NORTHWEST AIDS EDUCATION AND TRAINING CENTER

Curing HIV

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Presentation prepared by:
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Curing HIV

- Why do we want to cure people of HIV?
- What is the HIV Reservoir?
- The effect of HAART and initial predictions
- Sterilizing Vs functional cures
- What did Timothy Brown, the Berlin patient, and this baby from Mississippi teach us?
- HIV cure strategies
  - Activating and killing (purging) the reservoir
  - Therapeutic vaccination
  - Transplantation
  - Gene therapy

Curing HIV

Why do we want to cure people of HIV?

- Currently HIV treatment requires a lifetime of excellent adherence to treatment with expensive medications
- Life expectancy remains lower than the general population: 50% lower chance of reaching age 70 in one study
- ARV toxicities include lipodystrophy, hyperlipidemia, renal/bone disease
- Increase risk for vascular disease, liver disease, and malignancies even in people with well controlled HIV
- The stigma of HIV can still be crippling and contributes to social isolation, depression, substance abuse
- HIV disrupts the sexual health and reproductive future of patients and their partners

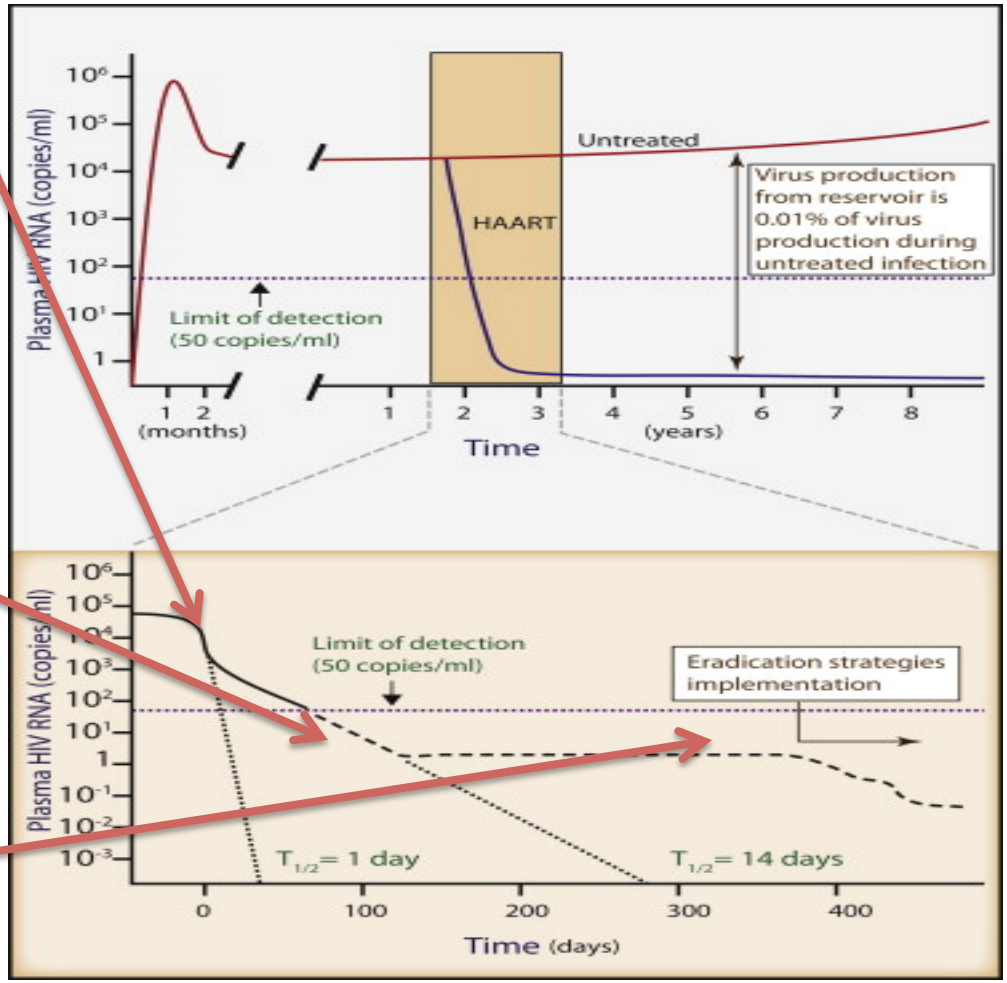
(Neuhaus, AIDS, 2010, ART-CC, Lancet 2008, Deeks, BMJ, 2009, Friis-Moller, AIDS, 2003)

