



**Eradication of HIV infection
Not in my lifetime?
or
Just around the Corner?**

Michael M. Lederman, MD

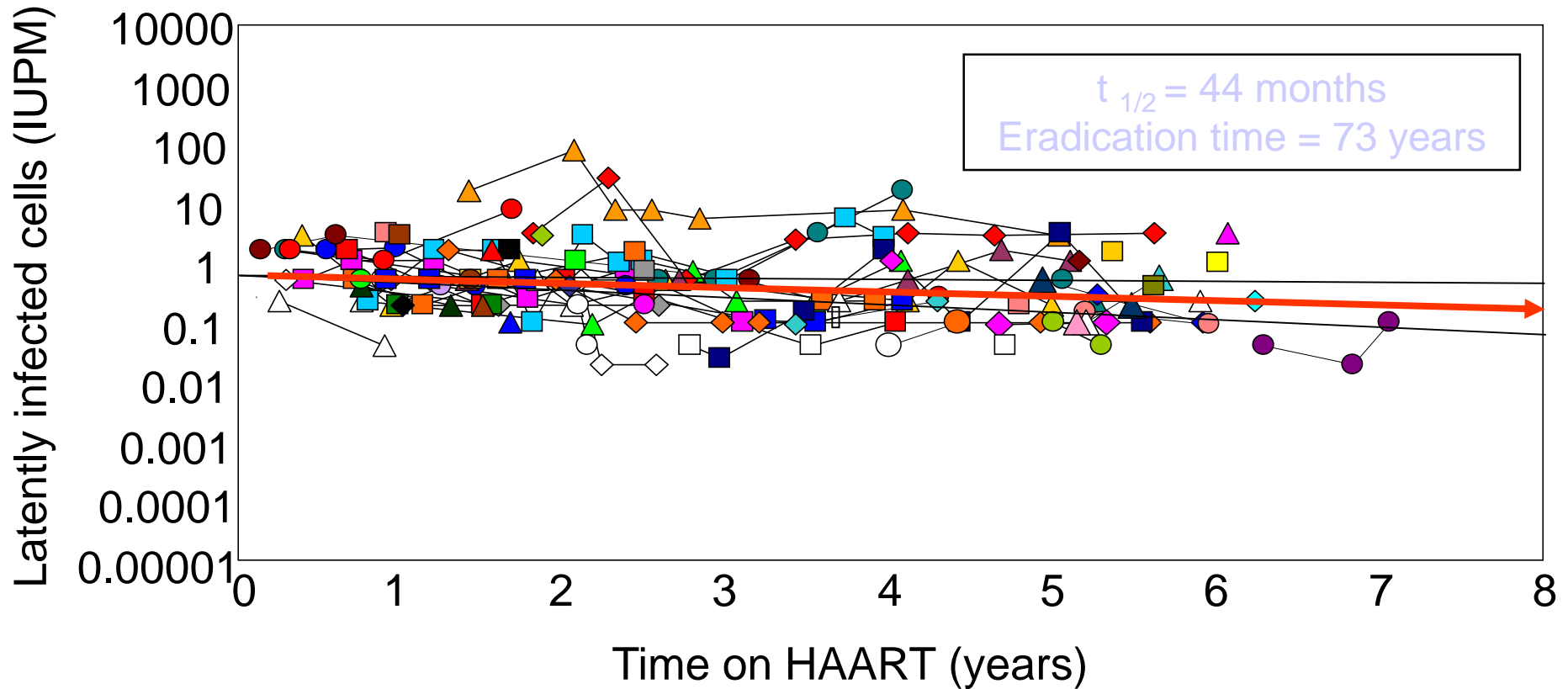


CASE

Conflicts of Interest

- The pending clinical trials discussed here are funded by grant awards from Gilead and the Federales

Stability of an HIV reservoir in Resting Memory CD4 T Cells



Key Barriers to HIV Cure

- Latency
 - Little death/turnover of latently infected cells
 - Poor targets for ART, for antiviral defenses
- Sites of persistence may be protected from ART, from host defenses
- Stupid virologists who can't measure replication competent virus without exsanguinating my patients!!



Strategies for cure

1. Promote viral expression from latency and knock off virus expressing cells (“shock and kill”)
2. Find a marker that selectively identifies latently infected cells and target those cells for destruction (eg using monoclonal antibodies).
3. Kill immune cells non-selectively, eliminating reservoir(s) allowing (or promoting) immune restoration under ART
4. Mobilize antiviral host immune defenses
5. Target host elements used for persistence
6. Render cells “resistant” to HIV



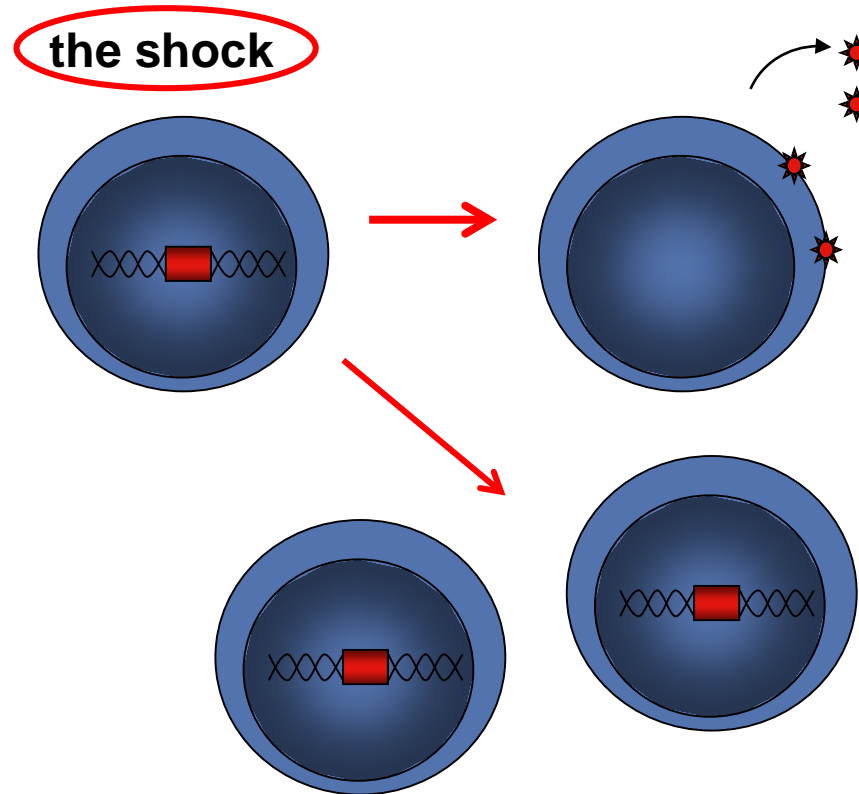
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Treatment strategies should be considered in the context of:

- Plausibility
 - Precedent
 - Scalability
 - Durability
 - Tolerability
 - Limitations
- “should it work?”
 - “does it work?”
 - “for how many could it work?”
 - “for how long does it work?”
 - “can we stand it?”
 - “what’s the downside?”

