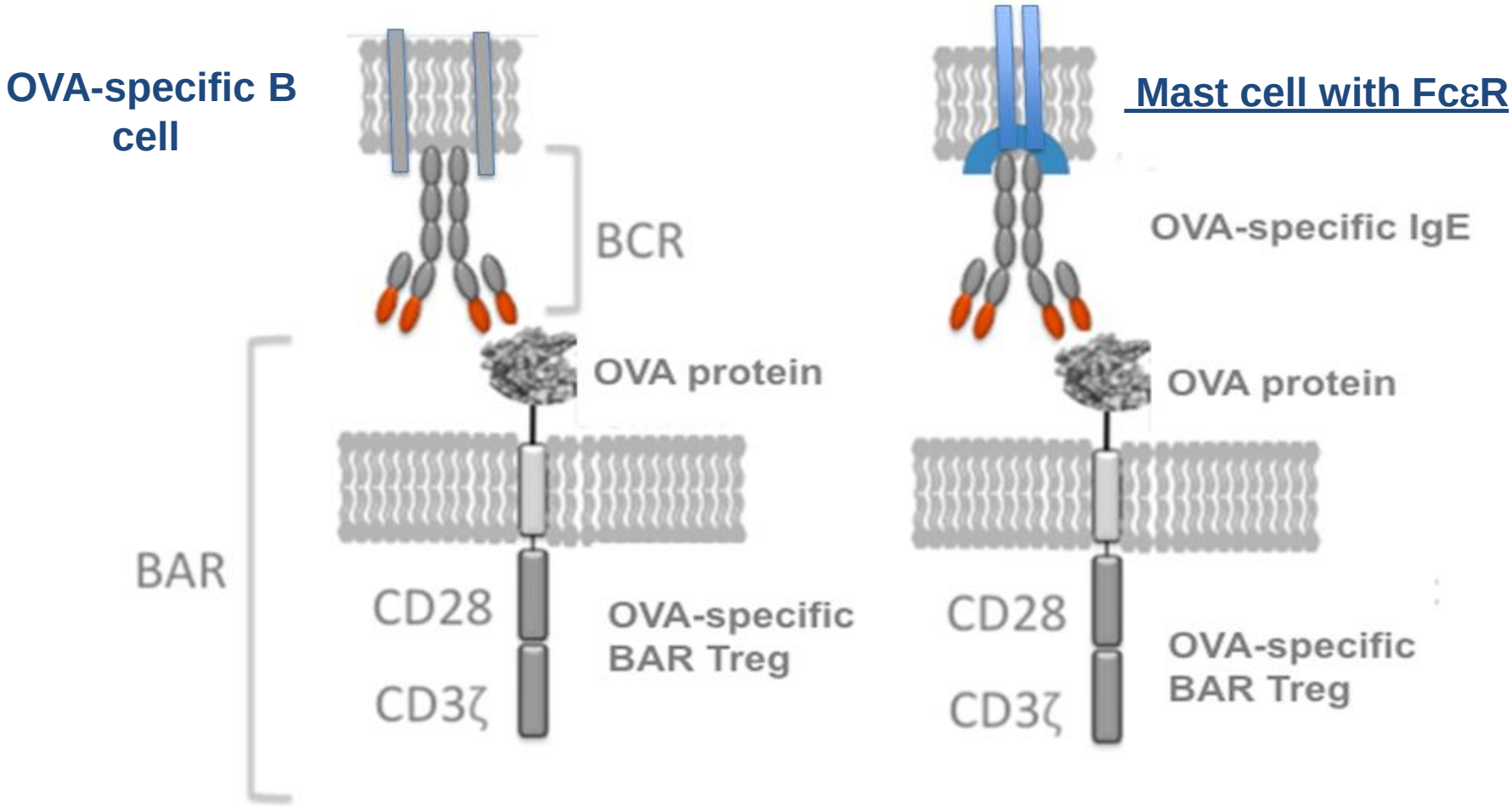


Systems and targets

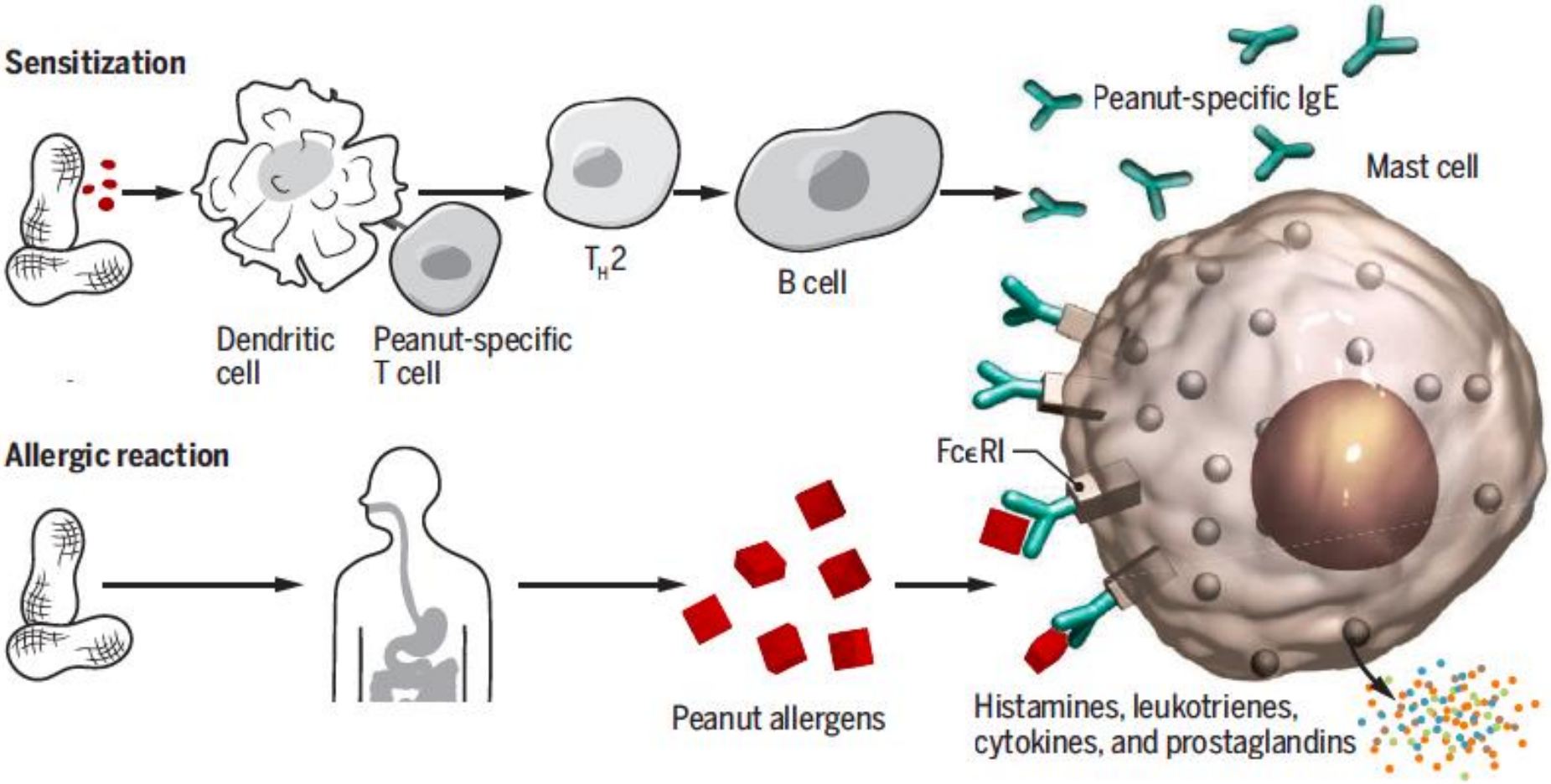
- Multiple systems:

  - ✓ Hemophilia inhibitors (FVIII)
  - ✓ Autoimmunity, e.g., MS (MBP) or Type 1 diabetes
  - ✓ **Allergy (OVA)**
  - ✓ Future targets (ADA's)