Curing HIV: What is the HIV Reservoir?

Most of the plasma virus is produced by activated CD4$^+$ T cells, which turn over very quickly ($t_{1/2} \approx 1$ day).
Ho et al., 1995; Perelson et al., 1996, 1997; Wei et al., 1995; Zhang et al., 1999

A minor population of virus-producing cells with $t_{1/2} \approx 2$ weeks- productively infected CD4$^+$ T cells with a resting phenotype.
Perelson et al., 1997; Zhang et al.1999

Stable reservoir - an infected cell population that allows the persistence of replication-competent HIV-1 in patients on optimal HAART - resting CD4$^+$ T cells
Noë et al., 2005; Persaud et al., 2000; Ruff et al., 2002 Nickle et al., 2003



Eisele and Silicano, Immunity Volume 37, Issue 3 2012 377 - 388

Curing HIV: What is the HIV Reservoir?

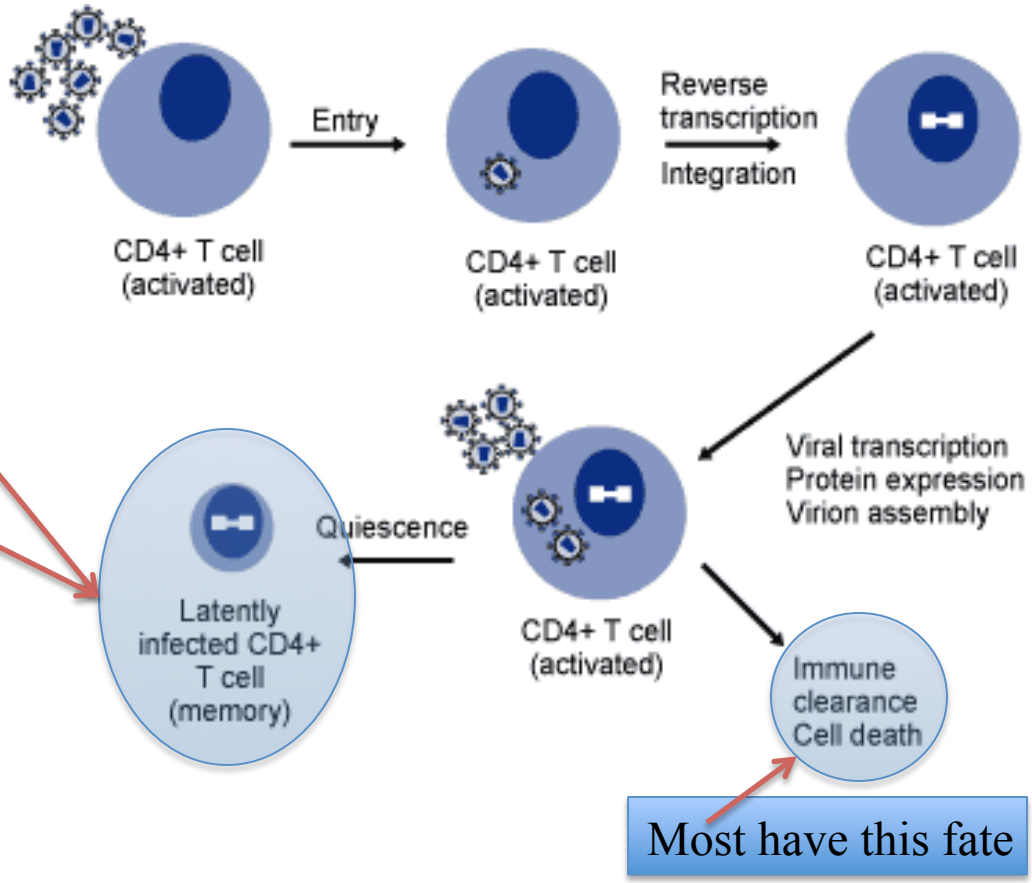
Its an accident! The result of some activated and infected T-cells that "turn off" and become quiescent memory T-cells