Can we induce HIV expression (shock) and deplete the latent reservoir (kill)?



A variety of shocking stimuli have explored:

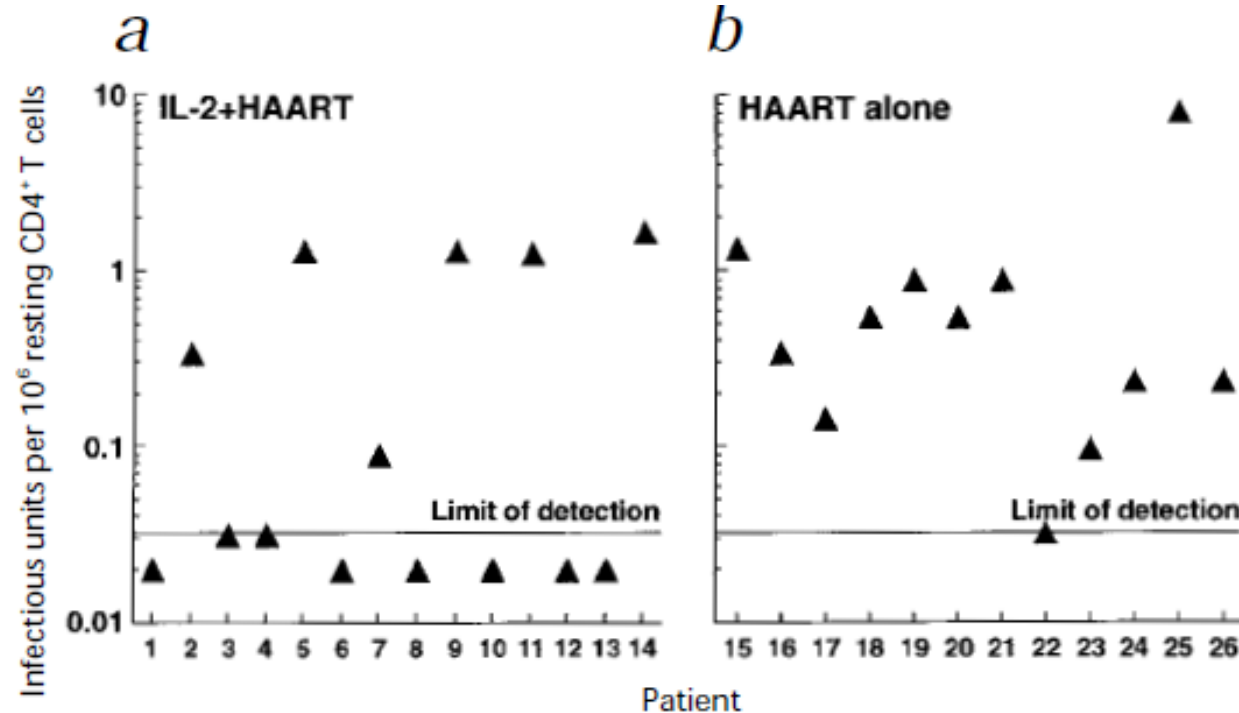
- HDAC inhibitors

- T cell receptor stimulation

- Cytokines: eg IL-2/IL-15/IL-7

Effect of interleukin-2 on the pool of latently infected, resting CD4+ T cells in HIV-1-infected patients receiving highly active anti-retroviral therapy

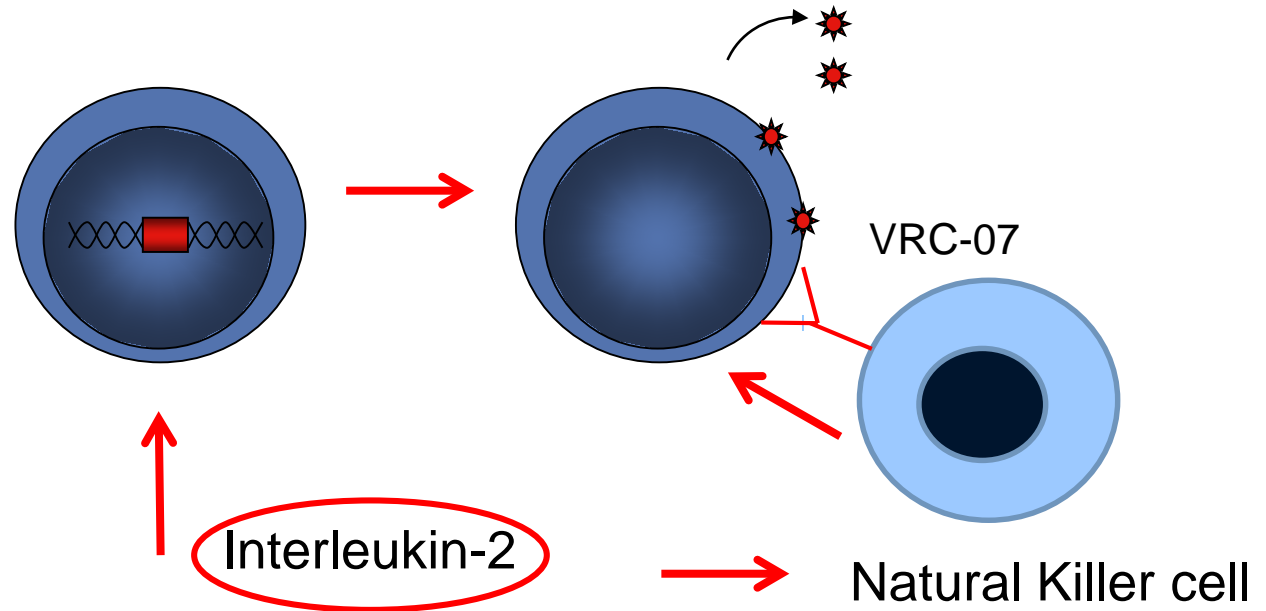
Chun et al., *Nature Medicine*, 5:651-655, 1999



3 pts had no infectious Virus detectable even with more sensitive assays
Two had no infectious virus in LN.

