

11<sup>TH</sup> EDITION

DECEMBER 10-13, 2024

# HIV PERSISTENCE DURING THERAPY

Reservoirs & Eradication Strategies Workshop



## A single shot of CCR5-modified stem-like CD4 T cells to limit HIV/SIV persistence

Ashish Sharma

*Emory University*

[www.hiv-persistence.com](http://www.hiv-persistence.com)

# CONFLICTS OF INTEREST

Co-founder of RORA Biologics:  
Start-up company that has licensed the patent used in the study below

# The Problem:

Latent HIV persists in long-lived memory CD4 T cells

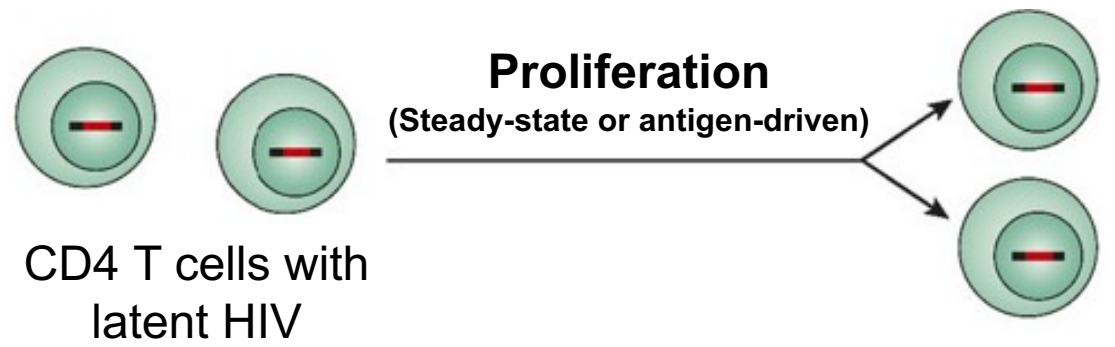
THE CHALLENGE



**Decay in reservoir:**  
Cells with latent HIV die upon effector differentiation

**Reservoir Persistence:**  
Cells with latent HIV are long-lived and self-renew

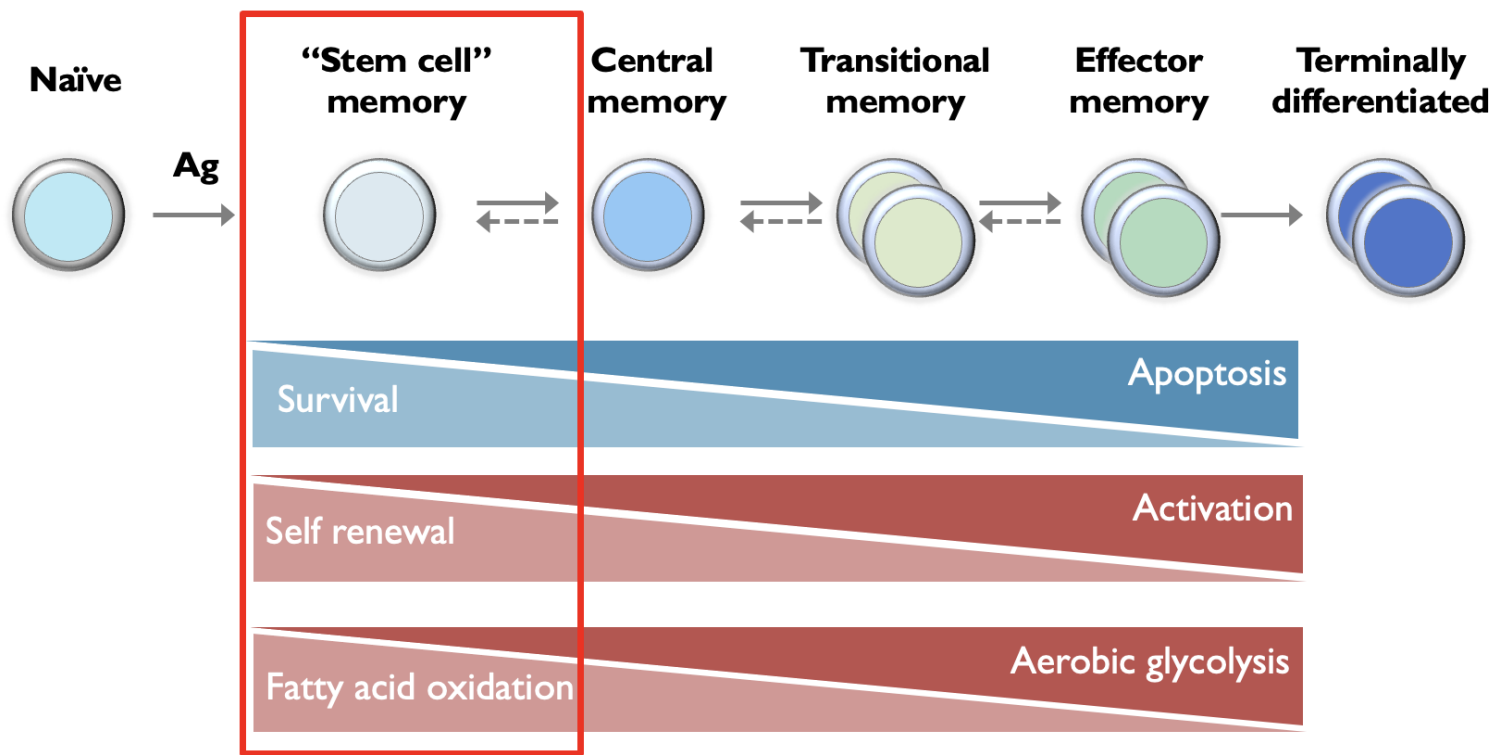
1. What are these cells?
2. How do we replace them?



# The Problem:

Stem-like CD4 T cells are self-renewing and last the longest

THE CHALLENGE



## Reservoir Persistence:

Cells with latent HIV are long-lived and self-renew

1. What are these cells?
  - a. Stem-like memory CD4s
  - b. Quiescent dysfunctional CD4s

## 2. How do we replace them?



## Make cell therapy products that:

1. Persist even better
2. And are HIV-resistant
  - a. Genetically modified (CCR5KO)
  - b. Anti-viral response

*Sallusto, Nature 1999 ; Riou, J Exp Med. 2007 ; Ahmed, Nat. Rev Immunol 2009 ; Gattinoni, Nat Med 2011 ; Farber, Nat. Rev Immunol 2014 ; Pearce, Nature 2009*



# Steps to address the challenge

## THE CHALLENGE

1. Identify long-lived HIV-resistant CD4 T cells
2. Generate long-lived HIV-resistant CD4 T cell product
3. Test products ability to control SIV in NHP model
4. Future: Combination therapies with IFN-I agonist to increase efficacy