The cellular composition of the reservoir is thought to be mostly the central-memory (and transitional-memory) T-cells

These number ~ 1 x 10^6

Other cell types *may* contribute to the reservoir including stem cells (CD 133+), gamma/delta-T-cells, macrophages, glial cells, neurons

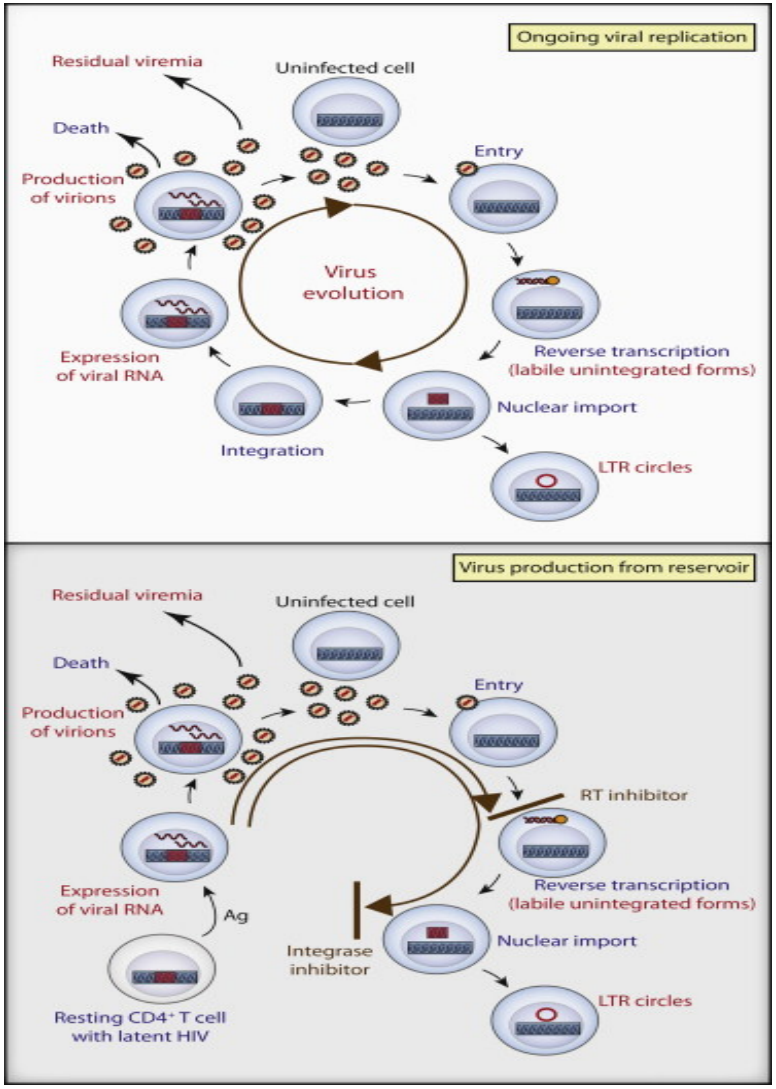
Anatomic sites include LNs, GALT, spleen, brain



(www.clinicaloptions.com, Eisele and Silicano 2012)