But: proviral sequences found in all three
And: 2 stopped ARVs and had virus rebound in plasma

**Can we induce HIV expression (shock)
and deplete the latent reservoir?
And can we kill infected cells?**



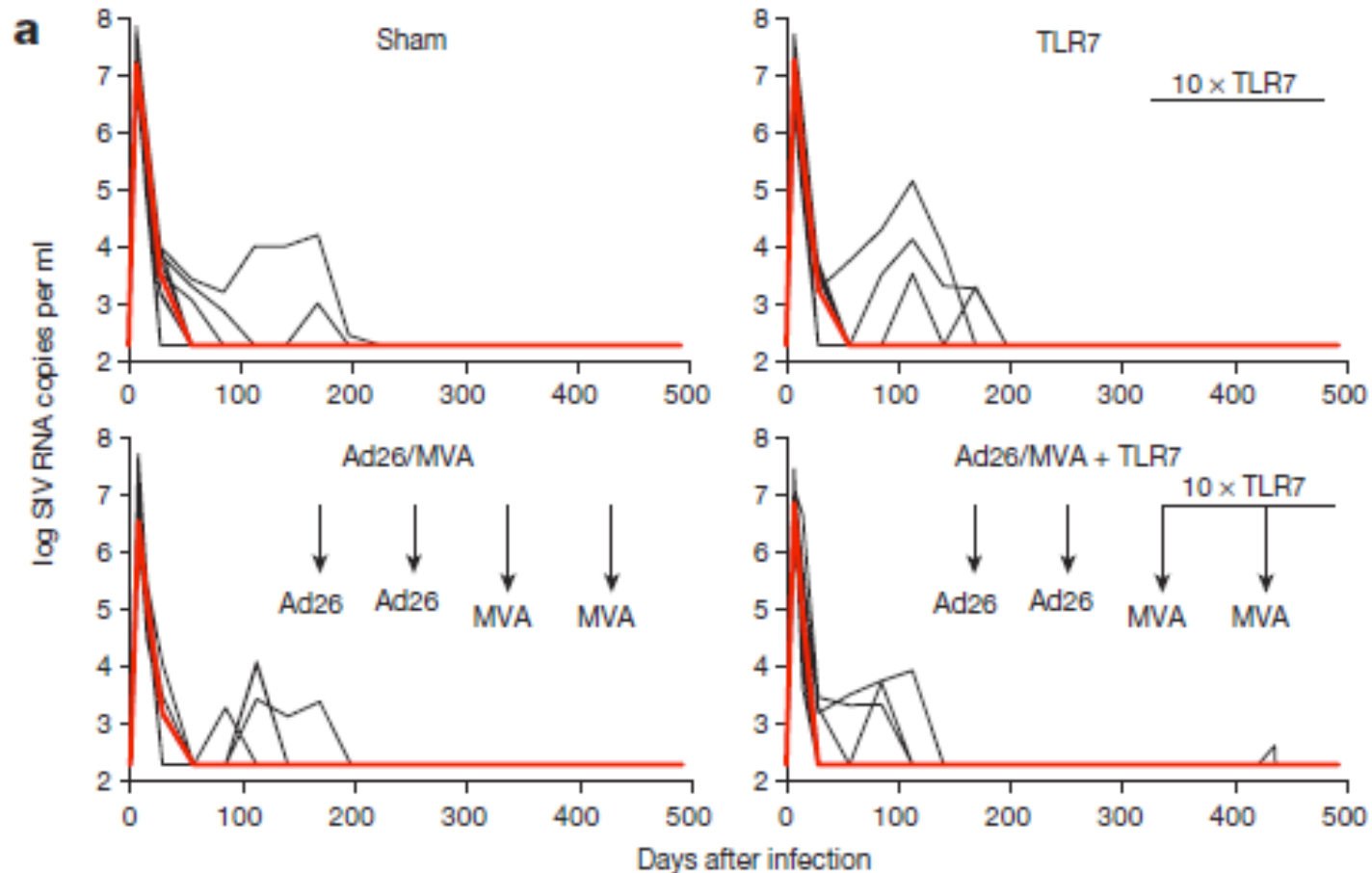
Our new trial will give IL-2 to induce HIV and activate NK cells to kill Infected targets.

Half the participants will also receive the Antibody VRC-07 to increase killing of infected cells

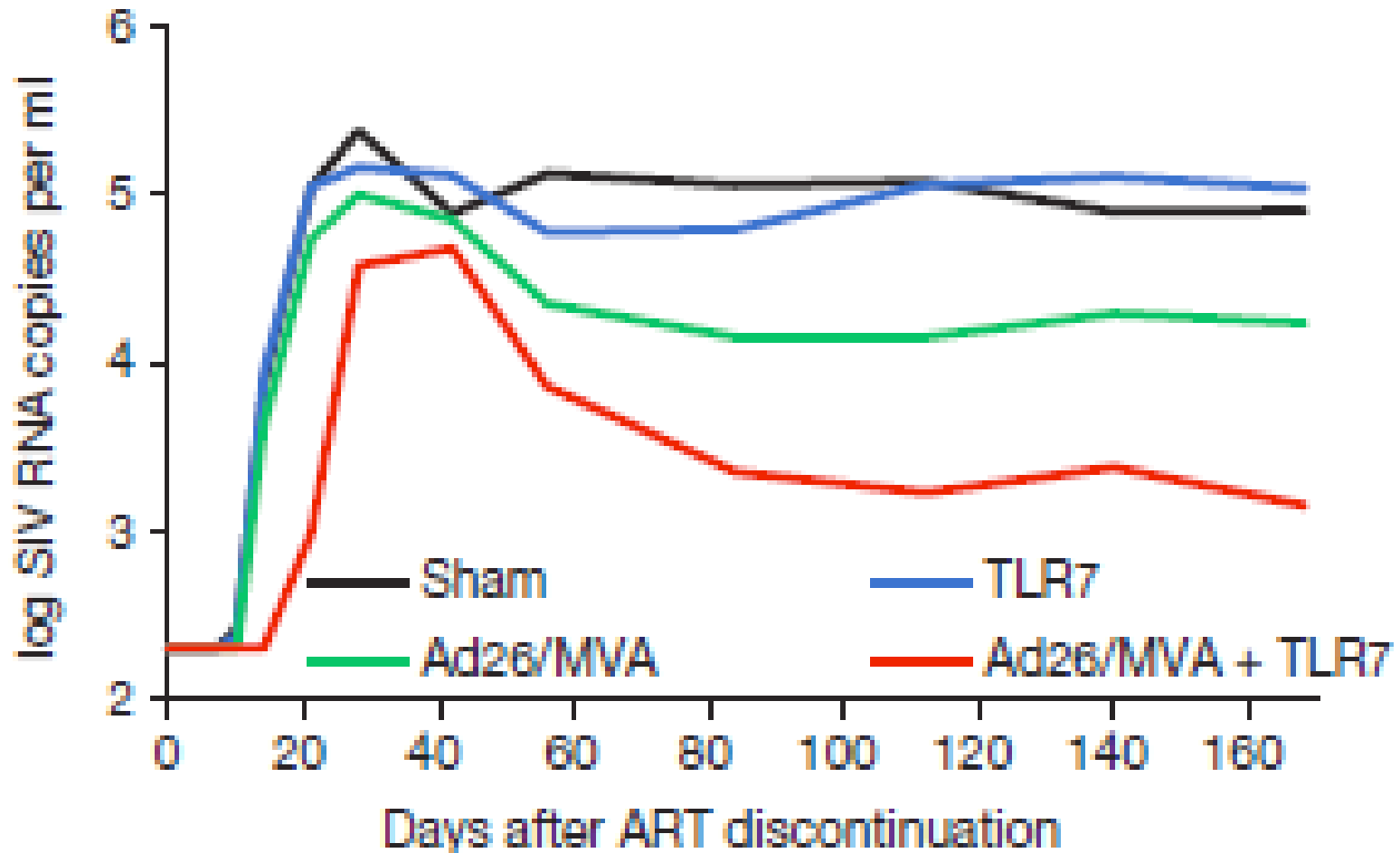
But if we're going to shock and hope to kill, we need soldiers