# Steps to address the challenge

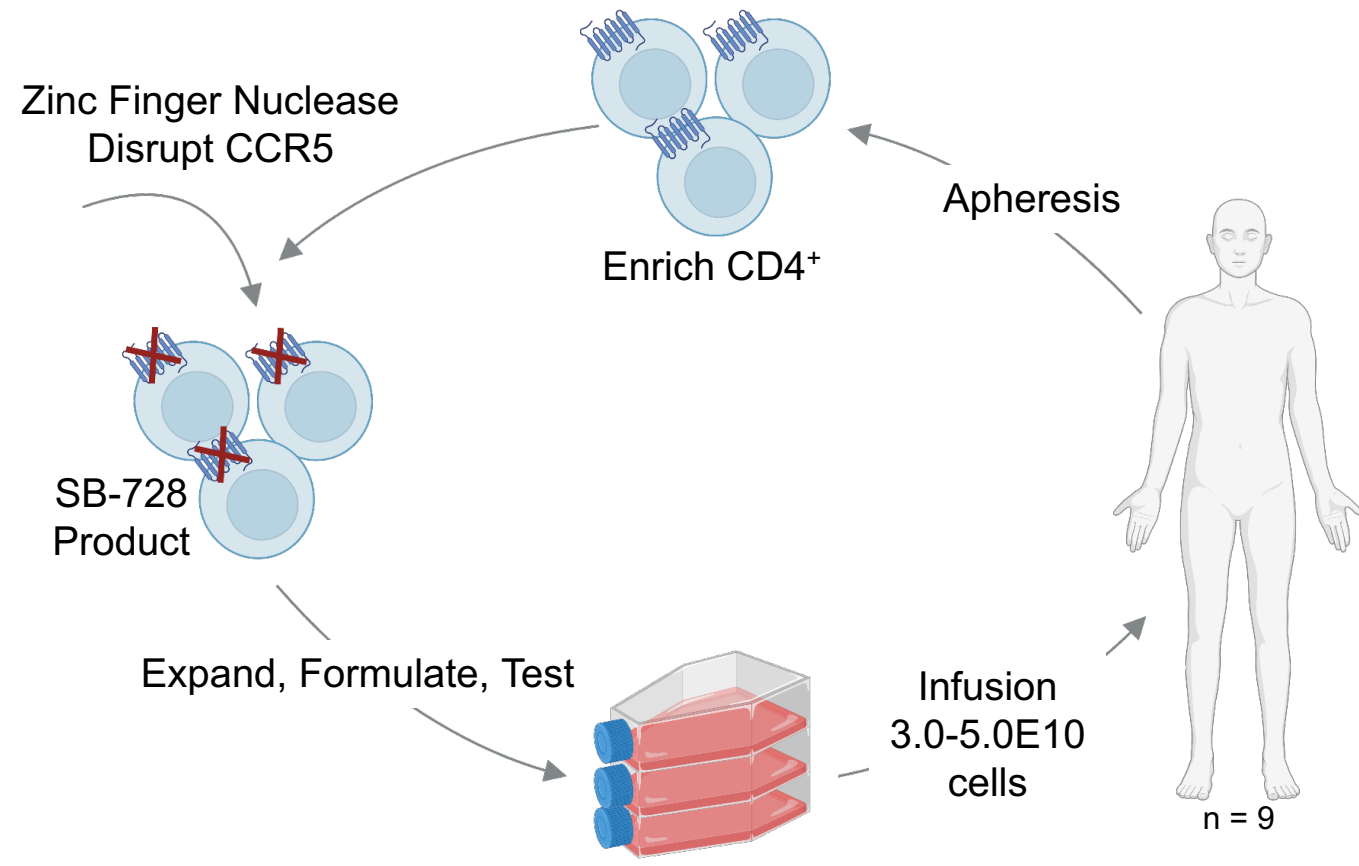
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# CCR5 Zinc finger nucleases (SB-728) Clinical Trials:

CCR5-modified CD4 T cells lead to lower reservoir (on ART) and control of viremia (ATI)

LONG-LASTING HIV RESISTANCE

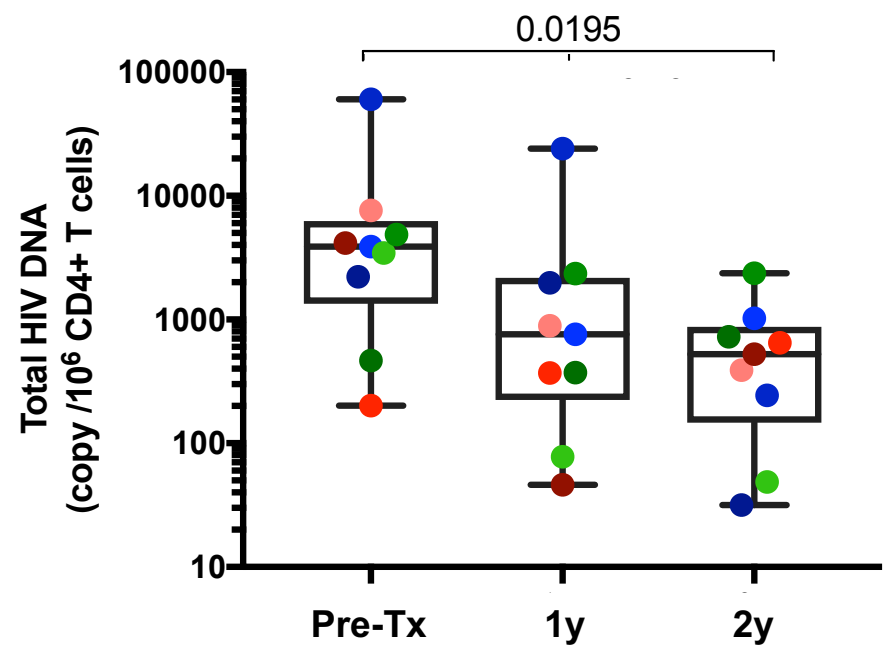


- **Product (SB-728):** Zinc-finger nuclease modified CCR5-modified CD4 T cells
- **902 Trial:** Reservoir decay on ART for 2-4 years
- **1101 Trial:** Viral control (<10,000 cp/mL) upon ATI in 5 of 9 PLWH for 1-year

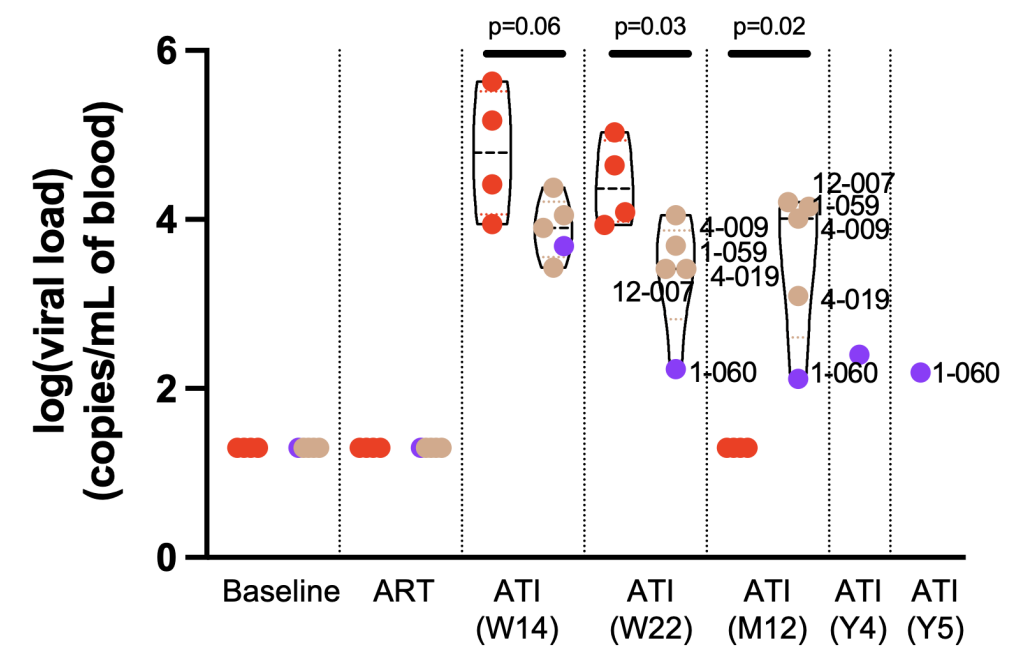
# CCR5 Zinc finger nucleases (SB-728) Clinical Trials: CCR5-modified CD4 T cells lead to lower reservoir (on ART) and control of viremia (ATI)