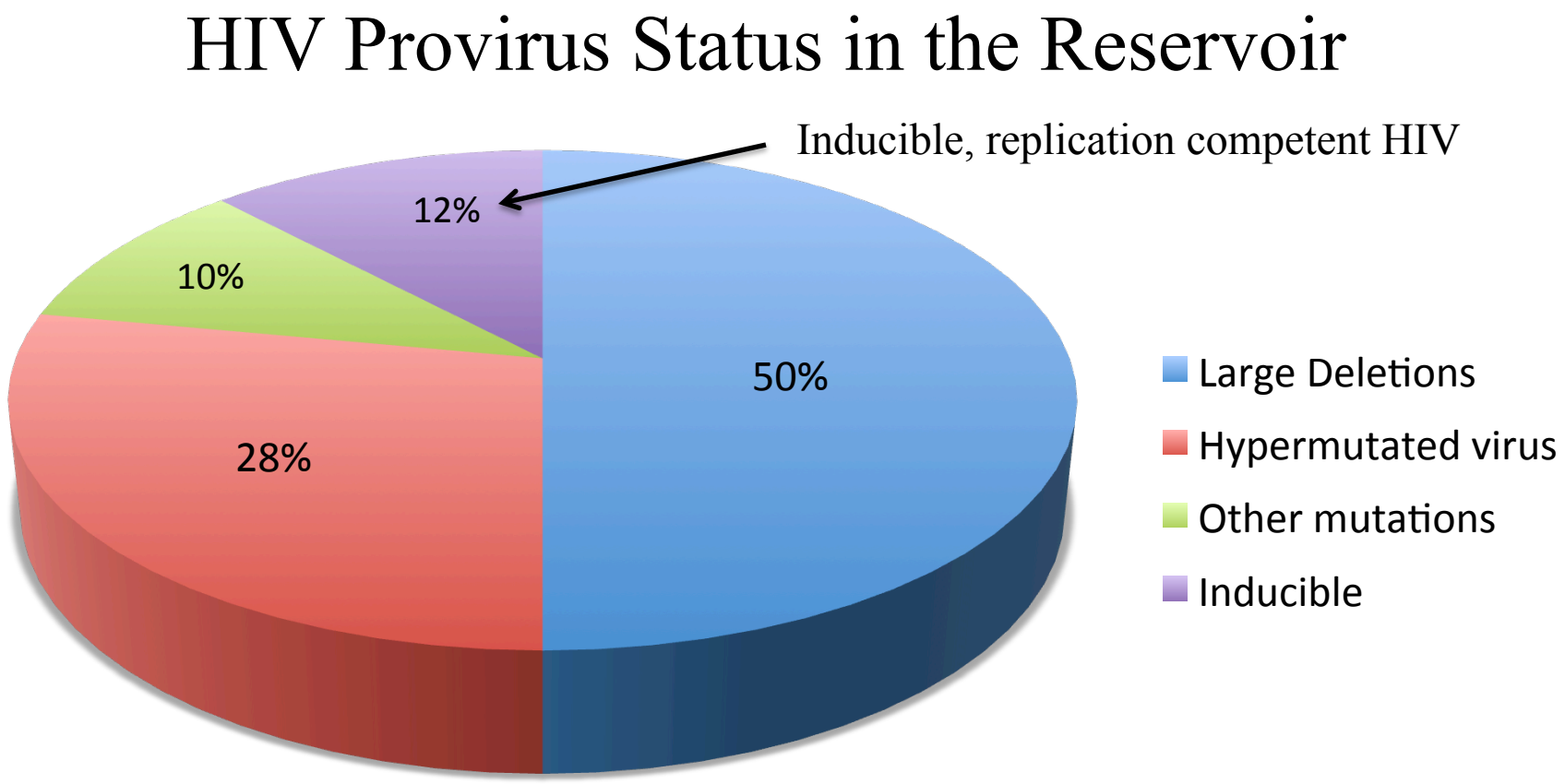
Curing HIV: What is the HIV Reservoir?