- CD8 T cell responses during HAART are weak
- Could improve with immunization.
 - Will any consensus sequence(s) suffice for the diversity of viruses in an infected person?
 - Non-dominant sequences?
 - Autologous sequences
 - Obtained when? And from where?
 - Will recent sequences target archived early viruses?
 - We could use more effective vaccination strategies

In Rhesus macaques treated with ART early in infection, combination therapeutic vaccine and TLR 7 agonist help to control viremia after treatment discontinuation



Rhesus macaques treated with ART early in infection, combination therapeutic vaccine and TLR 7 agonist help to control viremia after treatment discontinuation

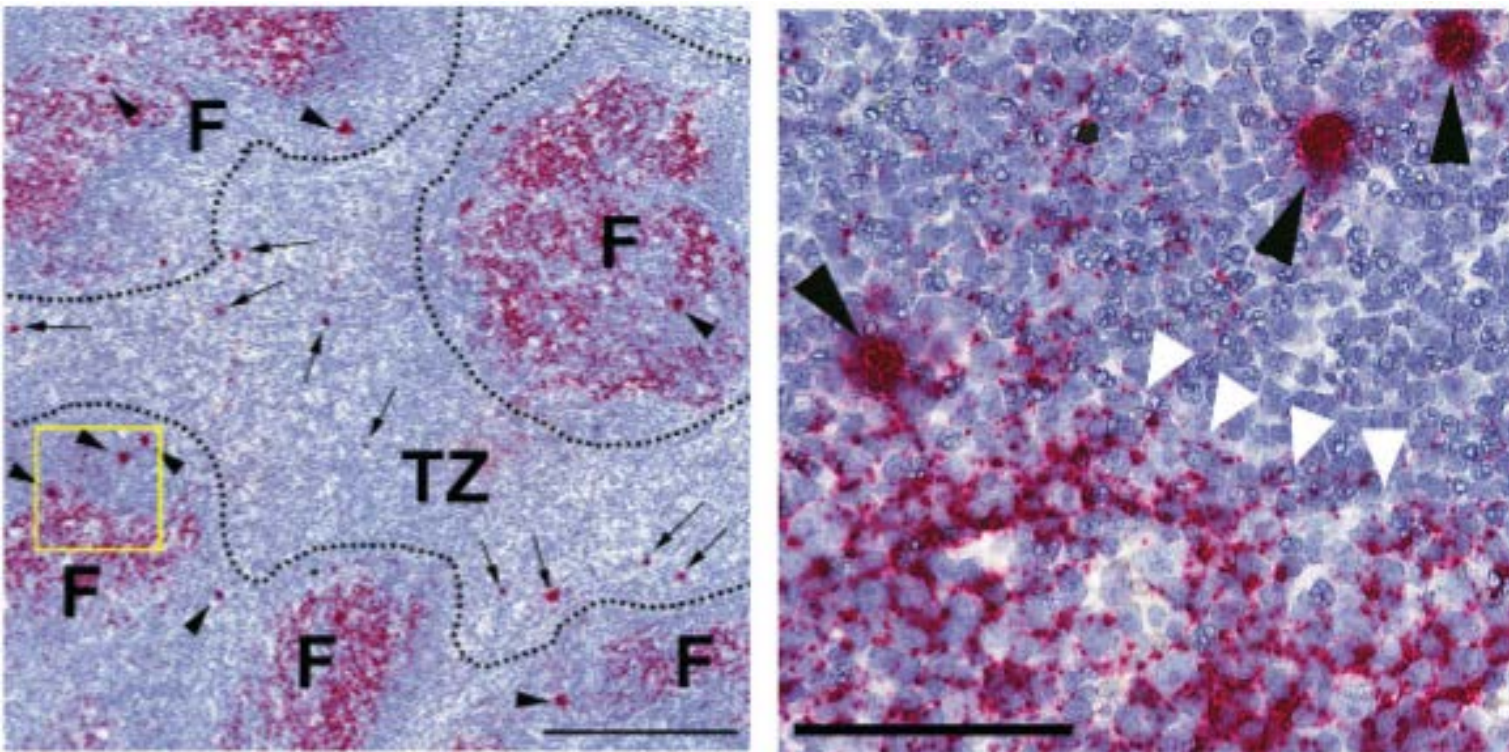


There are multiple sanctuaries where cells containing HIV may persist during antiretroviral therapy

- Lymph Nodes
- Gut Mucosa
- Central Nervous System

B cell follicle sanctuary permits persistent productive simian immunodeficiency virus infection in elite controllers

Yoshinori Fukazawa^{1,2}, Richard Lum^{1,2}, Afam A Okoye^{1,2}, Haesun Park^{1,2}, Kenta Matsuda³, Jin Young Bae^{1,2}, Shoko I Hagen^{1,2}, Rebecca Shoemaker⁴, Claire Deleage⁴, Carissa Lucero⁴, David Morcock⁴, Tonya Swanson^{1,2}, Alfred W Legasse^{1,2}, Michael K Axthelm^{1,2}, Joseph Hesselgesser⁵, Romas Geleziunas⁵, Vanessa M Hirsch³, Paul T Edlefsen⁶, Michael Piatak, Jr⁴, Jacob D Estes⁴, Jeffrey D Lifson⁴ & Louis J Picker^{1,2}