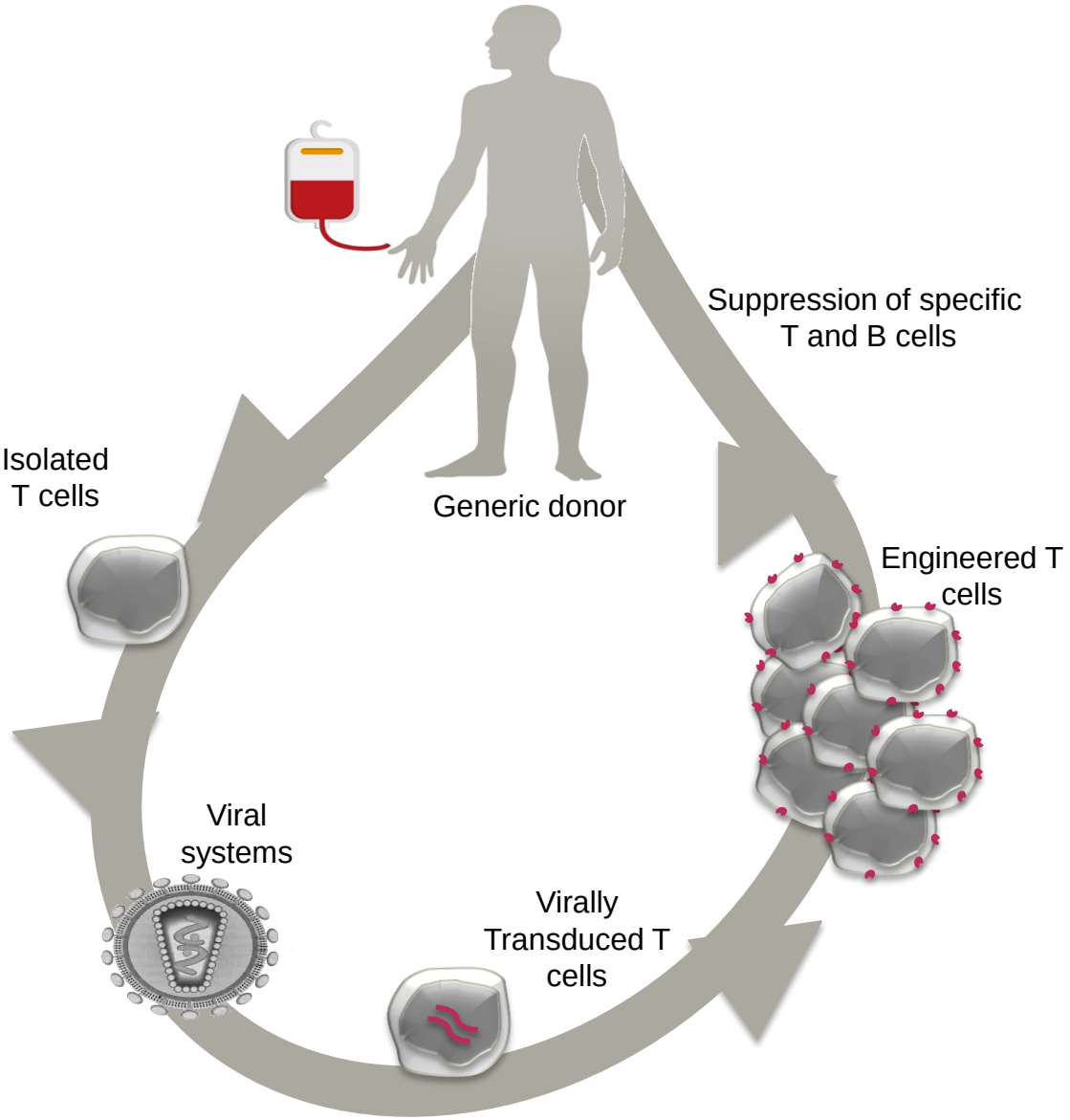
Can BAR Tregs be used to modulate allergy?



Allergic response



Future: CAR or BAR cell therapy not only for hemophilia, but also for allergy, transplantation, autoimmunity or other monogenic diseases (and ADA?)



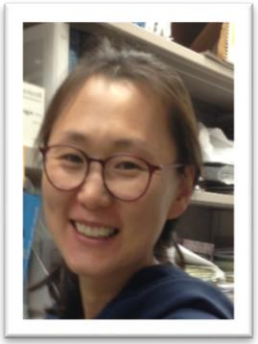
Summary

- Antigen-specific TCRs, single chain Fvs and antigen domains (BARs) have now been engineered for retroviral transduction into human T effectors and human (mouse) Tregs.
- These Tregs specifically suppressed both proliferation and cytokine production by antigen-specific T effectors, and antibody formation *in vitro* and *in vivo* in multiple model systems.
- Recent data with “BAR” CD8’s and Tregs (expressing antigen domains) may allow multiple approaches to regulate adverse immune responses.
  - ✓ e.g., Ovalbumin-BAR iTregs are able to suppress both active and passive anaphylaxis.
- Expansion of these studies to Tregs in a larger species (hemophilic dogs) is in progress, with human clinical studies on the horizon.

Acknowledgements

Yongchan Kim

Allan Zhang



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Obrigado
Gracias
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Dank u wel
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