LONG-LASTING HIV RESISTANCE

**Lower reservoir on ART**



**Control of viremia upon ATI**

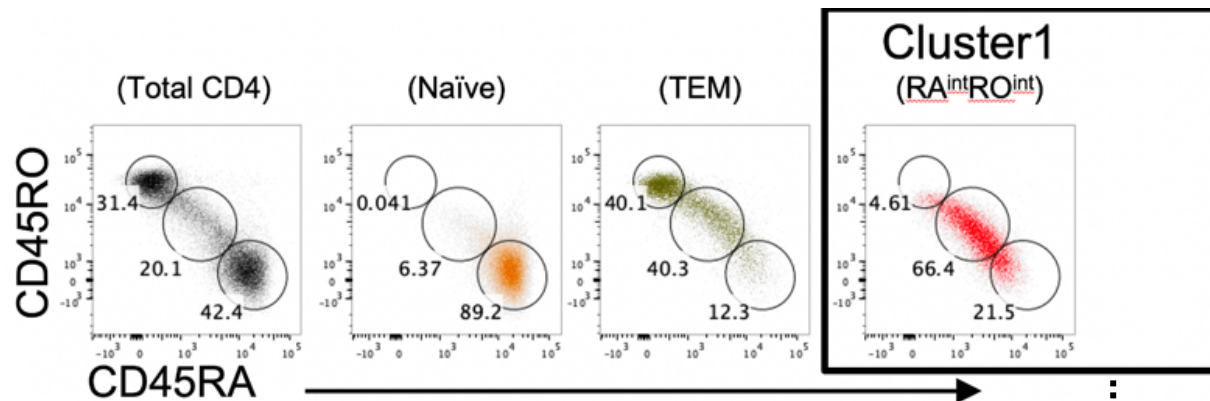


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# CCR5 Zinc finger nucleases (SB-728) Clinical Trials:

Novel subset of stem-like cells associated with outcomes

LONG-LASTING HIV RESISTANCE

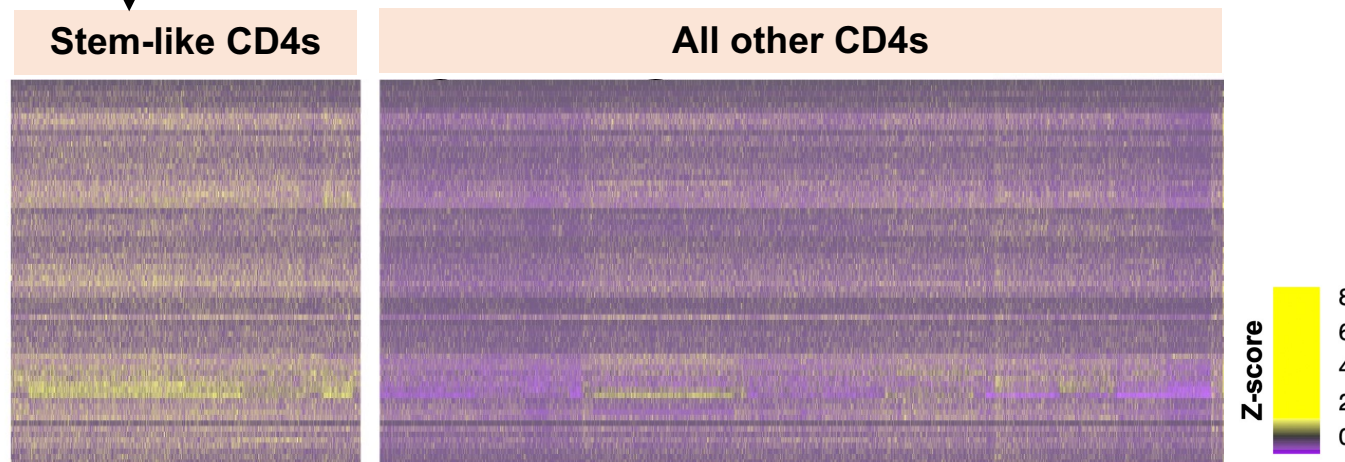


- **Novel Stem-like CD4 T cells abundant up to 4 years after cell therapy**
- Express intermediate CD45RA and RO (in combination with other stemness markers)

Novel Stem-like CD4s enriched in:

- WNT Signaling
- Expression of TCF1 targets
- STAT5 and NF-κB targets also enriched

Unique CCR5 mutation marks in stem-like cells found 3-4 years post-product infusion