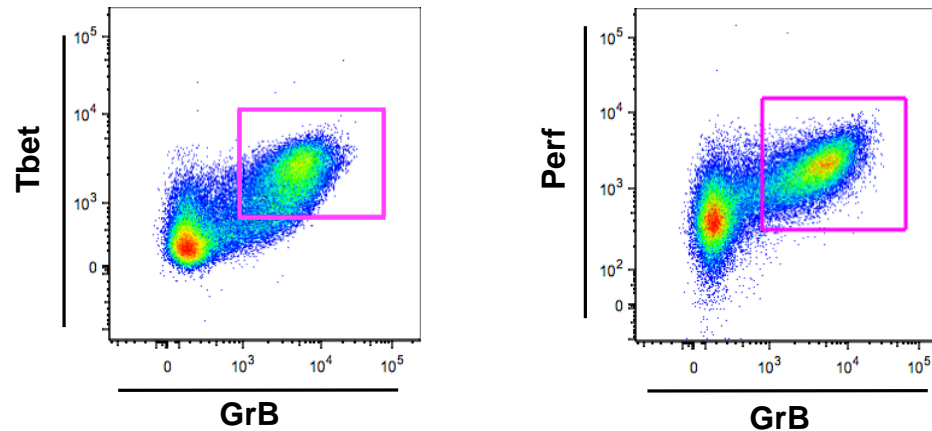


SIV RNAscope staining of Lymph Node

Immune Cells that target HIV gotta be in the right place at the right time

- How do we get Anti HIV killer cells (NK and CTL) into lymphoid sites of HIV replication?
 - Cytolytic cells typically excluded from nodes in health and during successful ART.
 - They migrate from nodes to blood via an S1P1-dependent mechanism
 - FTY 720 (Fingolimid) blocks S1P1, sequestering immune cells (including cytolytic effector T cells) in lymphoid tissues.
 - Approved for treatment of multiple sclerosis

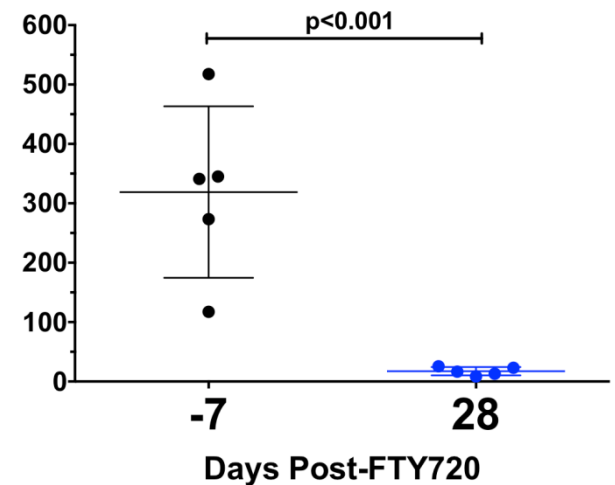
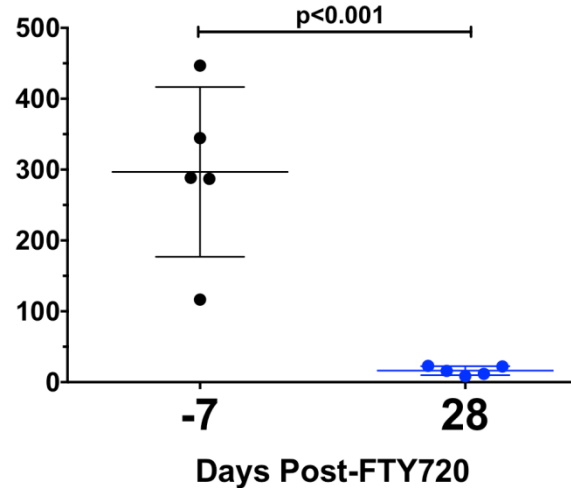
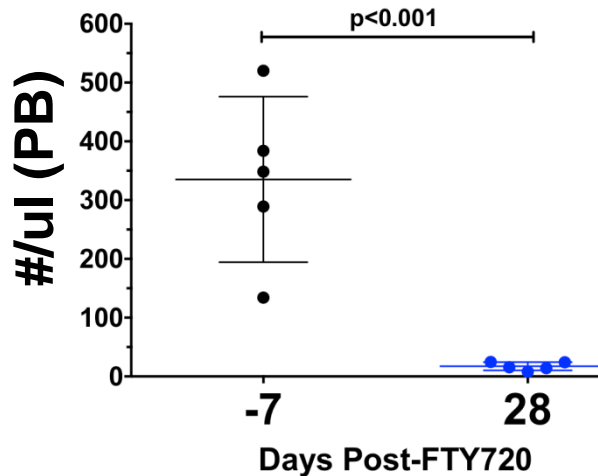
FTY720 induces a profound redistribution of cytolytic T cells from blood



