Treg therapy for IBD

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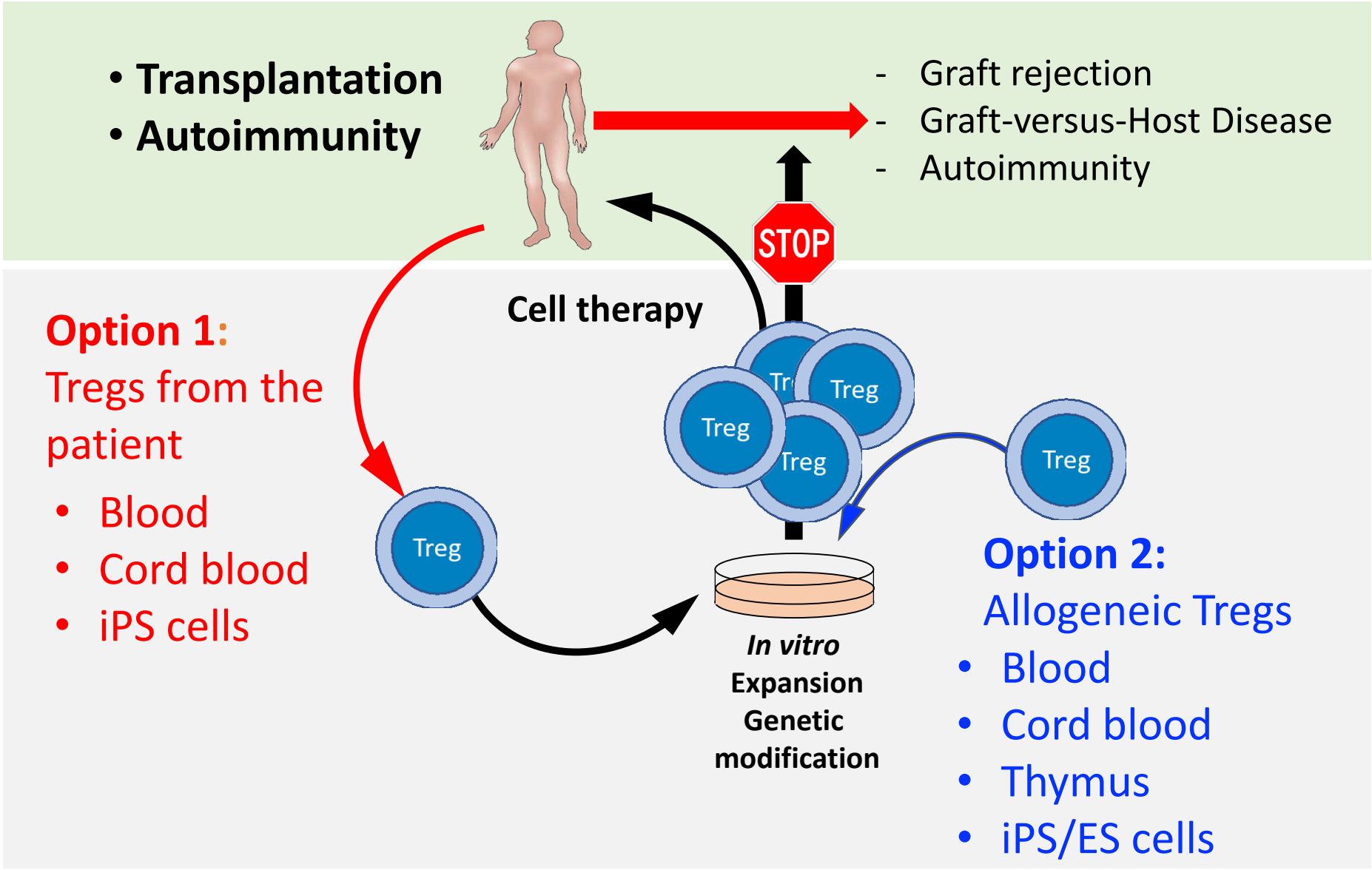
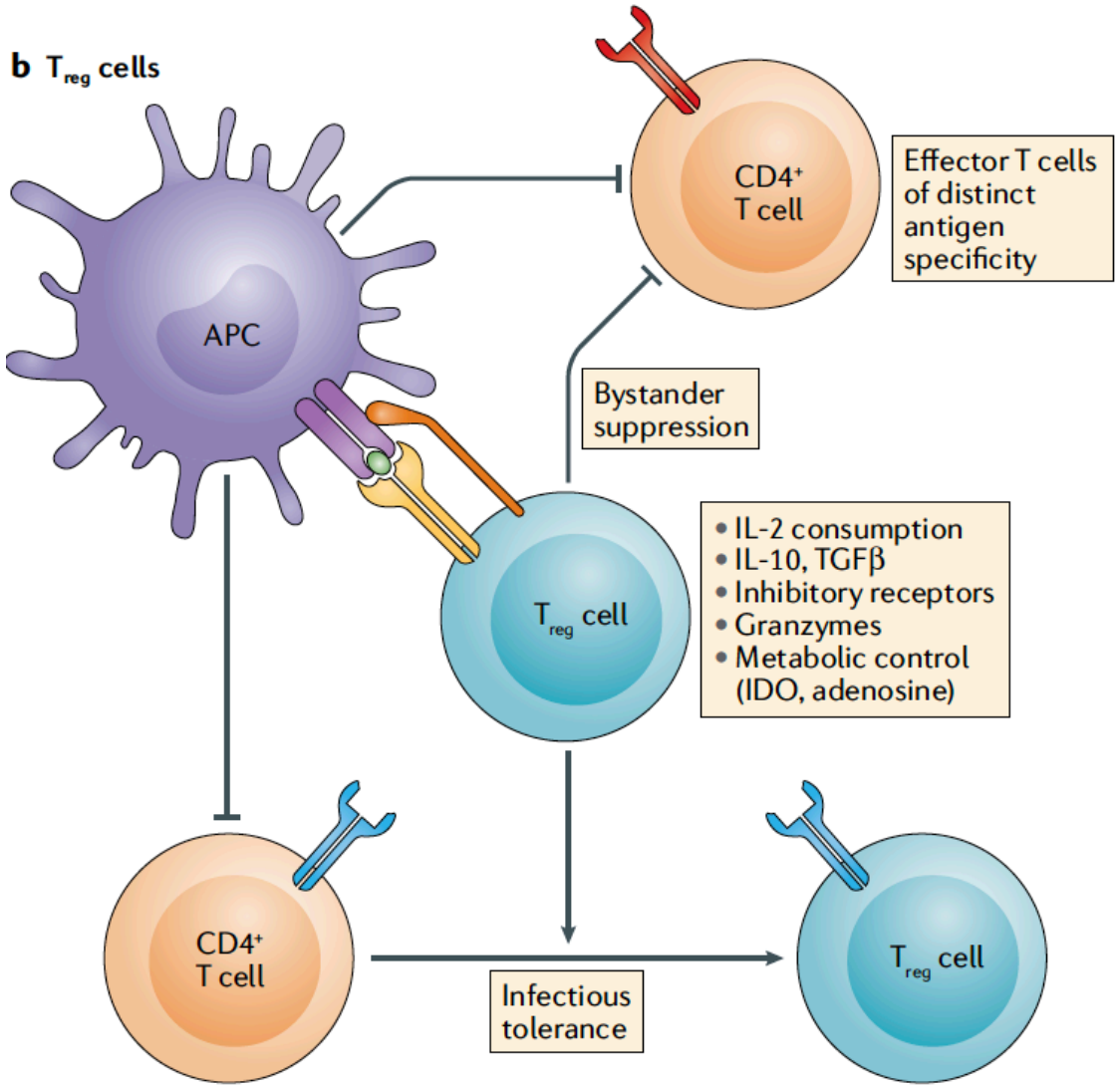
Disclosures

- Hold patents and a licence related to the use of A2-CAR Tregs

Regulatory T cell Therapy to Induce Tolerance



Tregs are living drugs with multiple mechanisms

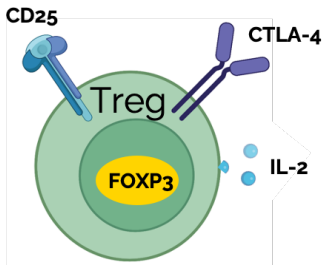


- Multiple modes of action, targeting many immune cell types

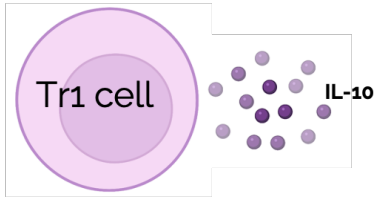
- Key mechanisms of action include:
  - CTLA-4-mediated transendocytosis of CD80/86
  - IL-10/TGF-β-mediated suppression of proliferation/activation
  - CD25-mediated IL-2 consumption
  - CD39-mediated adenosine production

Raffin, Vo, Bluestone, NRI 2020

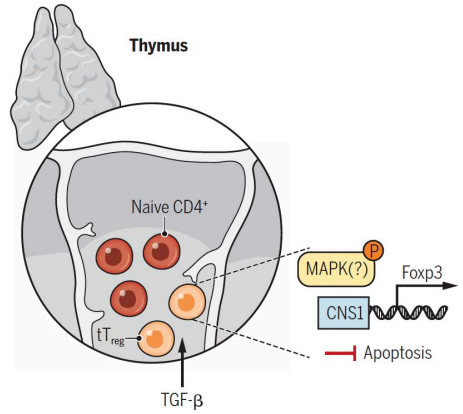
Two main types of Tregs



- FOXP3^{+}, most well-studied, CD25^{high}, CTLA-4+ and secrete low levels of cytokines



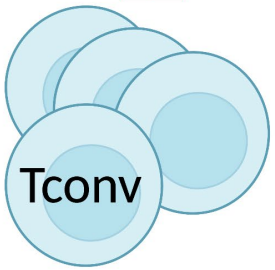
- Type 1 regulatory T cell (Tr1 cell), FOXP3^{neg}, CD25 upon activation, high levels of IL-10 and TGFβ