**STEMNESS CASCADE**

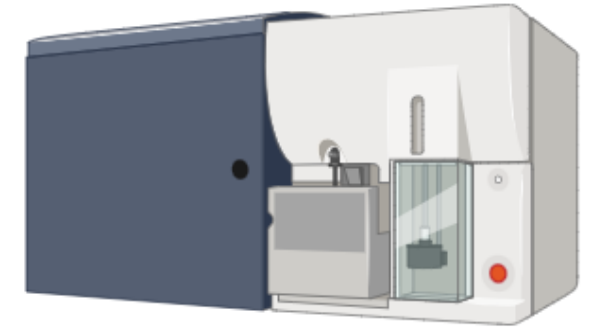
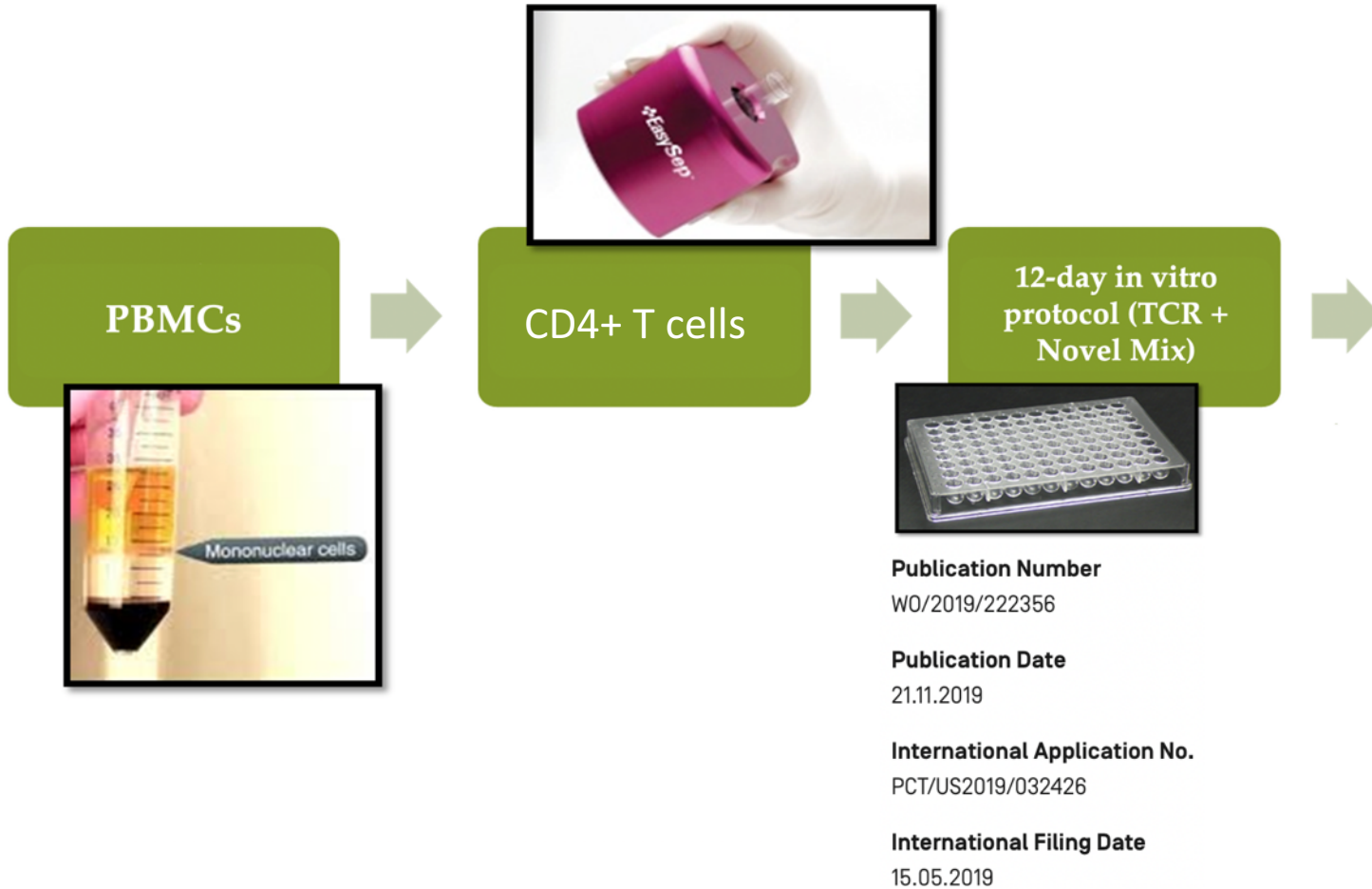
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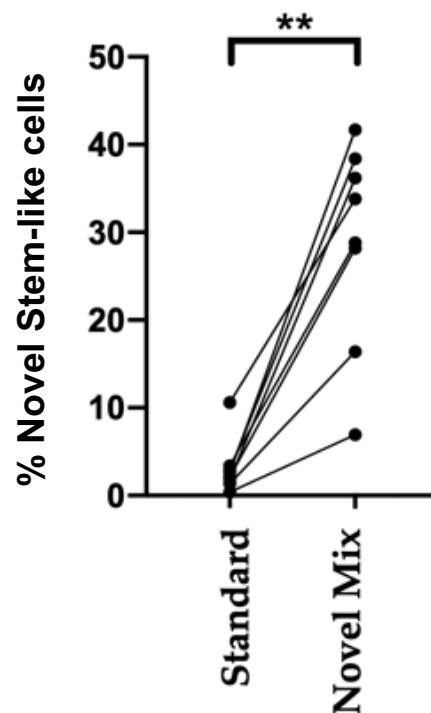
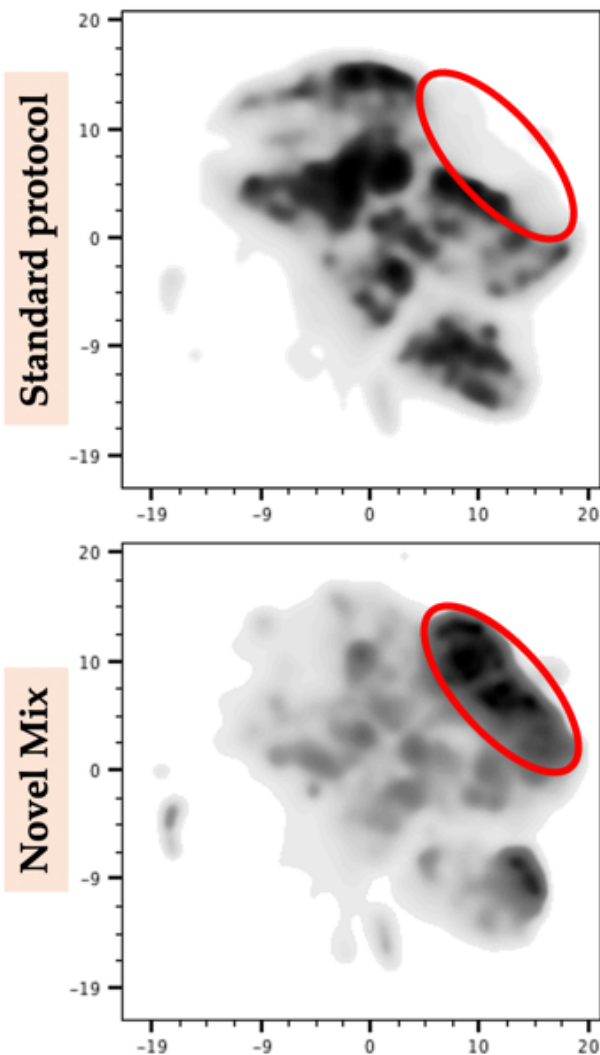


# Human and NHP cell products with stem-like profile



CD4 Gating		Subsets	
Marker	Fluorophore	Marker	Fluorophore
Live/Dead	APC-Cy7	CD45RA	BUV737
CD3	BV570	CD28	BV421
CD4	BV605	CD95/FAS	BUV615
CD8	BB700	CD27	BV650
<b>Stemness</b>		CD58	BUV805
		CCR7	AF647
		<b>Proliferation/KO</b>	
CD132/IL2RG	BV750	KI67	PECY5
CD127/IL7R	BV786	CCR5	PECF594
CD5	FITC		
CD6	BV510		
CD137/41BB	BUV661		
CD134/OX40	BUV395		
TCF1	PE		
NOTCH	BV711		
B-CATENIN	R730		

# Human and NHP cell products with stem-like profile



## Products made using novel protocol are:

- More homogenous
- Express stem-like profile markers observed in vivo
  - TCF1 + targets
  - WNT signaling

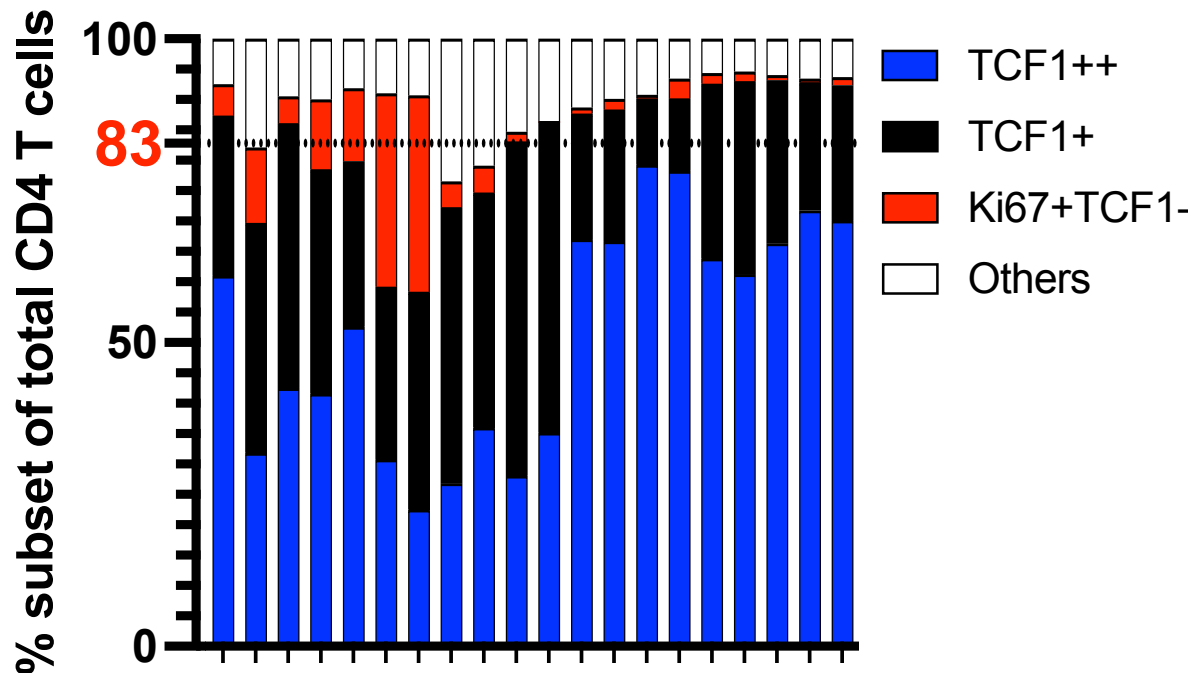
Products manufacturing protocol works for both NHPs and Humans