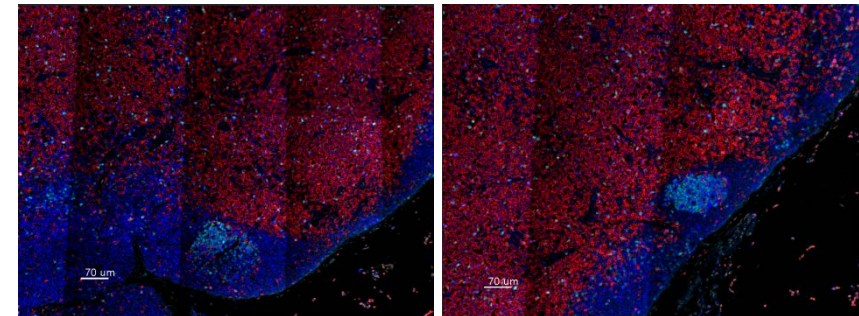
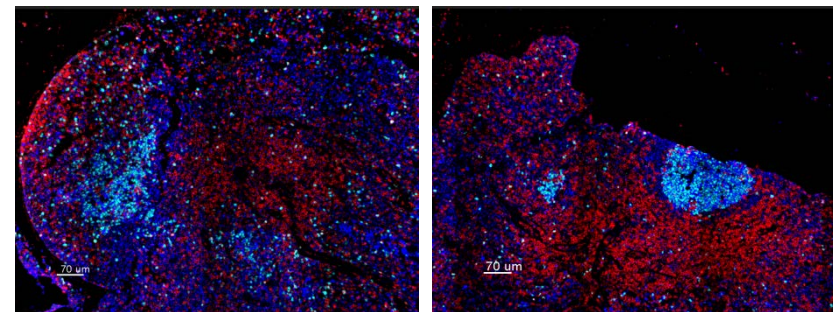
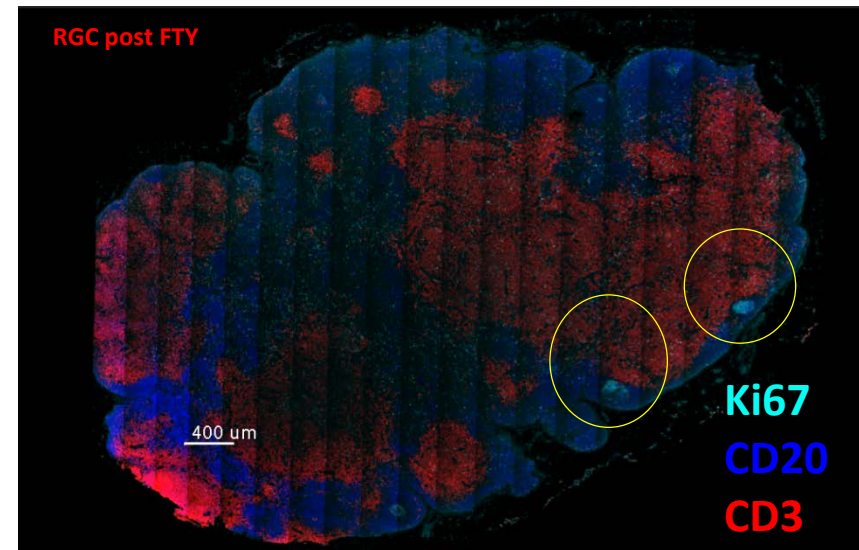
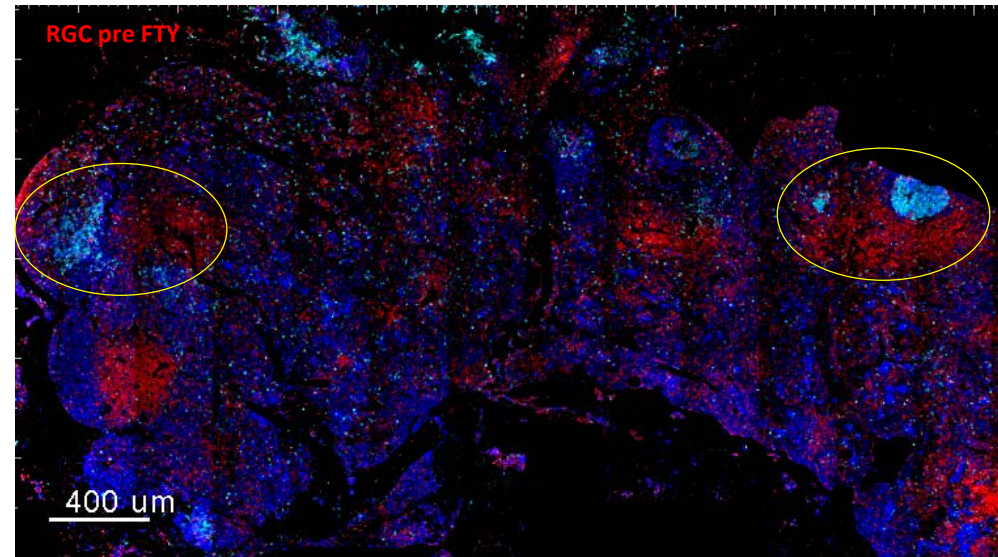
#CD8+Perforin+

#CD8+GrB+

#CD8+Tbet+



FTY720 retains CD4 and CD8 T cells in Lymph Nodes



Even though FTY720 keeps immune cells in lymph nodes, the Germinal Center may comprise an additional barrier to cytolytic immune cells that can destroy HIV infected targets.

And don't forget the central nervous system as a reservoir for HIV persistence

Can we make cells resistant to HIV infection? CCR5 and its “knockout”

- CCR5 a coreceptor for HIV cellular entry
- 32 bp deletion in the CCR5 gene results in defective protein
- Persons homozygous for this knockout are almost completely resistant to HIV infection
- The knockout occurred an estimated 10,000 years ago (only once), is in Hardy-Weinberg equilibrium and has an allele frequency of 10-20% in Northern Europe
- About 1% of Caucasians are born with two defective alleles

BRIEF REPORT

Long-Term Control of HIV by *CCR5* Delta32/ Delta32 Stem-Cell Transplantation

Gero Hütter, M.D., Daniel Nowak, M.D., Maximilian Mossner, B.S.,
Susanne Ganepola, M.D., Arne Müßig, M.D., Kristina Allers, Ph.D.,
Thomas Schneider, M.D., Ph.D., Jörg Hofmann, Ph.D., Claudia Kücherer, M.D.,
Olga Blau, M.D., Igor W. Blau, M.D., Wolf K. Hofmann, M.D.,
and Eckhard Thiel, M.D.

The Hutter Case Report

- HIV+ patient (CCR5 wt/ Δ 32) suppressed on HAART developed AML and failed chemotherapy
- During first induction, HAART held and viremia rebounded.
- After recurrence, conditioned with: TBI; amsacrine, fludarabine, cytarabine, cyclophosphamide, ATG)
- Allo stem cell transplant (CCR5 Δ 32/ Δ 32 donor); HAART held.
- Mild GVHD (treated with immune suppressants)
- Plasma levels of virus remained undetectable off HAART. No viral RNA or DNA in blood, BM, rectal biopsy, meningeal biopsy.
- CD4 T cell counts rose, serum Abs to gag/pol proteins decreased and T cell responses to HIV fell to background levels
- He remains well without evidence of virus recurrence more than 9 years after transplant and off antiretrovirals

Can this outcome be replicated?

Table 1. Men with Human Immunodeficiency Virus Type 1 (HIV-1) Infection Who Received an Allogeneic Transplant from a Stem-Cell Donor Who Was Homozygous for the CCR5 delta32/delta32 Mutation.*

Location of Transplantation	Age of Patient yr	Type of Cancer	Type of Graft	Outcome after Transplantation
Berlin†	40	Acute myeloid leukemia	HLA-matched unrelated	Alive after 7 yr, no viral rebound, no ART
Utrecht, the Netherlands‡	53	Myelodysplastic syndrome	Combined haploidentical bridge with umbilical-cord blood	Died from relapse of the myelodysplastic syndrome and pneumonia after 2 mo
Münster, Germany§	51	Non-Hodgkin's lymphoma	HLA-mismatched unrelated	Died from infection after 4 mo
Essen, Germany¶	30	Non-Hodgkin's lymphoma	HLA-matched unrelated	CXCR4-tropic HIV-1 rebound, died from relapse of non-Hodgkin's lymphoma after 12 mo
Minneapolis§	12	Acute lymphoblastic leukemia	Umbilical-cord blood	Died from GVHD after 3 mo
Santiago, Chile§	46	Non-Hodgkin's lymphoma	HLA-matched related	Died from pneumonia shortly afterward
Barcelona§	37	Non-Hodgkin's lymphoma	Combined haploidentical bridge with umbilical-cord blood	Died from relapse of non-Hodgkin's lymphoma after 3 mo

* ART denotes antiretroviral therapy, and GVHD graft-versus-host disease.