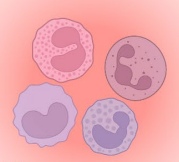


Suppress adaptive immunity

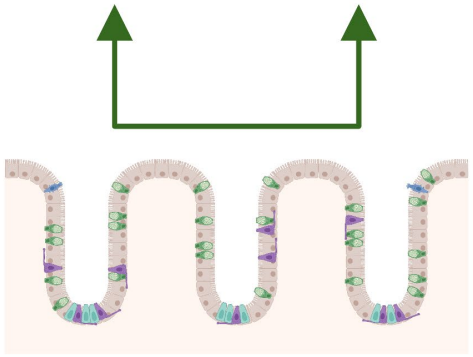
Suppress innate immunity

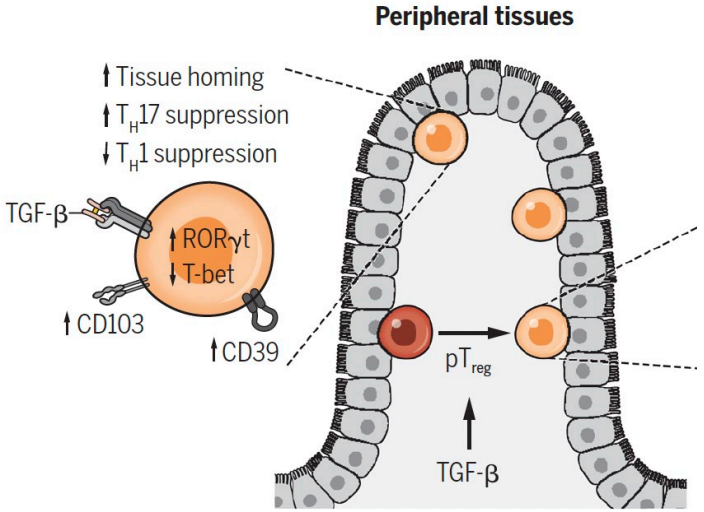
Promote intestinal barrier function



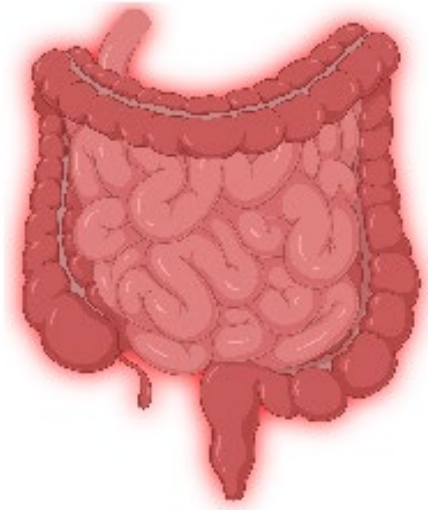


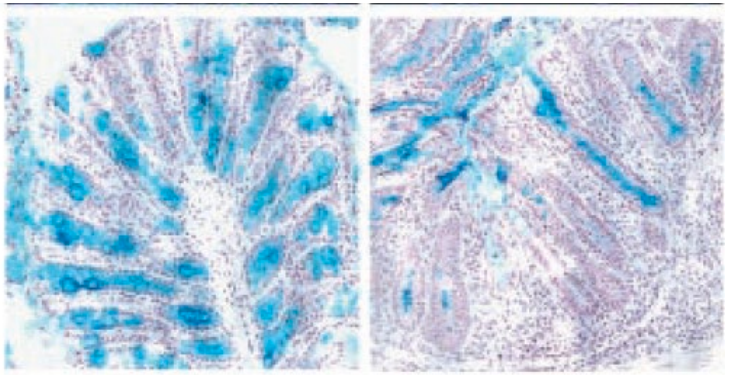
Inflammation



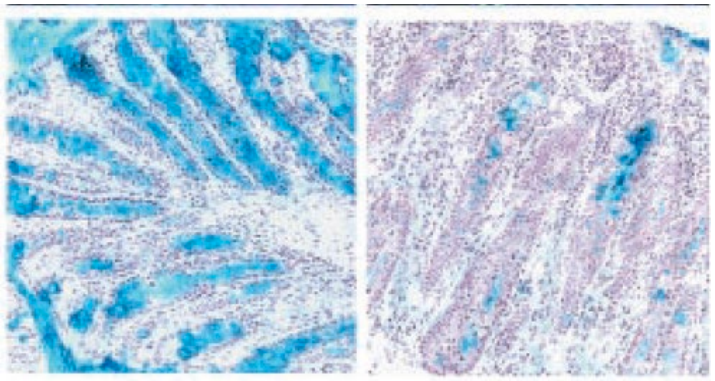


Treg therapy in IBD





+Tregs
Day 0



+Tregs
Weeks 5-14

Mottet, Uhlig, Powrie
J Immunol 2003

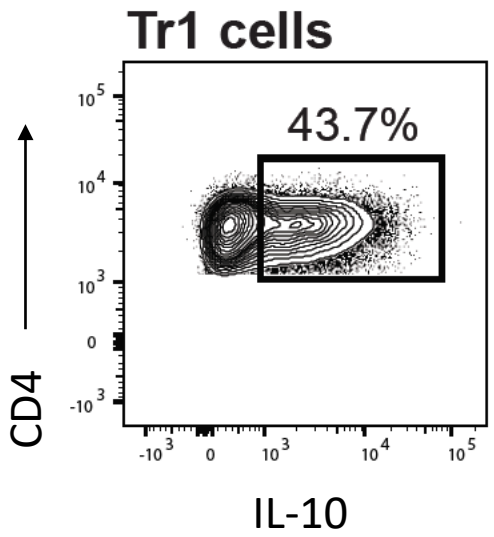
- Treg (Tr1) therapy is safe and potentially efficacious in CD patients (N=20, response in 40%) (Desreumaux, Colombel et al, Gastro 2012, PMID: 22885333)
- N=1 successful Treg therapy in UC+PSC (Atreya, Neurath, Gut 2022 PMID: 35428657)
- TRIBUTE RCT trial of Tregs (NCT03185000) due to start (Lord et al)

Application of Tr1 cells as a therapy for IBD

Tr1 cells as an IBD therapy:

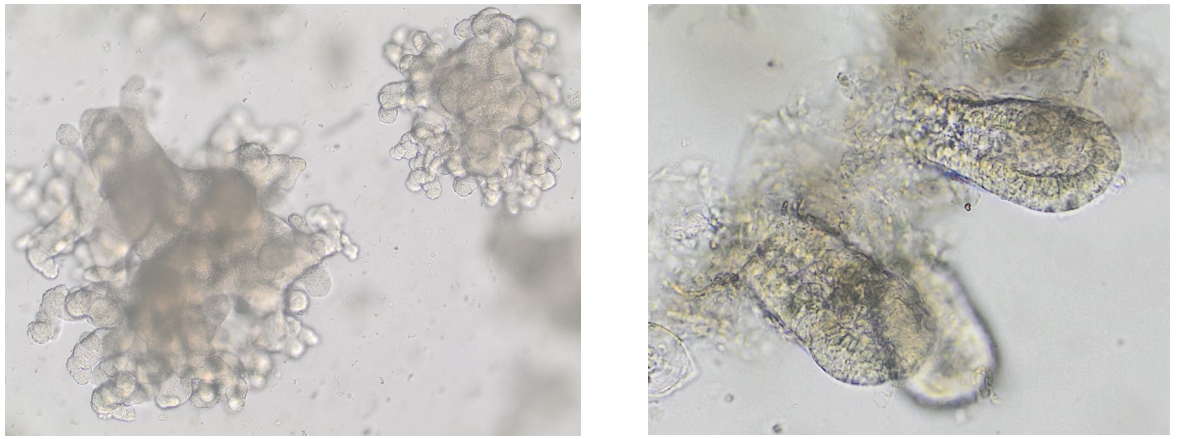
- ✓ Robust method for isolation
- ✓ Can be expanded without loss of phenotype
- ✓ Suppression of adaptive immune responses (T cell proliferation)
- ✓ Suppression of innate immune responses (LPS/ATP-activated monocytes)

Cook, Steiner, Levings et al, Gastroenterology 2019



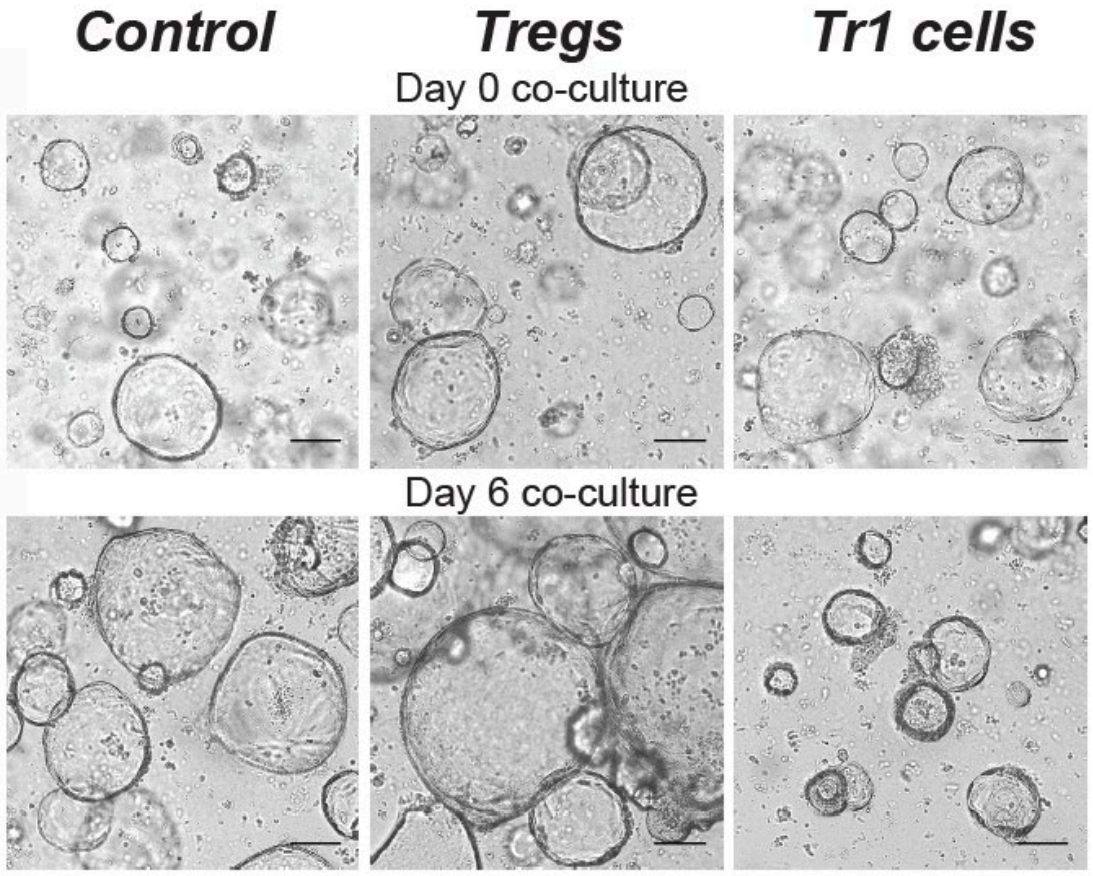
Effect on non-immune intestinal epithelial cells??