# Steps to address the challenge

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# NHP product enriched in stem-like CD4 T cells



- Products were 60-90% stem-like
  - TCF1+, Ki67-
  - Similar trend for other stemness markers
- CCR5-KO efficiency was ~80%
- 5 million product cells per kg were infused
  - Log-folds lower than prior human and NHP studies

# Stem-like CCR5-edited CD4 T cells for control of SIV



N = 20

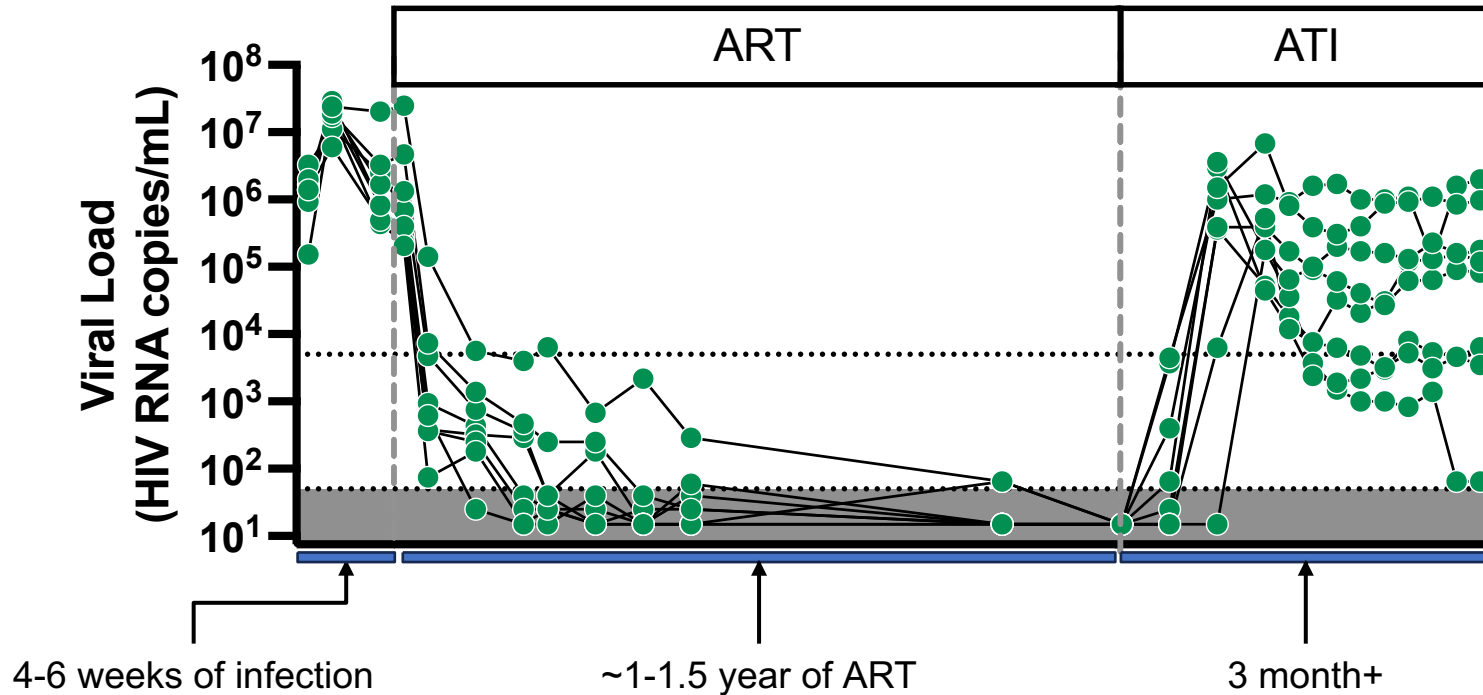
- **Week 0: SIV-mac239 infection**
- **Week 4: ART initiation**
- **Week 45: Chemo-conditioning**
- **Week 46: Autologous CCR5-modified stem-like CD4 product infusion**
- **Week 46-52: Anti-CD20 in 10 of 20 NHPs**
- **Week 62: ATI**
- **Week 74: Necropsy**

In collaboration with  
Justin Harper and Mirko Paiardini



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# Standard SIV-mac239 model kinetics: No cell therapy



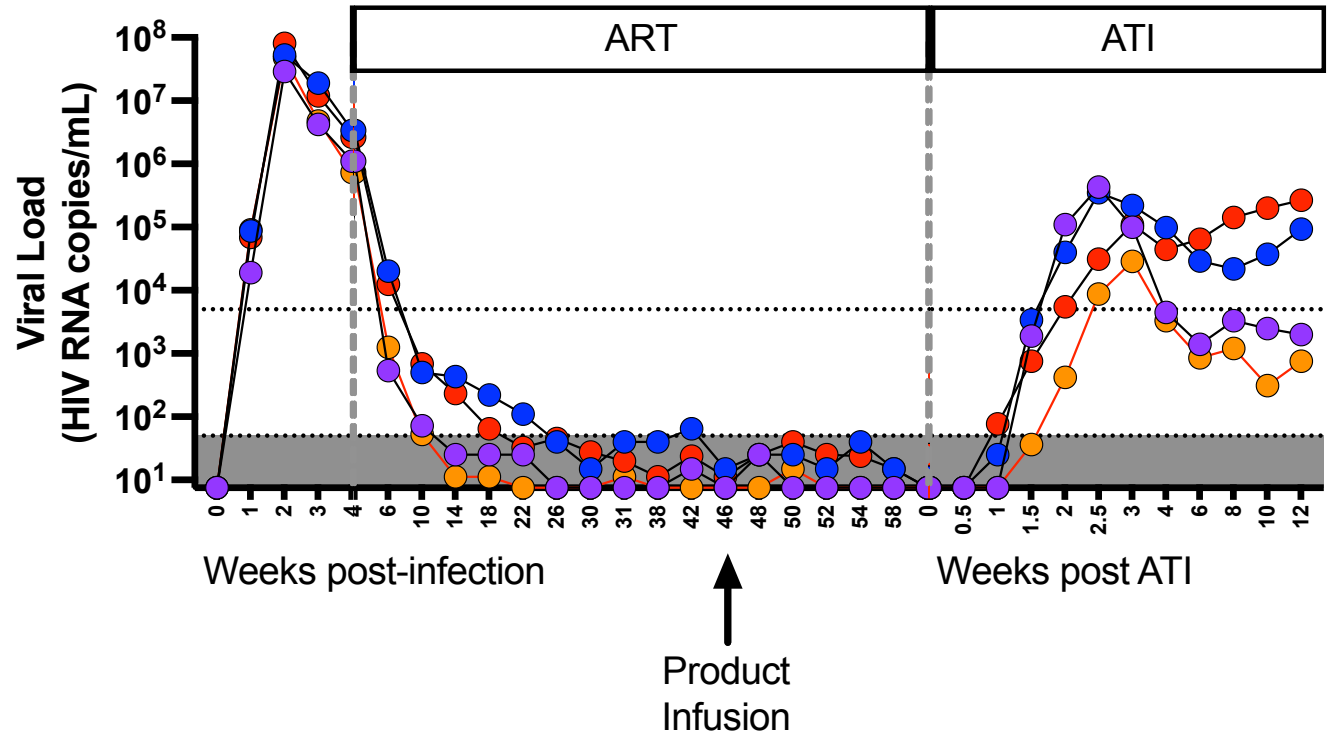
- Most NHPs rebound upon ATI and maintain viremia over 5000 cps/mL
- Spontaneous post-treatment control more likely in NHPs with quicker suppression