† Data are from Hütter et al.¹

‡ Data are from Kwon et al.³

§ Data are from a personal communication with the transplantation center.

¶ Data are from Kordelas et al.²

Antiretroviral-Free HIV-1 Remission and Viral Rebound After Allogeneic Stem Cell Transplantation

Report of 2 Cases

Timothy J. Henrich, MD; Emily Hanhauser, BS; Francisco M. Marty, MD; Michael N. Sitrignano, BS; Sheila Keating, PhD; Tzong-Hae Lee, MD, PhD; Yvonne P. Robles, BA; Benjamin T. Davis, MD; Jonathan Z. Li, MD; Andrea Helsey, BS; Allison L. Hill, PhD; Michael P. Busch, MD, PhD; Philippe Armand, MD, PhD; Robert J. Solfer, MD; Marcus Altfeld, MD, PhD; and Daniel R. Kurtzkes, MD

- 2 HIV+ pts with lymphoma
- Allogeneic stem cell transplantation (donors CCR5 wt/wt)
- On HAART 2.6 and 4.3 yrs with
 - No HIV detected in plasma
 - No HIV DNA found in multiple assays of 5 million CD4 cells
 - No HIV DNA in rectal bx of one patient
 - ART withdrawn
 - Initially, no virus or viral sequences in blood or tissues. HIV Antibody levels fell
- But viral rebound after 12 and 32 weeks off ART
- Virus persisted somewhere at low frequency

What does this mean??

- Allo stem cell transplants for malignancy carry risk.
- Cure in this setting is possible but not predictable
- You can decrease reservoir with myeloablation but cure may need replacement with **resistant** cells
- Large blood or tissue sampling is needed for eradication studies
 - We can't sample a whole person to find a rare infectious virus
- Stochastic awakening of even very few (even one?) infectious virus may be enough to restart full blown infection

So why was Tim Brown (the Berlin Patient) cured

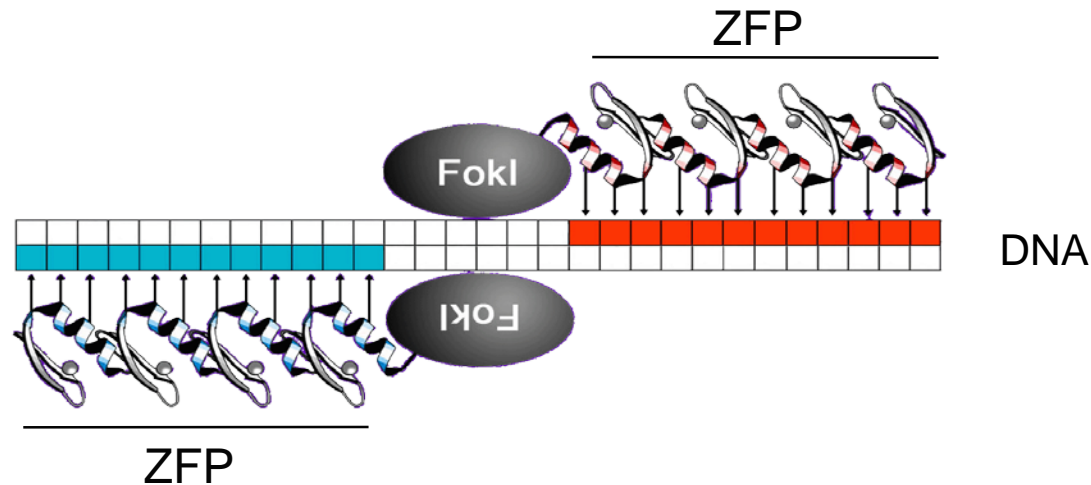


- A combination of non-selective myeloablation and replacement of the immune repertoire with HIV resistant cells resulted in a “Cure”
- Can’t find virus or viral sequences
- Are all HIV-infected cells gone?
- Maybe, Maybe not: His CD4 T cells are all HIV resistant, any HIV reactivation cannot amplify to produce detectable rebound

Can a similar effect be achieved genetically?

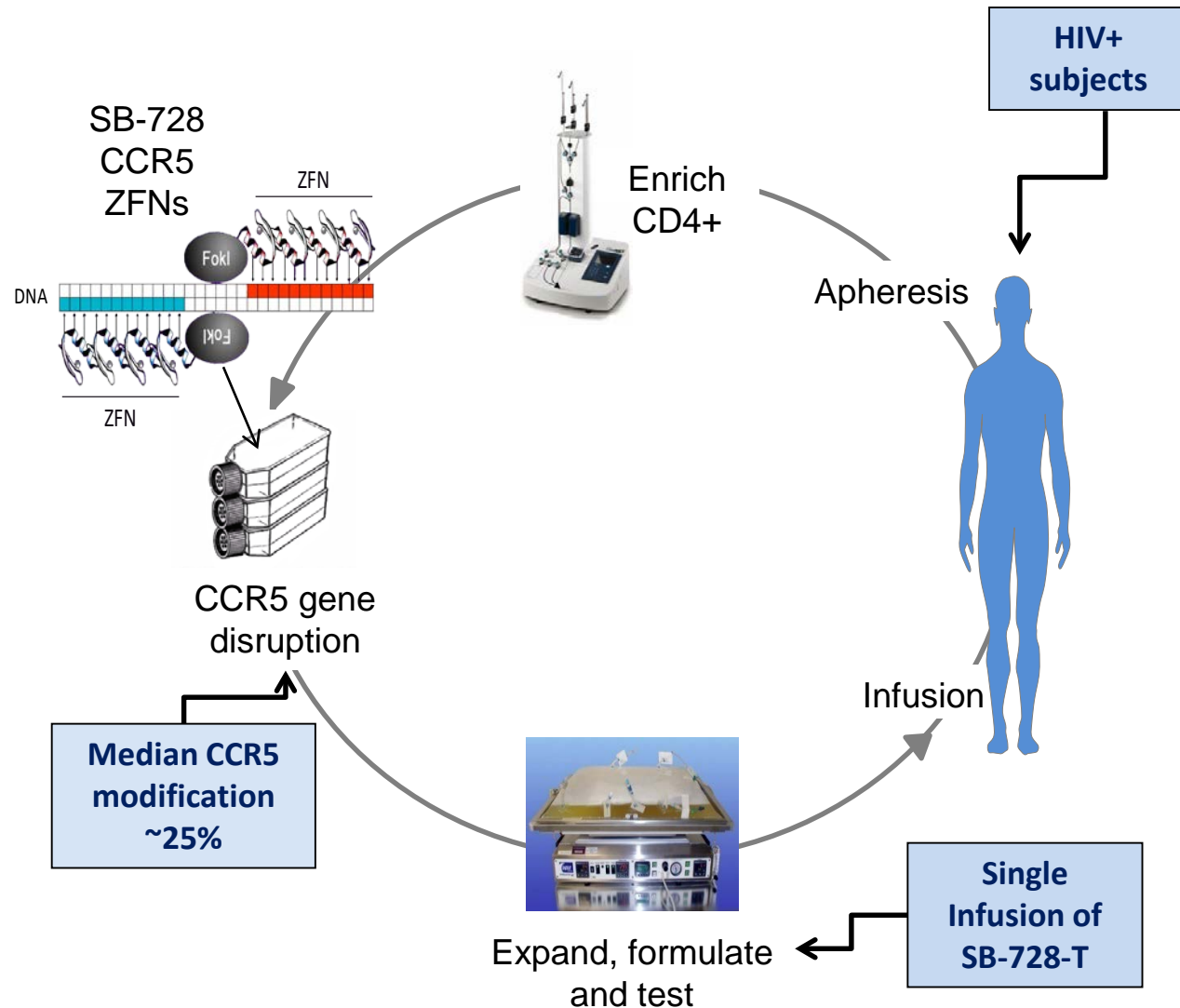
- Without risking morbidity of allo transplantation?