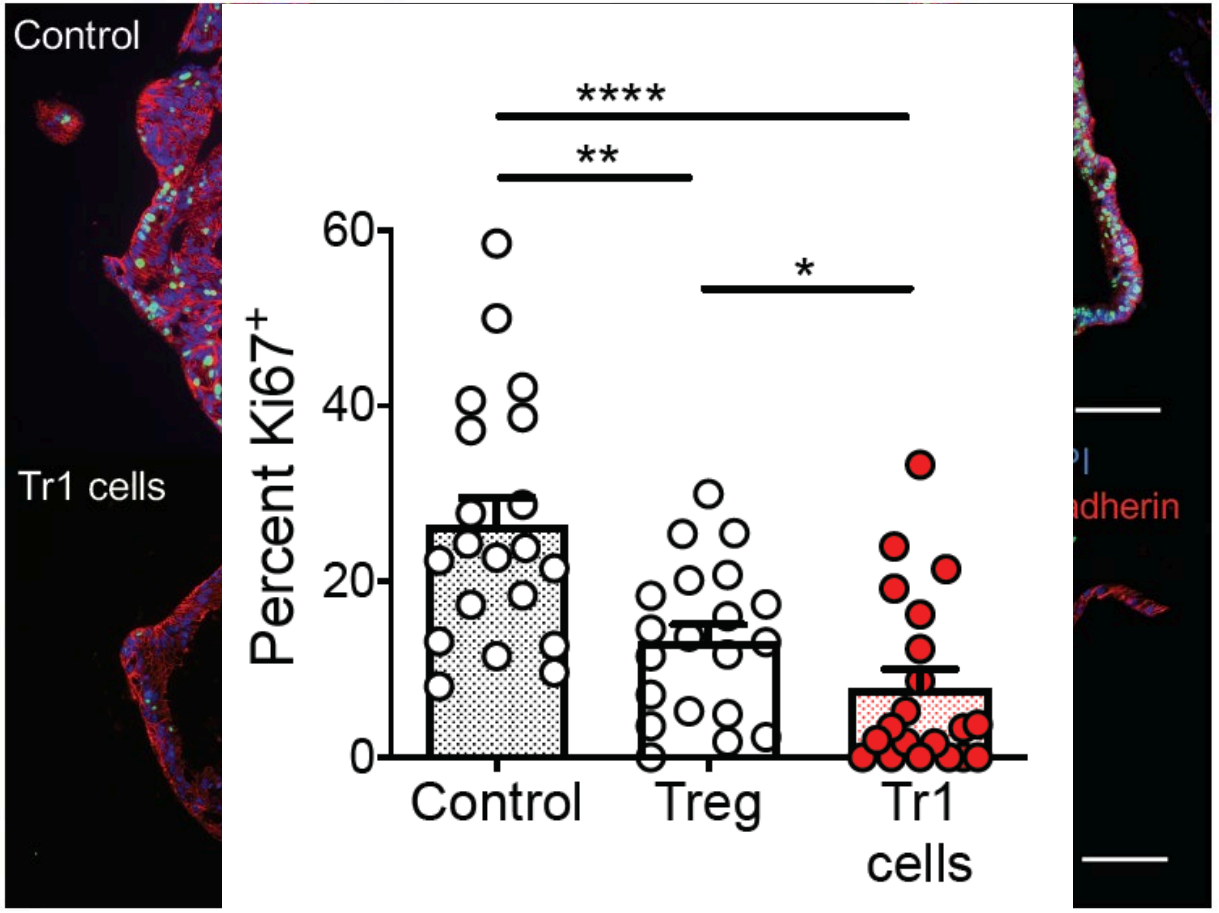
Effects of Tr1 cells on colonic organoids



Collaboration with B. Vallance @ UBC

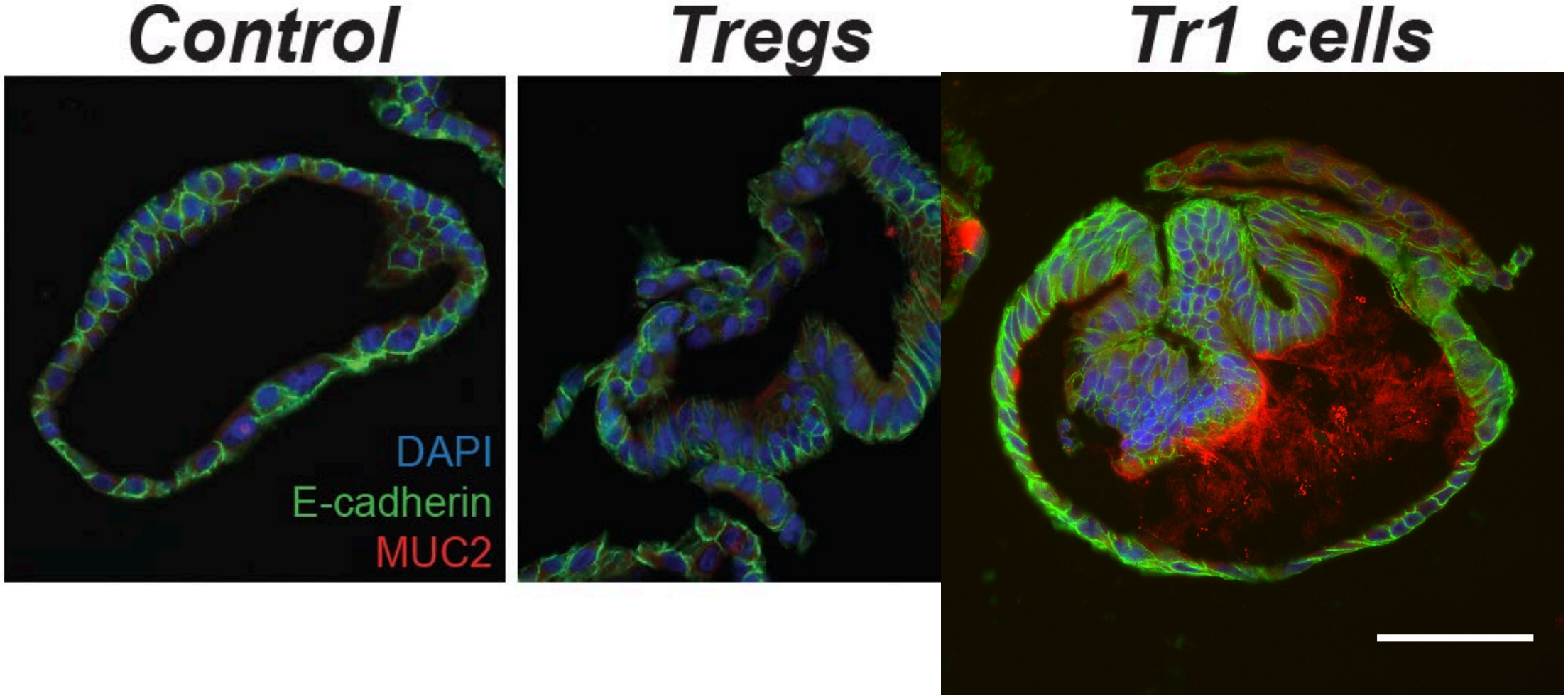
Tr1 cells promote intestinal epithelial cell differentiation