# 9 of 20 NHPs show partial control of viremia upon ATI

NHP STUDY

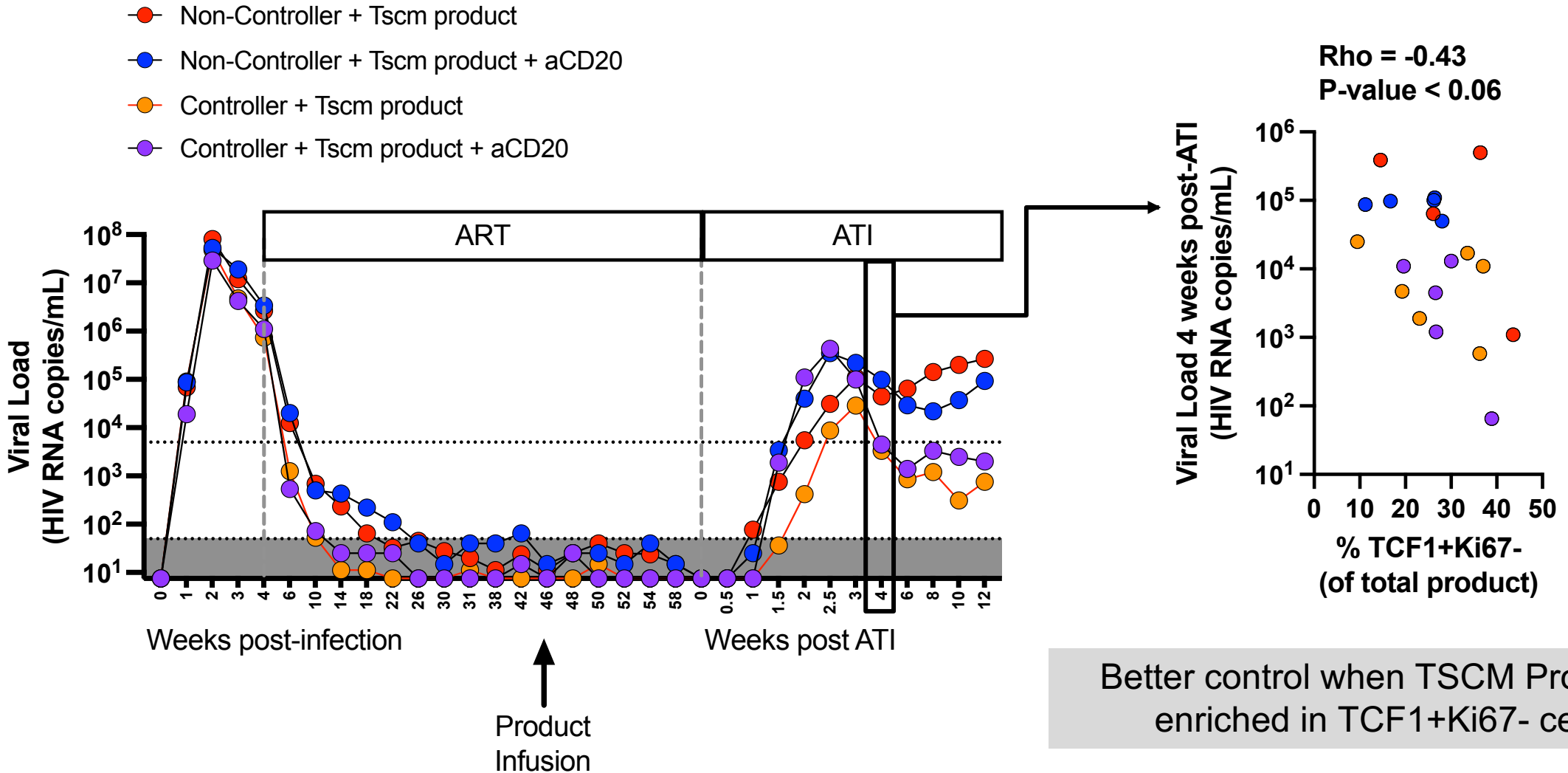
- Non-Controller + Tscm product
- Non-Controller + Tscm product + aCD20
- Controller + Tscm product
- Controller + Tscm product + aCD20



- 9 of 20 NHPs limit viremia to less than 5000 cps/mL at 3-month follow-up
- No significant change when anti-CD20 administered