Zinc Finger Nucleases can target host elements and delete them



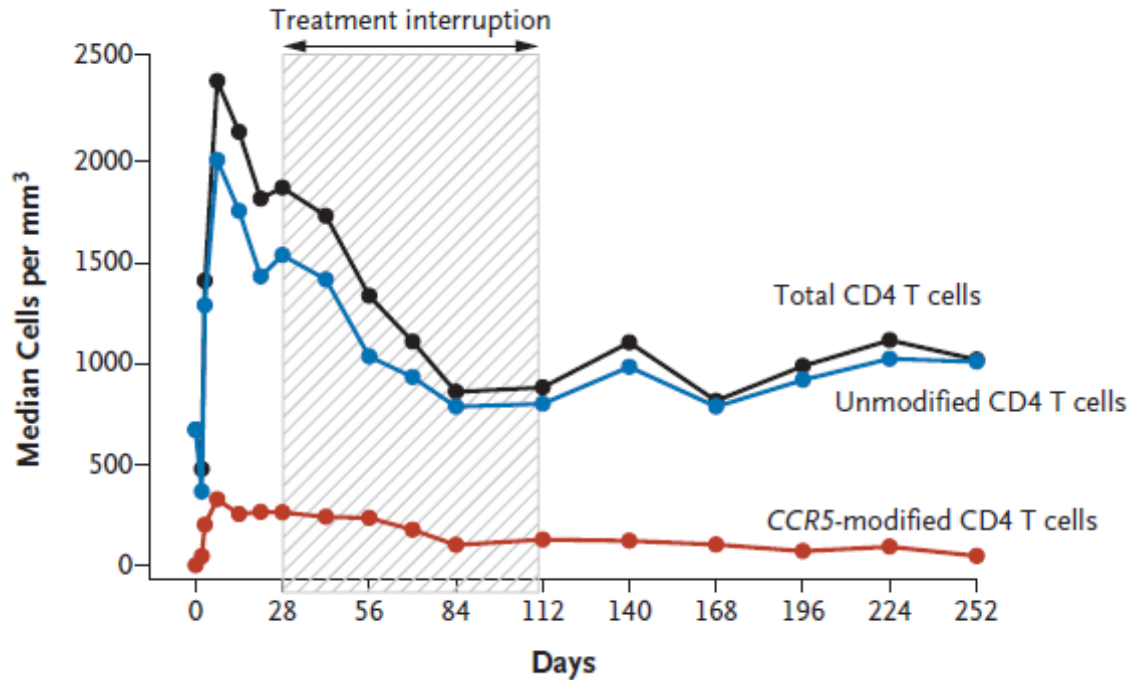
- Comprised of two domains:
 - Nuclease domain of FokI restriction enzyme
 - Engineered zinc finger protein (ZFP) provides DNA binding specificity
 - Targets 12 nucleotides each for a total of 24-bps of DNA
- ZFN cleaves genomic DNA as a heterodimer within a 5-6 bp gap between the two binding domains
- Repair typically renders a deletion in the target gene
- Introduction of this vector into host target cells or host stem cells can render the target or stem cell progeny resistant to HIV infection (if both gene copies are knocked out)

Adoptive Transfer of CCR5 Gene Modified Autologous CD4⁺ T-cells



- This immunotherapy is **minimally invasive**, with no severe adverse events
- It is **more accessible than HSCT**; it removes the need of searching for compatible donors and the risks associated with GVHD

Infusion of autologous CD4 cells with modified CCR5



Dramatic CD4 rises – mechanism?
Modified cells persisted durably (were they protected?)

We can expand autologous CD4 T cells in treated HIV infection and render some of them resistant to HIV infection by CCR5 knockout

- Is the CD4 T cell expansion related to the CCR5 knockout or is it related to the ex vivo CD4 T cell expansion?
- How many “resistant” CD4 T cells are needed to allow control of HIV infection off ART?
- A clinical trial of CCR5 knockout to begin here at CWRU in late 2018
- Can we learn how to knockout CCR5 in autologous stem cells (HSC) and protect their progeny (without the risks of allogeneic HSC transplant?)

What if we can render all HSC and their progeny resistant to HIV

- Will knocking out CCR5 work for everyone?
 - There are other ways to help cells become HIV resistant
- Will brain microglia be protected by resistant HSC?
- Are there other sites of viral persistence?
- Will HIV eradication “fix” the residual fibrosis, inflammation and coagulation characterize treated HIV infection?

Engaging participants in Cure Studies?

- Eradication – a high priority for many persons with HIV infection
- Be careful not to promise too much
- Journalistic and Institutional Sensationalism hurt the field
- Remember that ART regimens are now safe, effective, well tolerated and life expectancy can be normal or near normal
- **The bar is high!!**

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