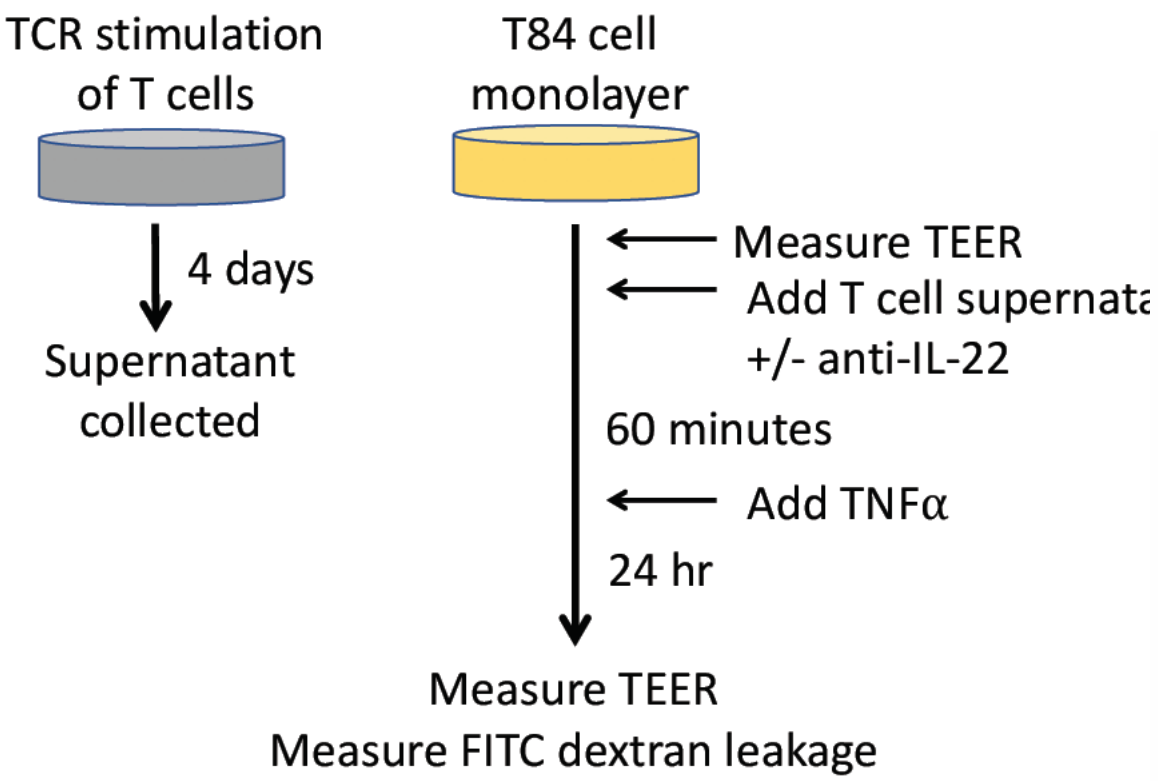
Cook et al, Gastroenterology 2019

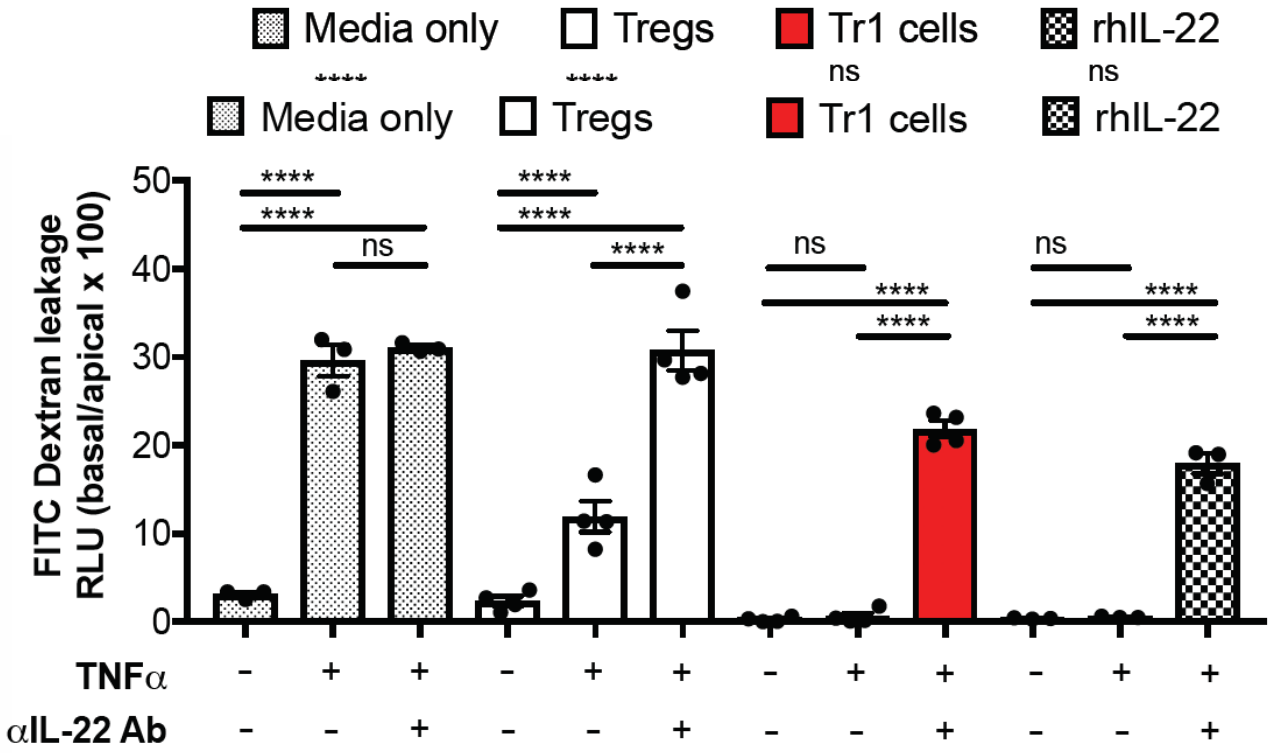
Tr1 cells promote goblet cell differentiation



Cook et al, Gastroenterology 2019

Tr1 cells promote barrier function



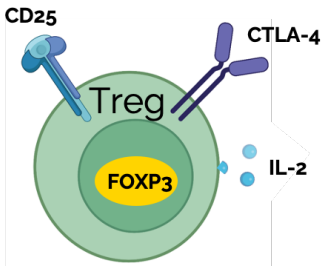


Cook et al, Gastroenterology 2019

Summary Part 1

- In vivo differentiated human Tr1 cells can be isolated on the basis of their rapid IL-10 production
- Ex vivo expanded Tr1 cells retain their cytokine phenotype
- Both Tr1 and Tregs suppress T cell proliferation and IFN-γ production
- As with mouse Tr1 cells, human Tr1 cells suppress innate immune responses
- Tr1 cells secrete factors which affect epithelial cells in colonic organoids and monolayers by:
  - increasing mucus production (primarily MUC-2, effects consistent with goblet cell differentiation)
  - promoting barrier function via IL-22

Two main types of Tregs



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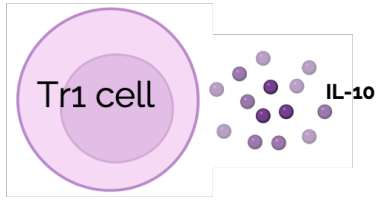

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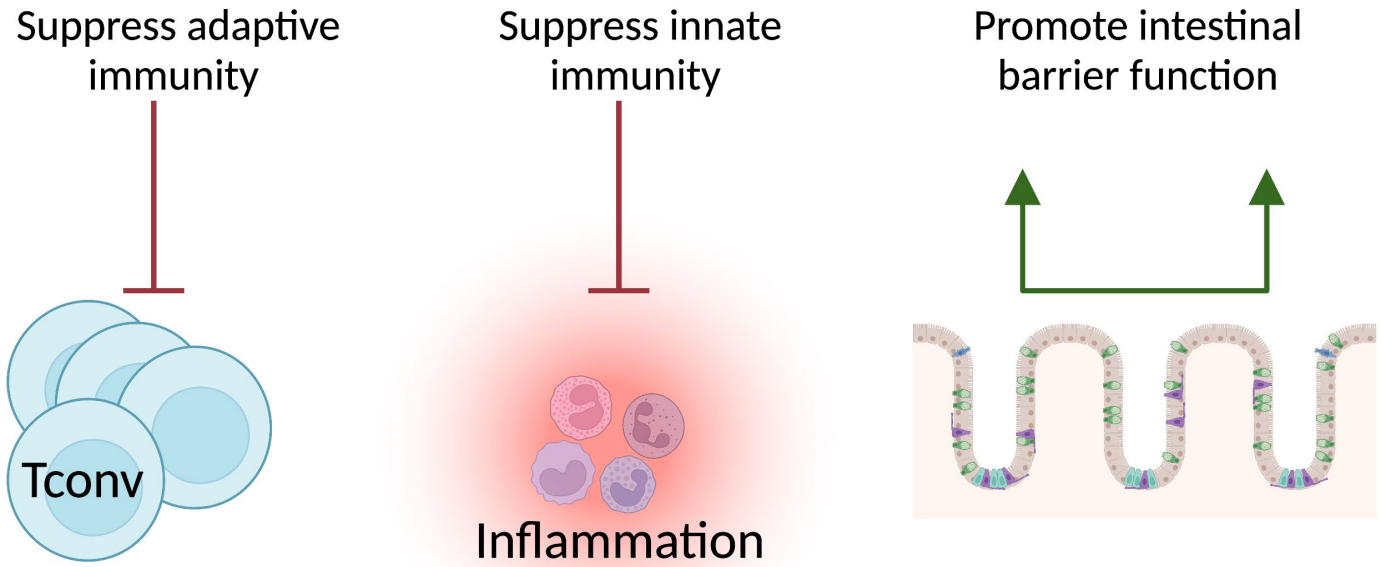
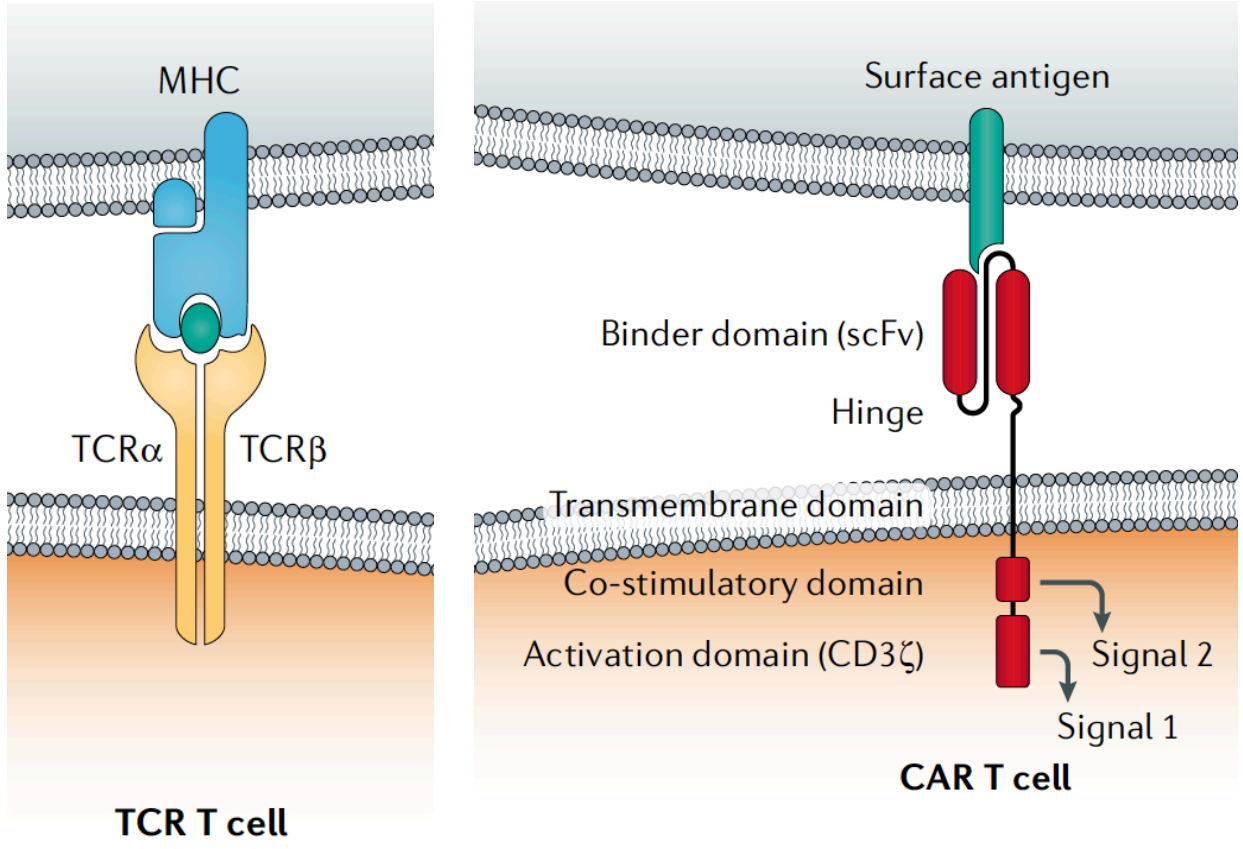


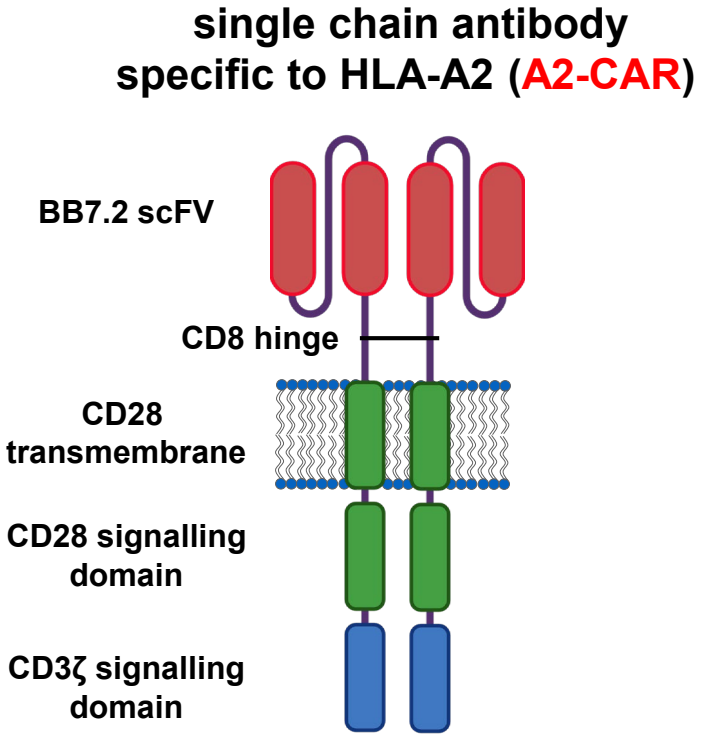
Controlling Treg activity: TCRs and CARs