# Product Stemness associated with control post-ATI

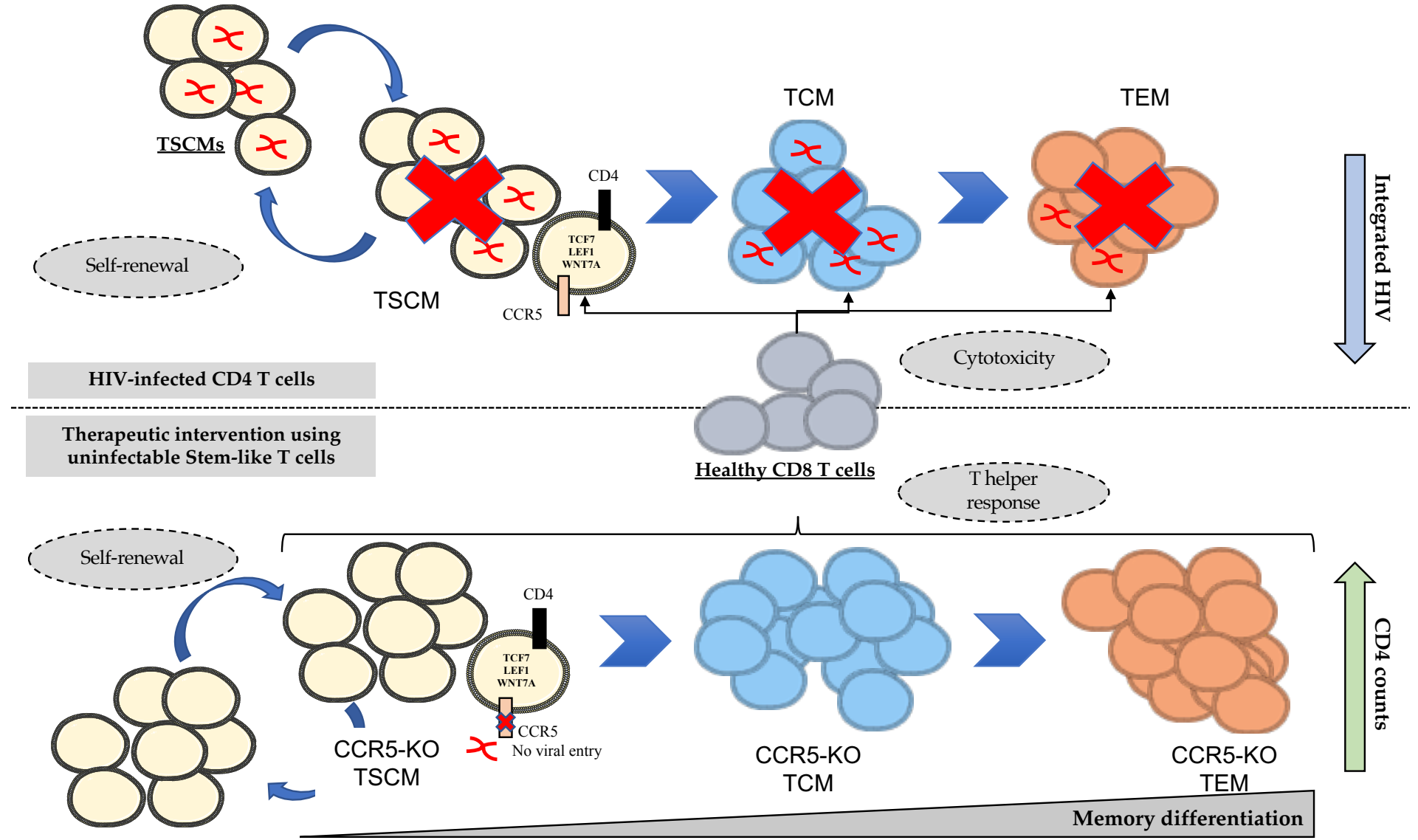
NHP STUDY



Better control when TSCM Products enriched in TCF1+Ki67- cells

# HIV-resistant long-lived CD4 T cells reinvigorate the immune system

THE CHALLENGE



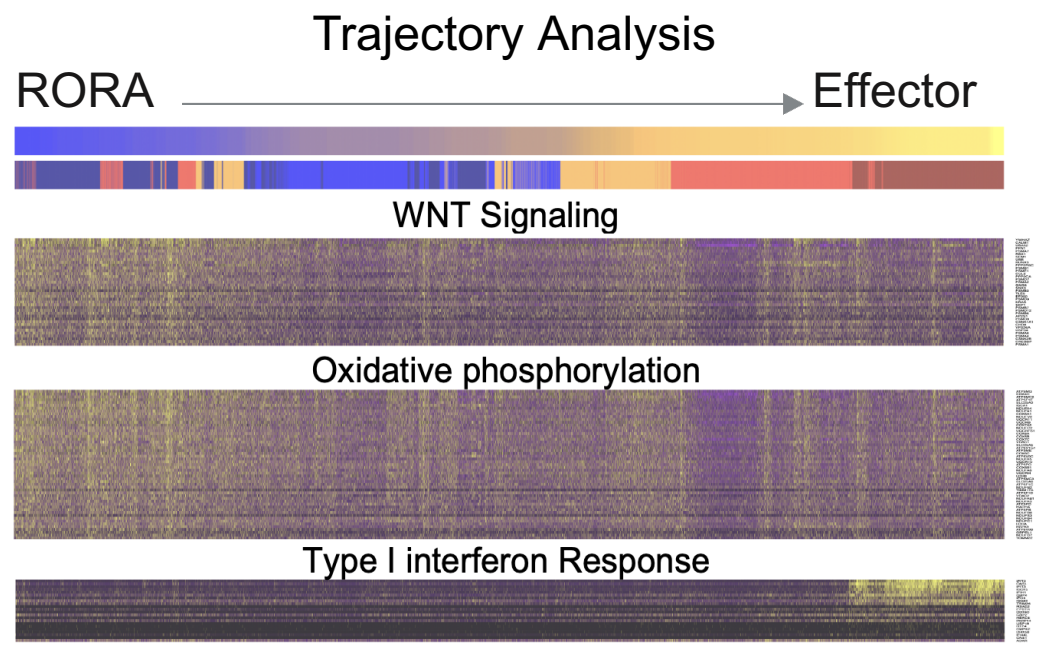
# Steps to address the challenge

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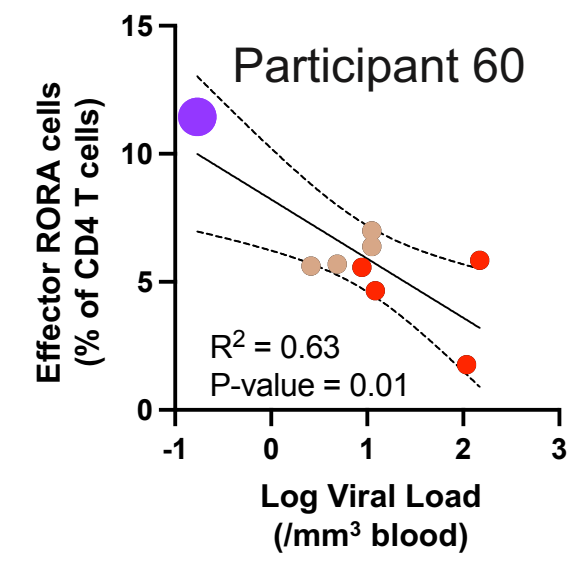
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# CCR5 Zinc finger nucleases (SB-728) Clinical Trials: Effector differentiation of novel stem-like CD4 cells associated with control of viral load

ISG EXPRESSION IN VIVO

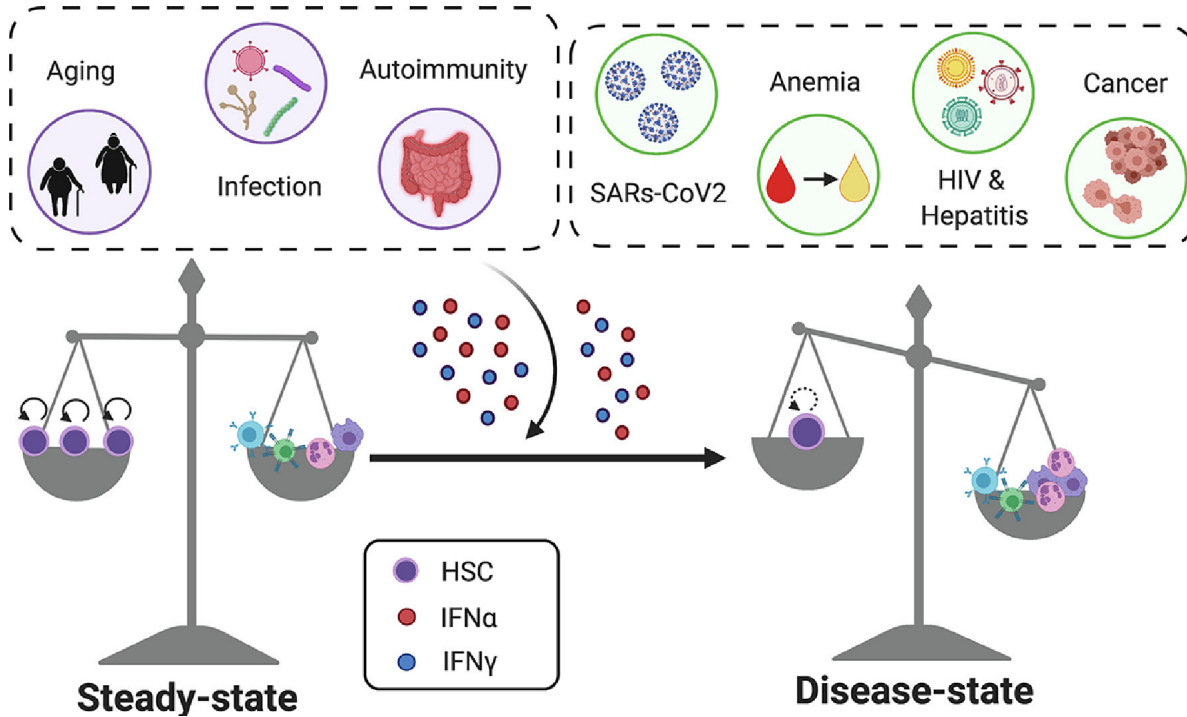


Novel stem-like cells have higher interferon response capacity and express ISGs when virus comes back