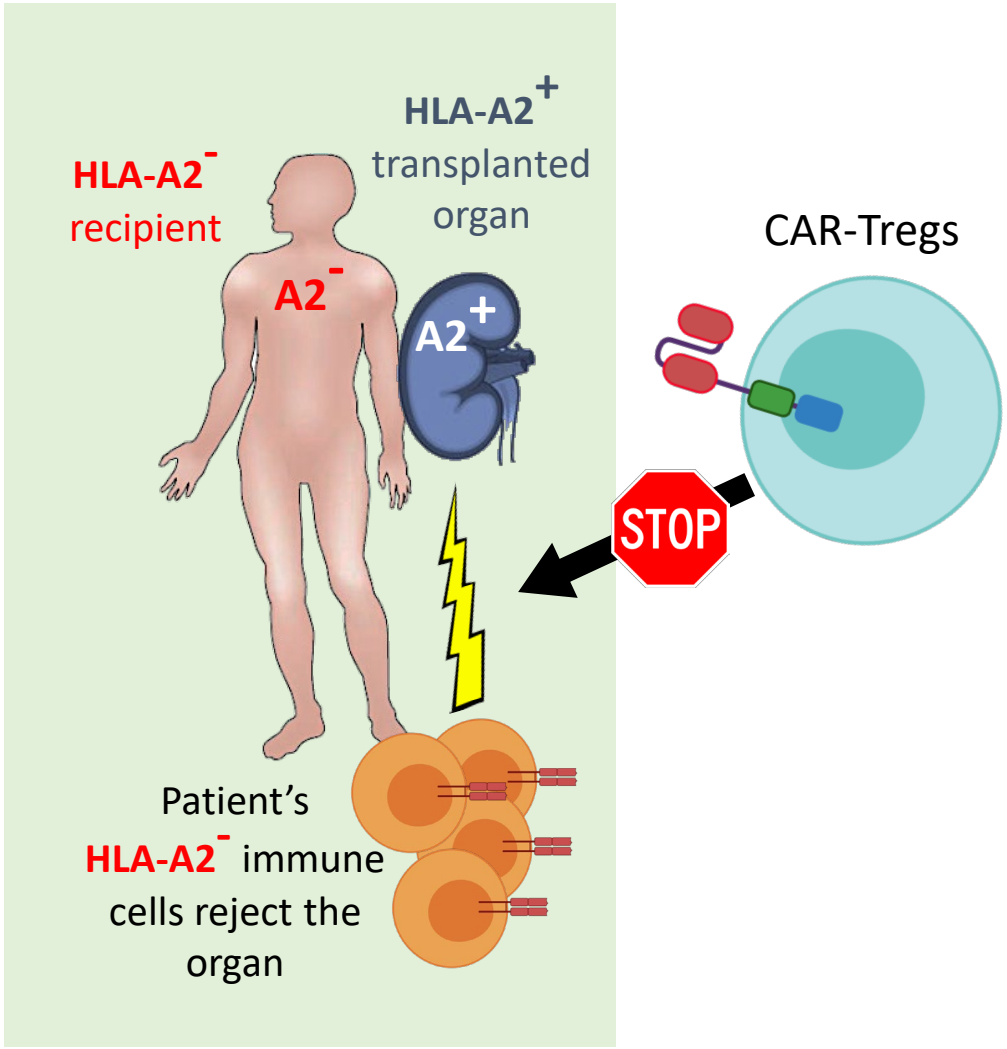


	TCR	CAR
Affinity	Low	High/Variable
Co-stim	Dependent	Independent
Specificity	Cross-reactive	High
Endogenous TCR	Mis-pairing	Independent (usually)
Types of Ags	Intracellular + extracellular	Membrane bound/oligomeric
MHC	Restricted	Usually not

Ellis, Sheppard, Riley, Nat Rev Genetics 2021

Alloantigen-specific CAR Tregs

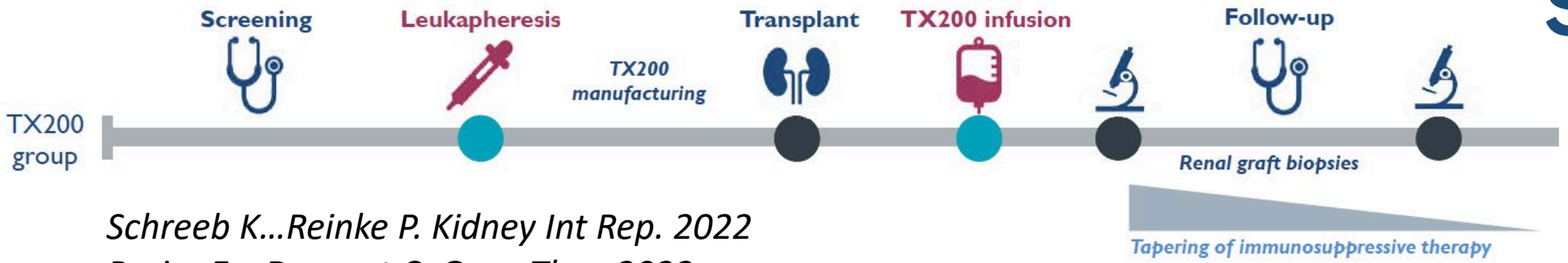




- *MacDonald et al, JCI 2016; Boardman et al, AJT 2017, Noyan et al AJT 2017.*
- *Dawson, Lamarche et al, JCI Insight 2019*
- *Dawson et al, STM 2020*
- *Sicard, Lamarche et al, AJT 2020*

Clinical trials of A2-CAR Tregs

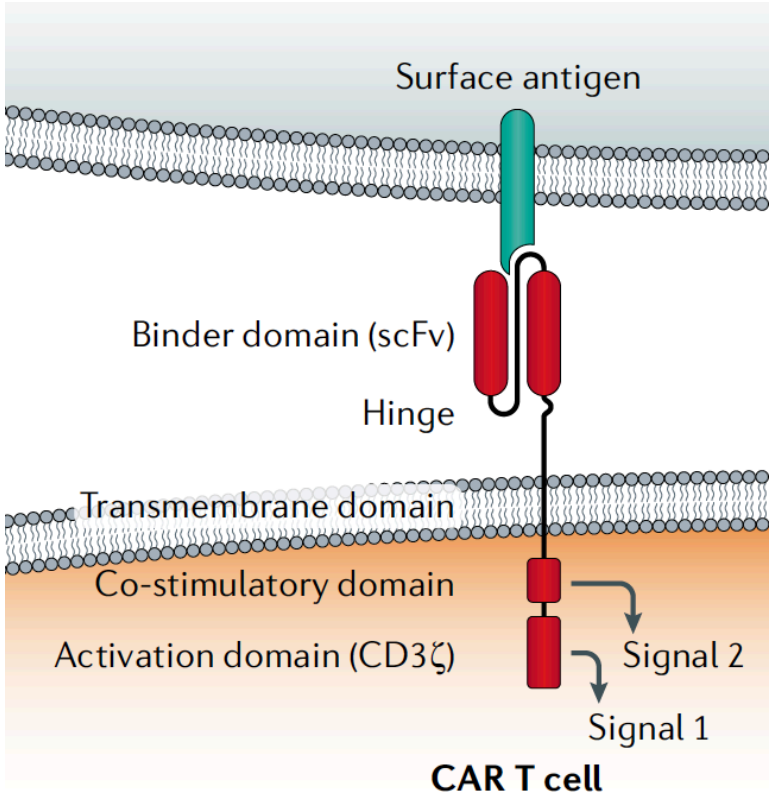
- **STEADFAST Phase I/IIa Study: Multicentre, open-label, single ascending dose, dose-ranging (NCT04817774)**



Schreeb K…Reinke P. Kidney Int Rep. 2022
Proics E….Dumont C, Gene Ther. 2022

LIBERATE Phase 1/2 clinical trial: investigate the safety and efficacy of QEL-001, in 33 HLA-A2 mismatched liver transplant patients (NCT05234190)

Antigen-specific Tregs for use in IBD: *proof of concept*



TNP-specific CAR Tregs

- Adoptive transfer protects mice from TNBS colitis
- Cells migrate rapidly (hours) to TNBS-induced mucosal lesions

Elinav, Eshhar, Gastro 2008 and 2009

CEA-specific CAR Tregs

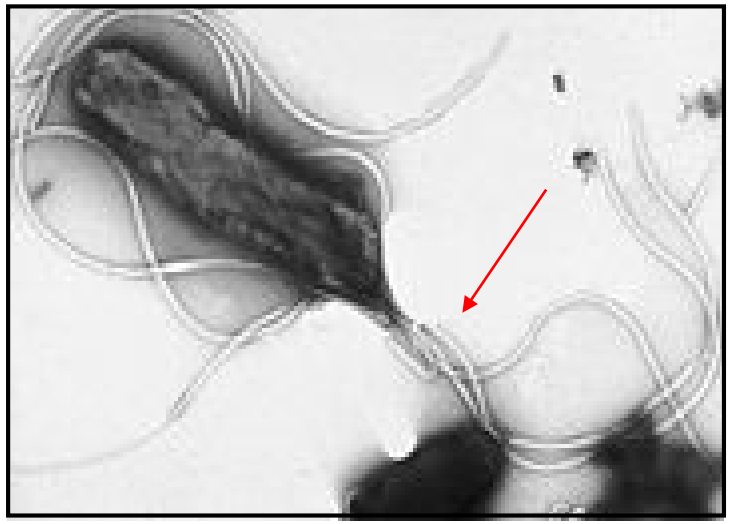
- Suppress T-cell-transfer colitis
- Suppress azoxymethane-dextran sodium sulfate model of colitis-associated colorectal cancer (bystander)

Blat, Eshhar, Molecular Therapy 2014

Antigens driving maladaptive immunity in IBD

Bacterial antigens: Flagellin

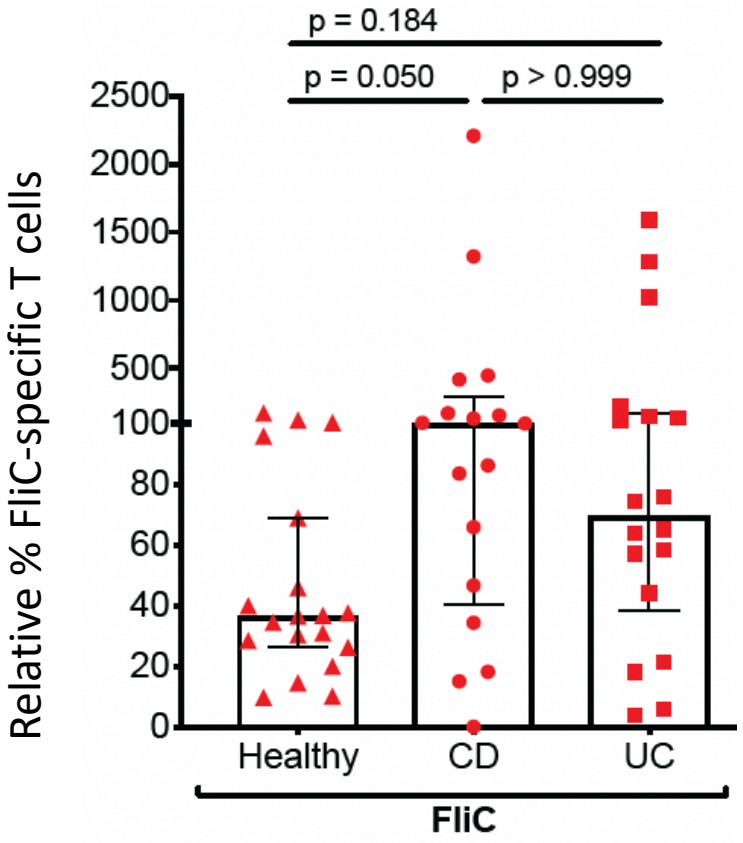
- Present on all flagellated bacteria
- Readily shed into gut environment
- Translocates across epithelial cell barrier
- Naturally oligomeric
- *Some* forms stimulate TLR5



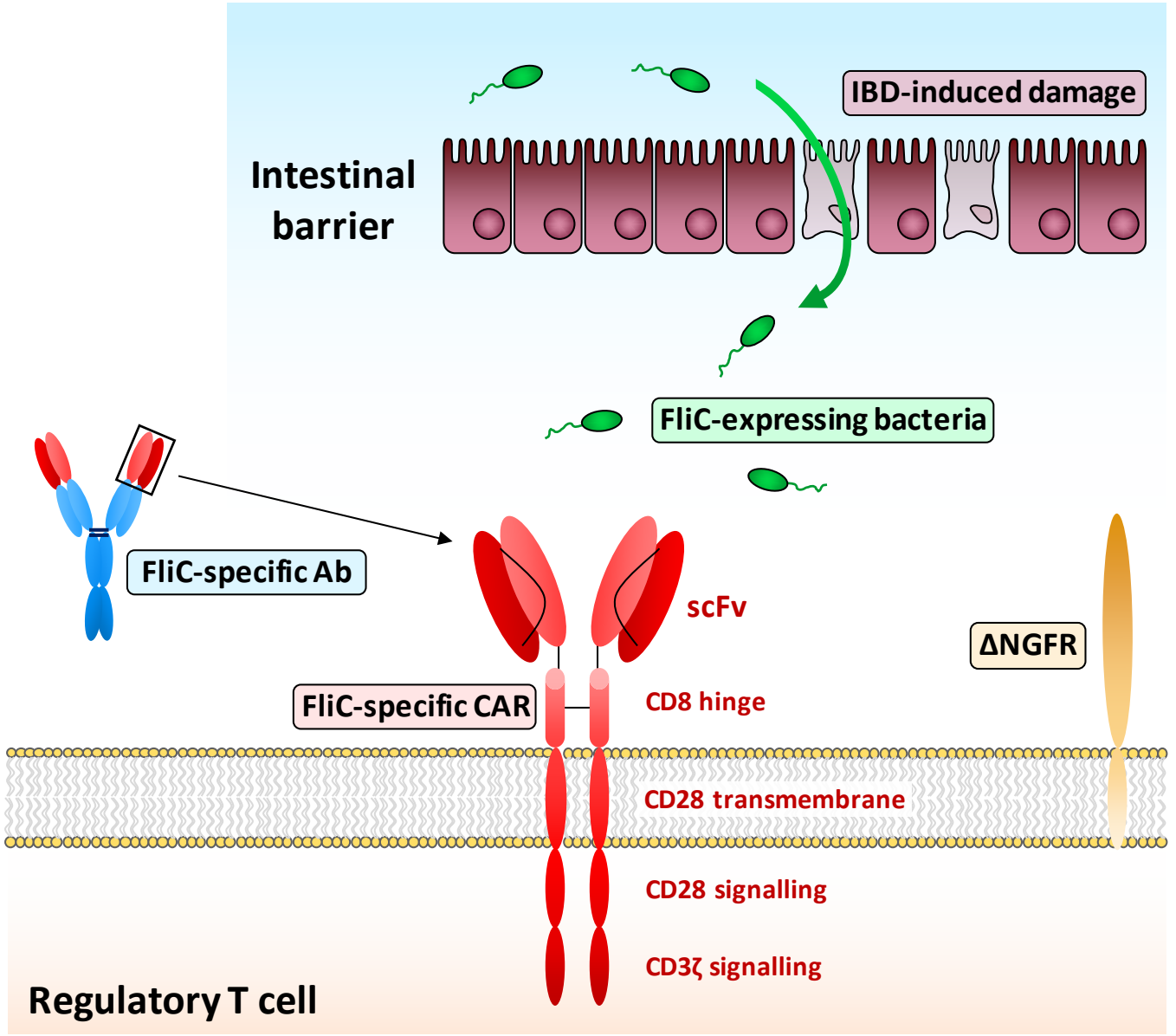
Subset of patients with Crohn's disease:

- Elevated levels of flagellin reactive antibodies *(Lodes 2004; Gewirtz 2005)*
- Anti-flagellin Abs from Clostridium subphylum XIVa including Cbir, FlaX and A4-Fla2

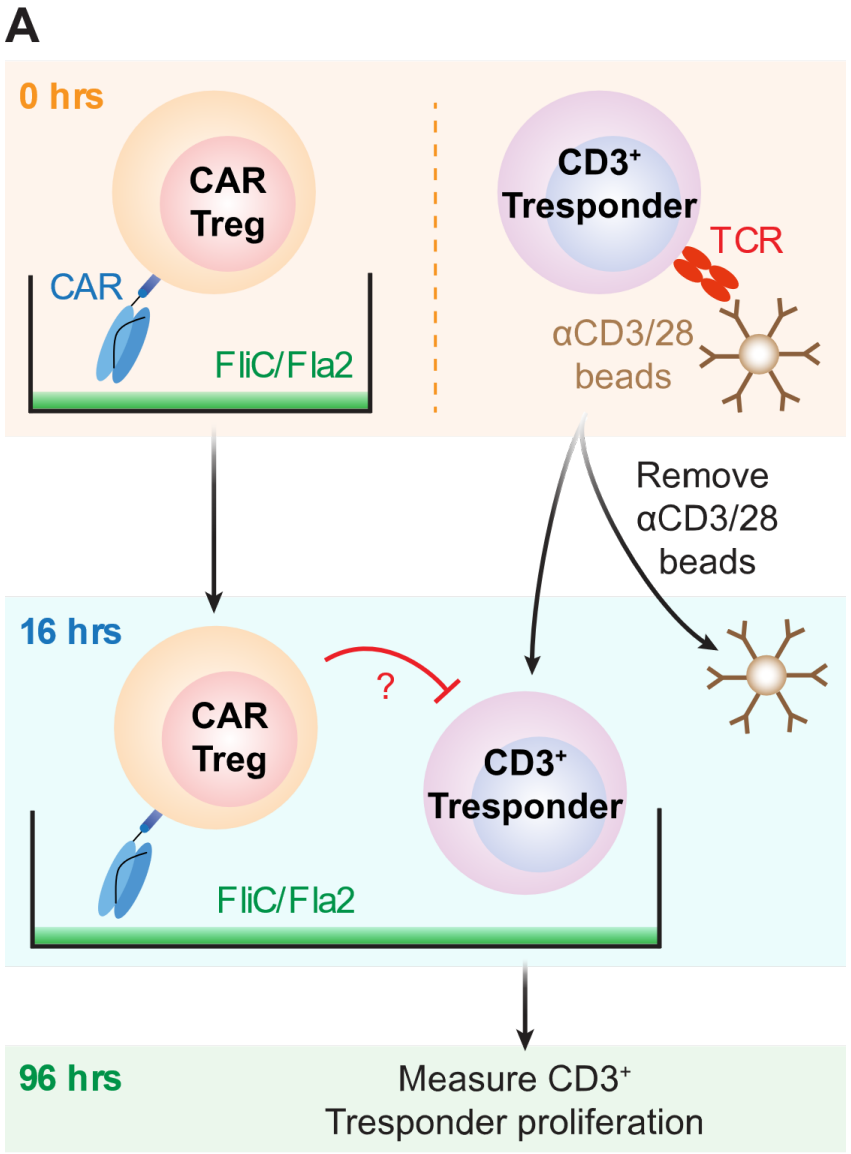
Antigen-specific Tregs for use in IBD – humans