Viral load post-ATI reduced with increased ISG+ CD4 T cell differentiation

# HSCs: Intrinsically anti-viral and differentiate into ISG+ effectors



Article

## Cell

### Intrinsic Immunity Shapes Viral Resistance of Stem Cells

#### Authors

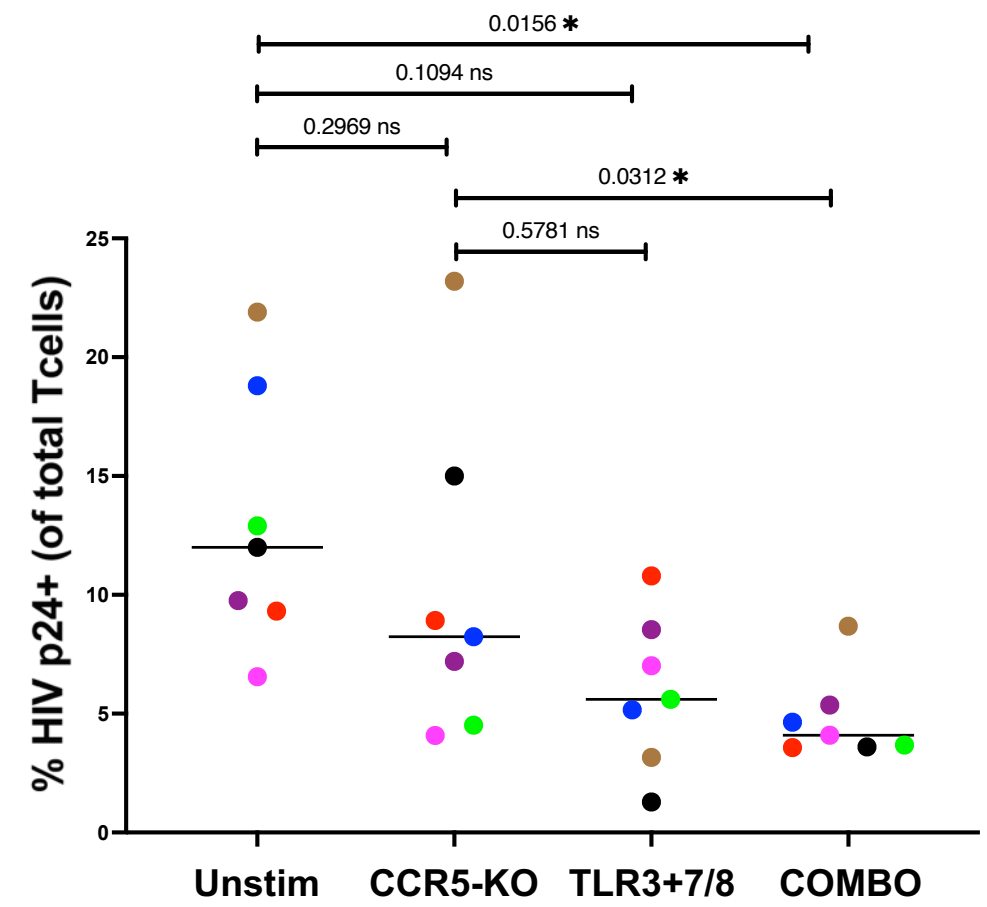
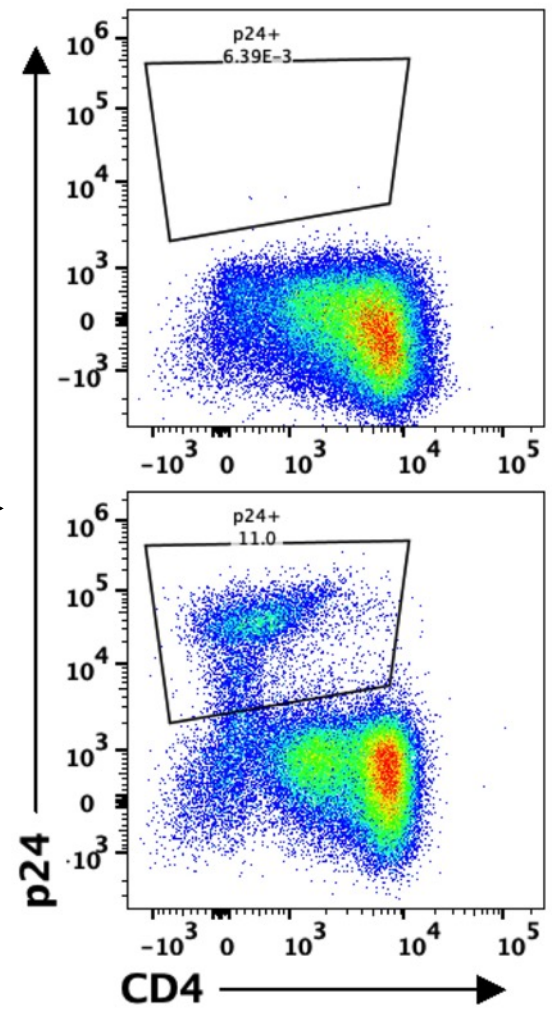
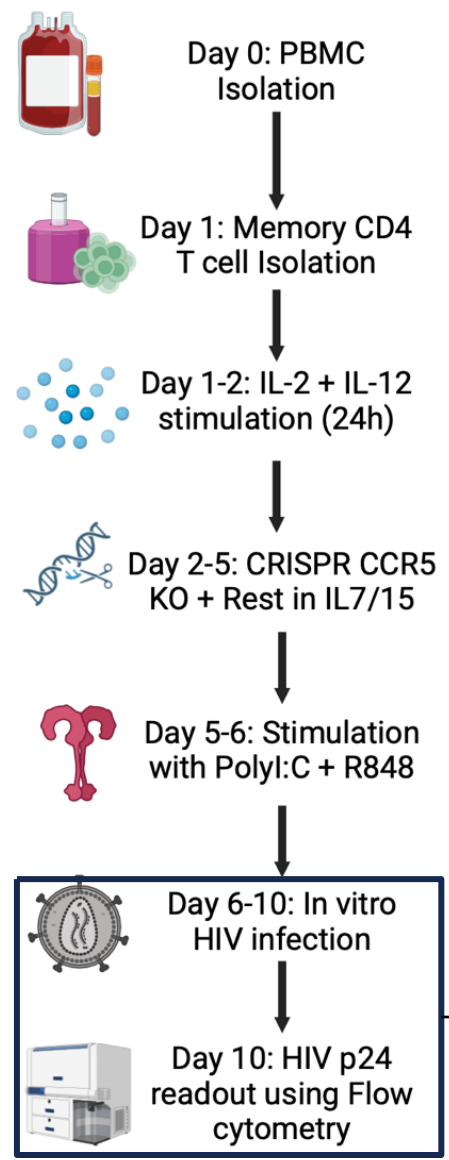
Xianfang Wu, Viet Loan Dao Thi, Yumin Huang, ..., Xiuli An, Brad R. Rosenberg, Charles M. Rice

- Novel stem-like CD4s have high interferon response capacity and differentiate into ISG+ effectors to resist viremia
- Interferon response capacity vs CCR5 KO
- Interventions to train the immune system/HSCs – conserve high interferon response capacity



# CCR5-KO synergizes with interferon signaling to limit HIV infection in CD4 T cells in vitro

ISG-Expression + CCR5KO



# Conclusions and Next Steps

## Recap

- Identified novel subset of stem-like CD4s
- Developed and infused CCR5 KO stem-like products for NHPs
- Viral control in 8 of 20 NHPs upon ATI
- Viral control associated with frequencies of stem-like cells in product

## Ongoing work

- Tracking the product in vivo: pre- and post-ATI
- Virology: Intactness, integration site analyses
- Immunology: SIV-specific responses, single-cell and spatial omic (in reservoir sites)

## Path to Clinical Trial

- HIV-resistant stem-like product for long-term viral control
- Use in combination with other therapies (like ISGs inducing compounds) for better efficacy

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