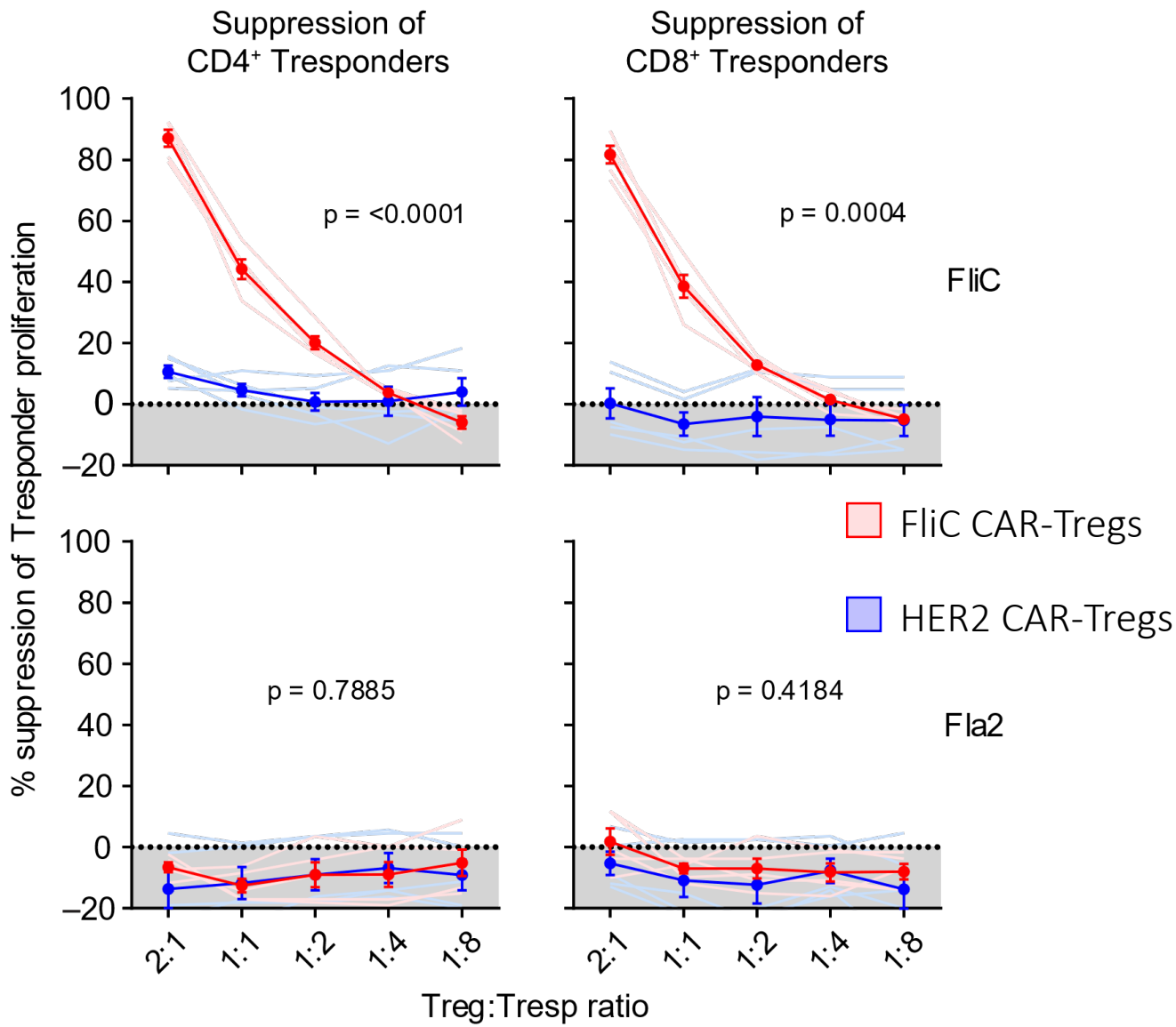


Cook L, Levings, Steiner.
CMGH (2019) 9(3):485-506

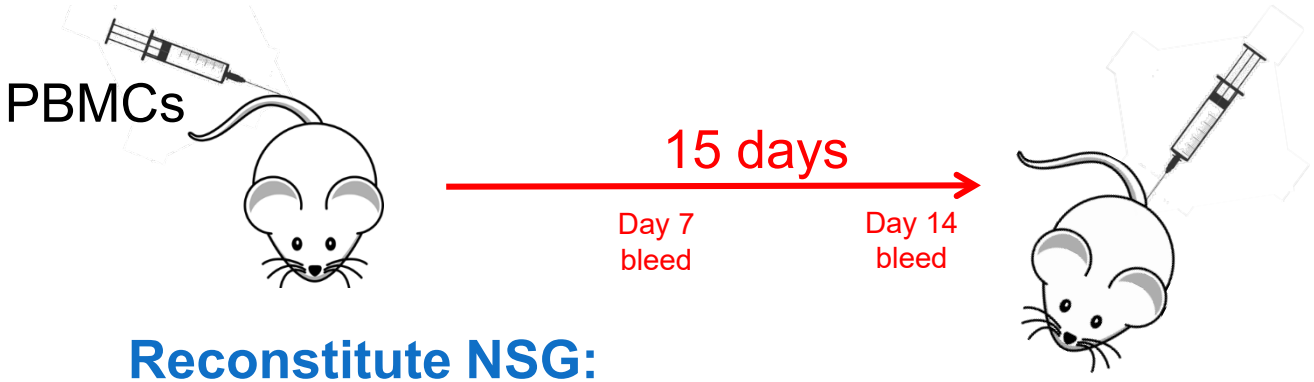


Flagellin-specific CAR Tregs – T cell suppression





Do flagellin-specific CARs sense Ag in vivo?



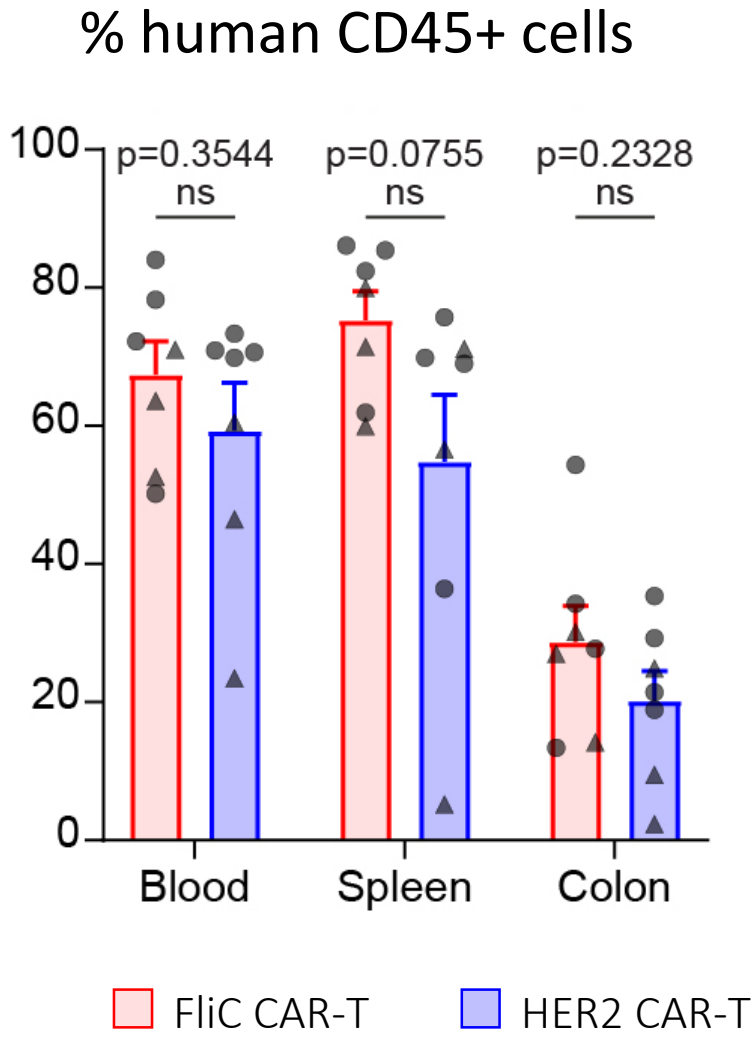
Reconstitute NSG:

- **5x10⁶ PBMCs**
- **5x10⁶ FliC or control CAR-T cells**

DNBS colitis induction

3 days

Sacrifice and analyse (histology)



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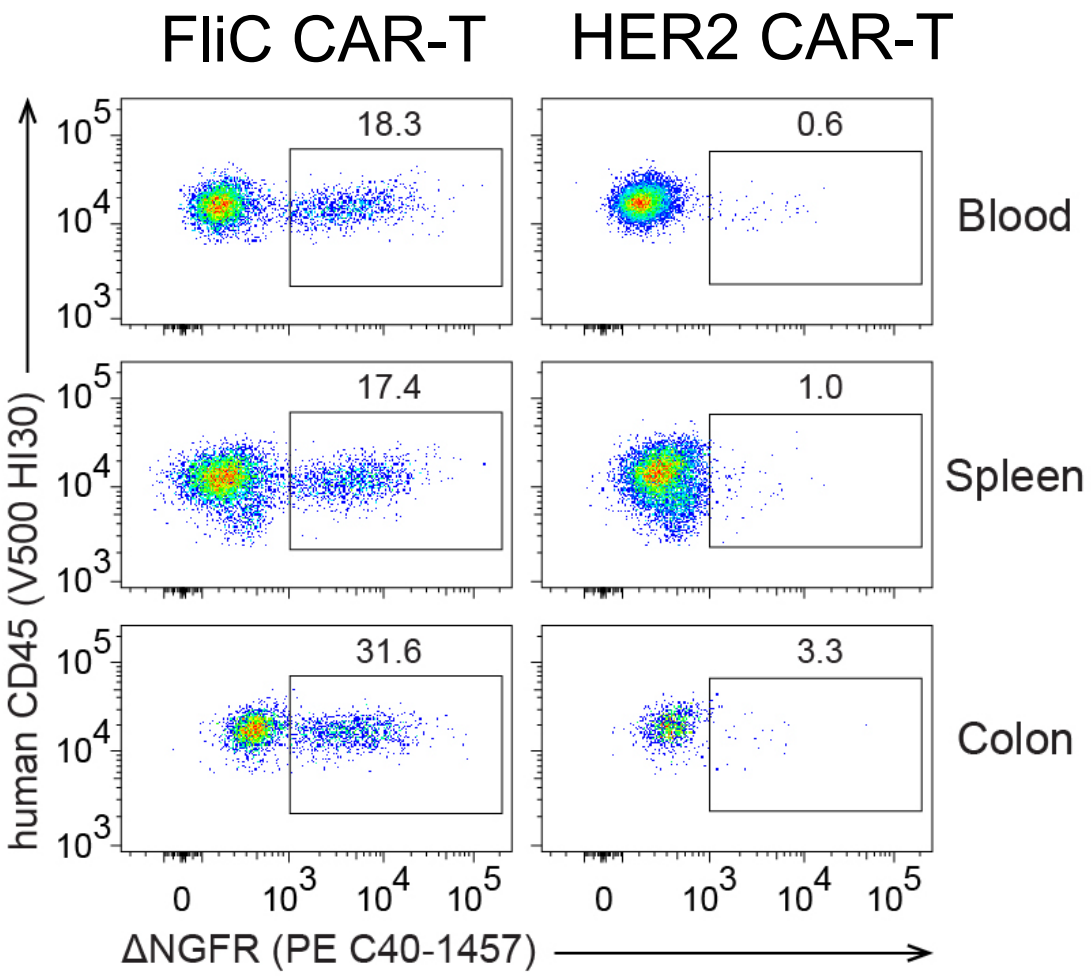
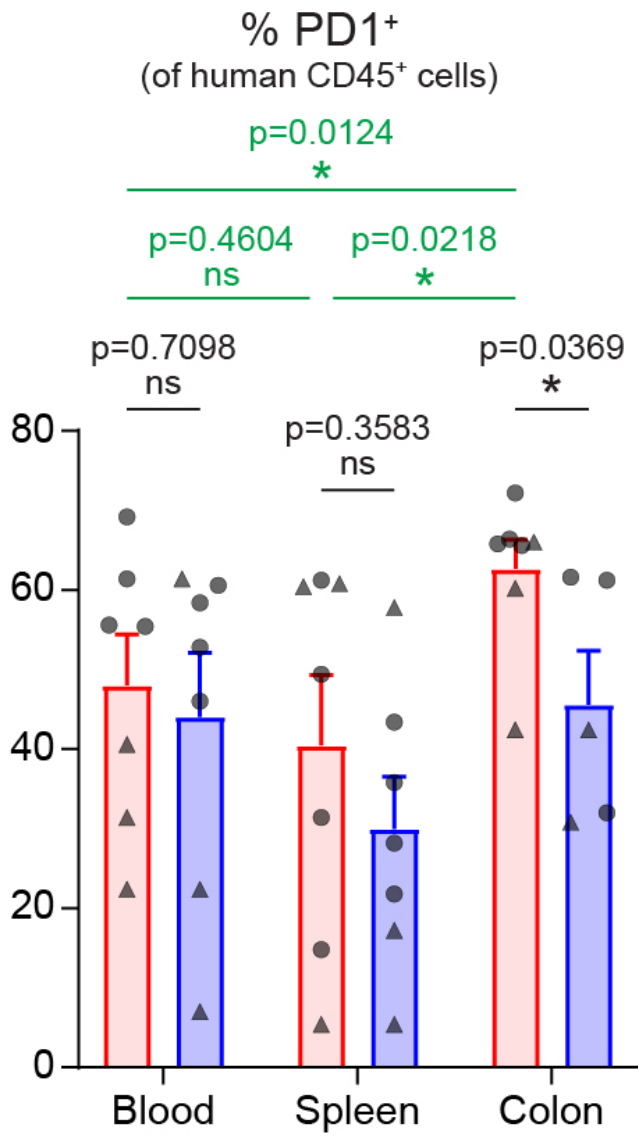


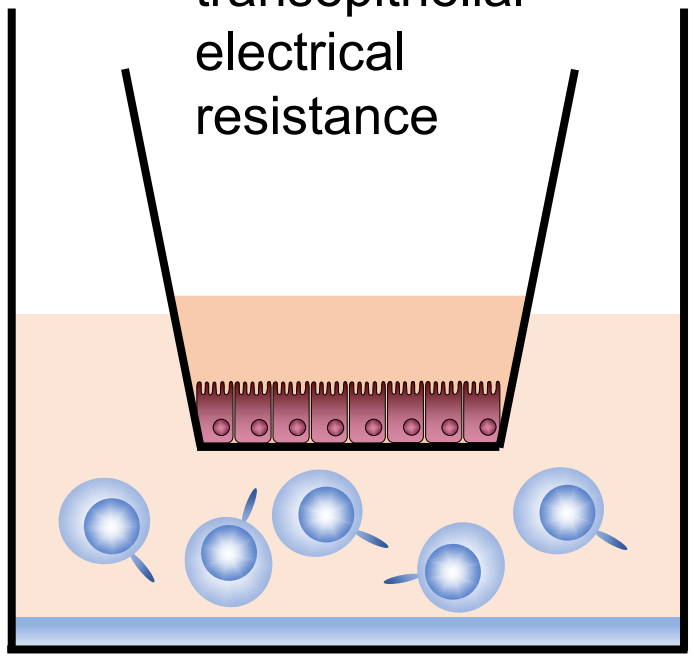


FliC CAR-T

HER2 CAR-T

FliC CAR Tregs promote barrier function

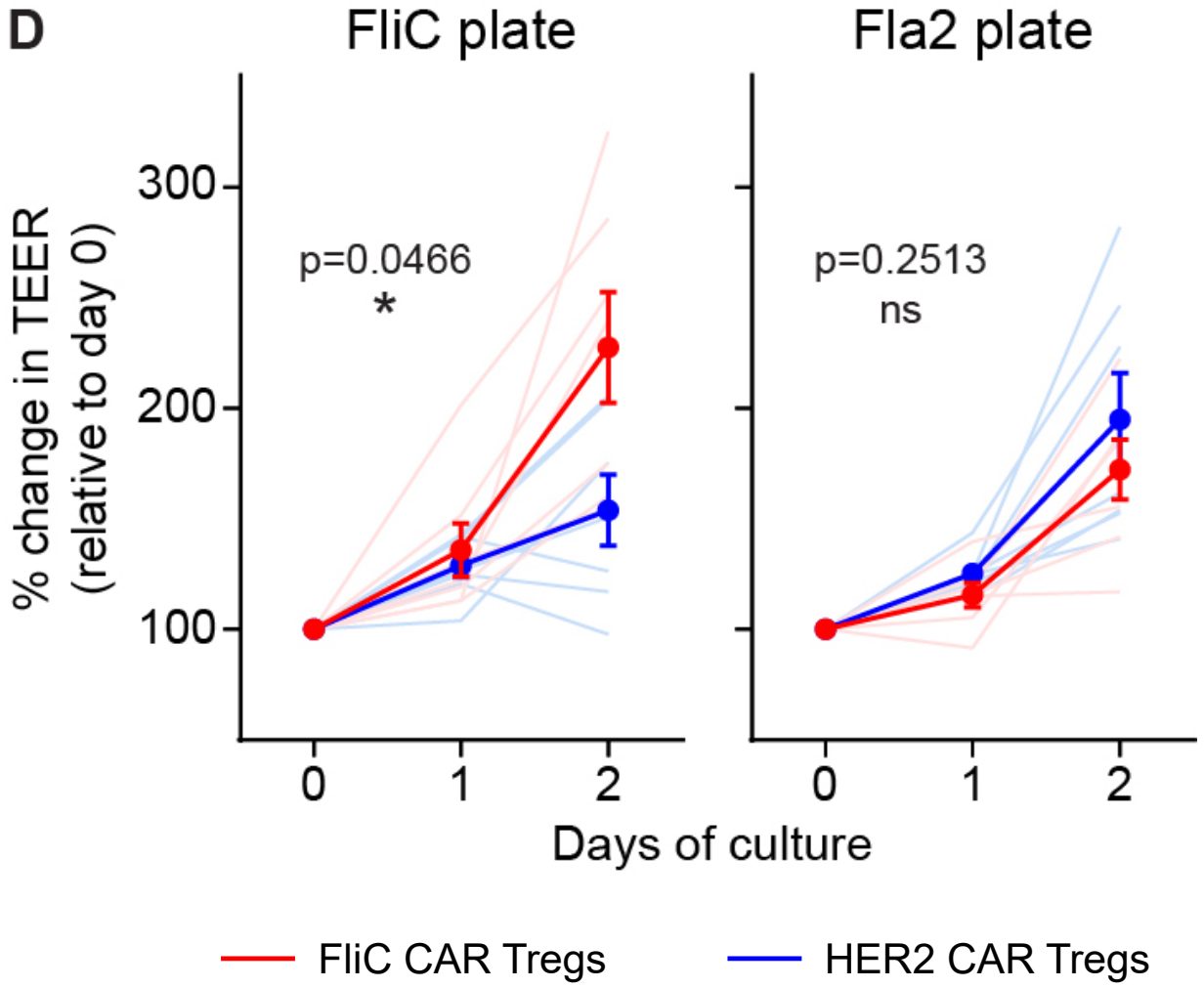
TEER = transepithelial electrical resistance



Non-confluent organoid monolayer

1x10^6 CAR Tregs

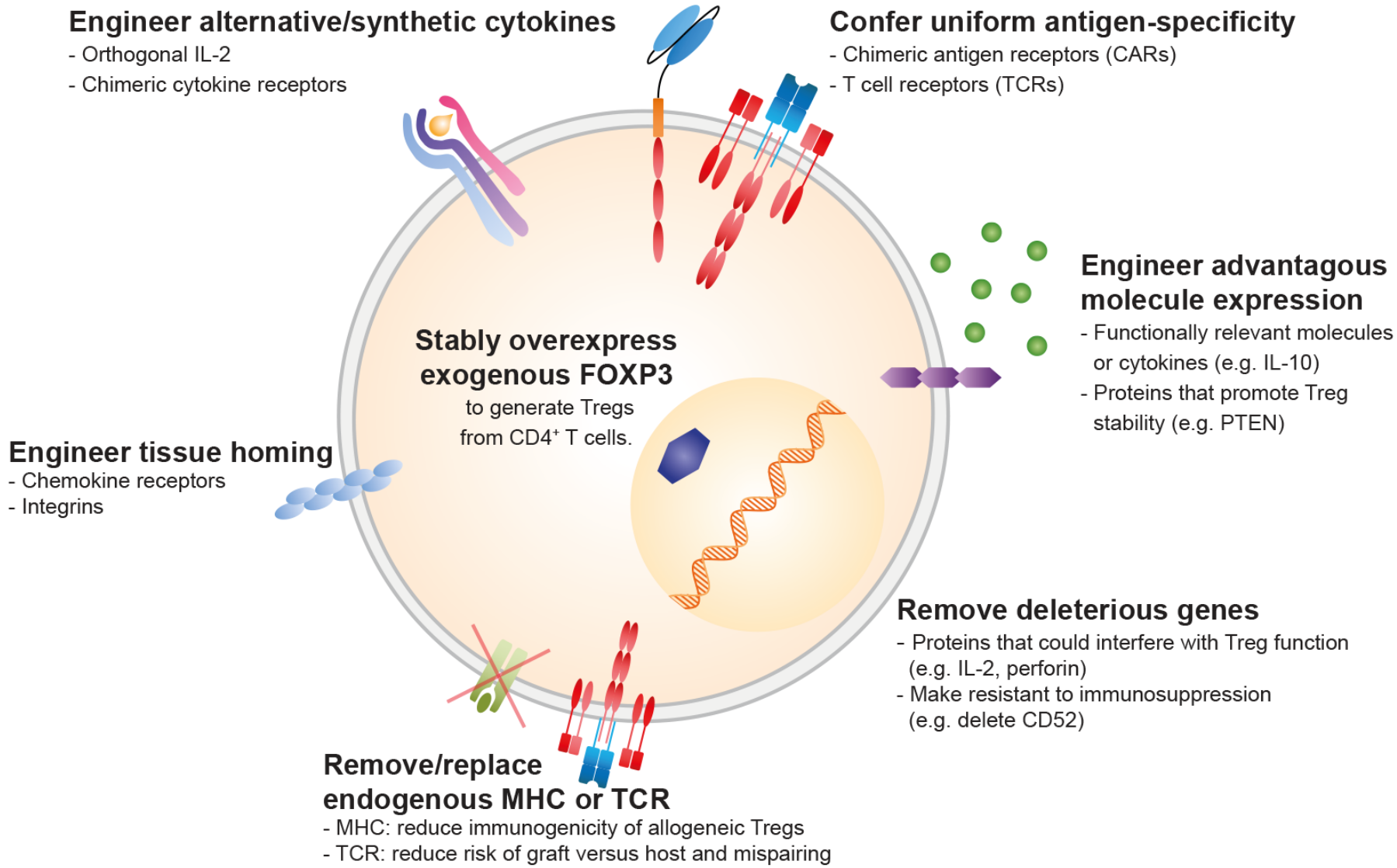
5 µg/mL FliC or Fla2



Summary Part 2

- Flagellin is a dominant antigen in IBD
  - True for flagella for several different bacterial strains
- CARs can re-direct the specificity of human Tregs towards flagellin
- As with A2-CAR Tregs, flagellin-specific CAR-Tregs:
  - Mediate antigen-specific suppression of T cell proliferation
  - Preferentially traffic to sites of Ag in vivo, where they are activated
- Flagellin-specific CAR-Tregs enhance epithelial cell proliferation
- Hold promise as a new therapy for IBD

The future of Treg engineering



Boardman & Levings, JACI 2022

Crohn's and Colitis Foundation of Canada

Fondation canadienne des maladies inflammatoires de l'intestin

CIHR IRSC

Canadian Institutes of Health Research

Instituts de recherche en santé du Canada

Broad Medical Research Program

<u>Tr1 cells</u>

Laura Cook

<u>Flagellin Tregs</u>

Dominic Boardman

UBC/BCCHRI

- Ted Steiner
- May Wong
- Martin Stahl
- Bruce Vallance

McMaster

- Charu Kaushic
- Aisha Nazli
- Sara Dizzell