- Is the reservoir replenishing itself (panel A) or can virus 'leak out' but not lead to the infection of other cells that aid in the persistence of the reservoir (panel B)?
- Most data suggest 'B'is correct
  - Limited evolution of the reservoir over time
  - Intensification has no effect of residual viremia



(Eisele and Silicano, Immunity, 2012, Nickle, J Virol 2003, Baily J Exp Med 2006, Kieffer JID 2004, Nettles JAMA 2005, Dinoso PNAS 2009, Ghandi Plos Med 2010, Yukl JID 2010)

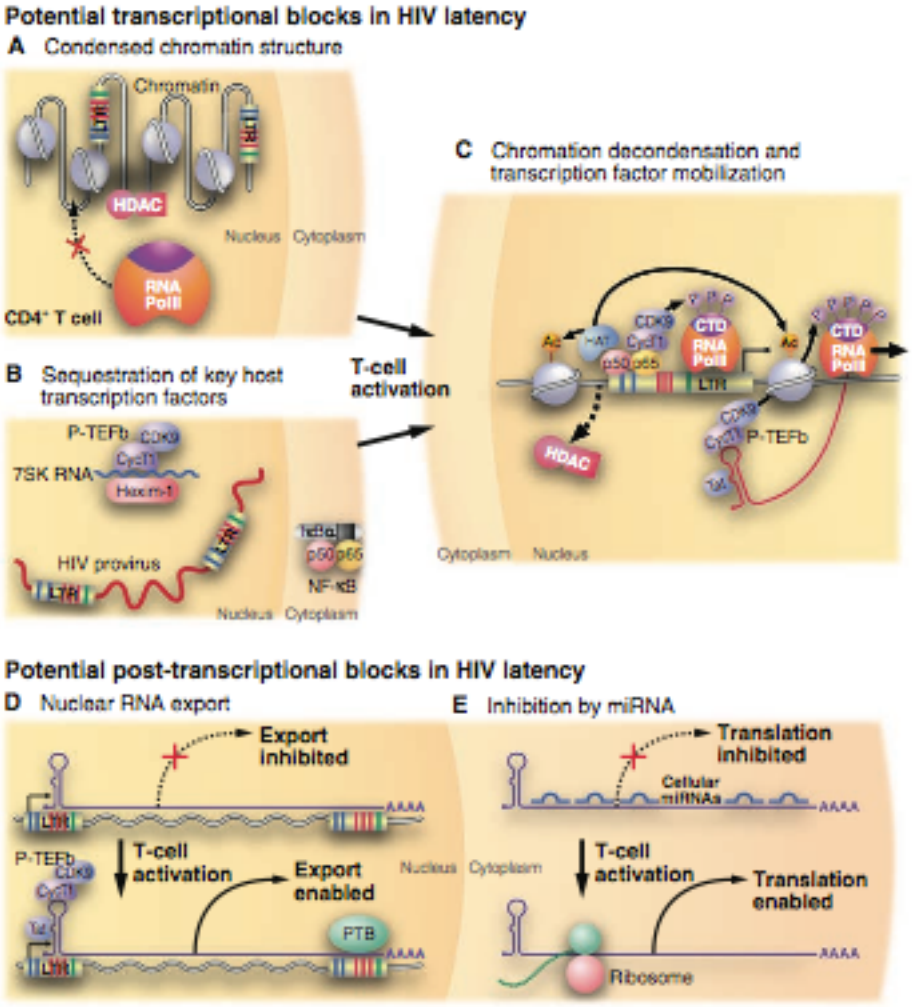
Curing HIV: What is the HIV Reservoir?



Silicano, CROI, 2013

Curing HIV: What is going on in latently infected T-cells?

- HIV provirus is crowded by de-acetylated histones
- Transcription (NFkB, NFAT) and elongation factors are sequestered and/ or in limited supply
- Methylation of DNA to prevent transcription
- mRNA transport out of the nucleus and translation are also inhibited

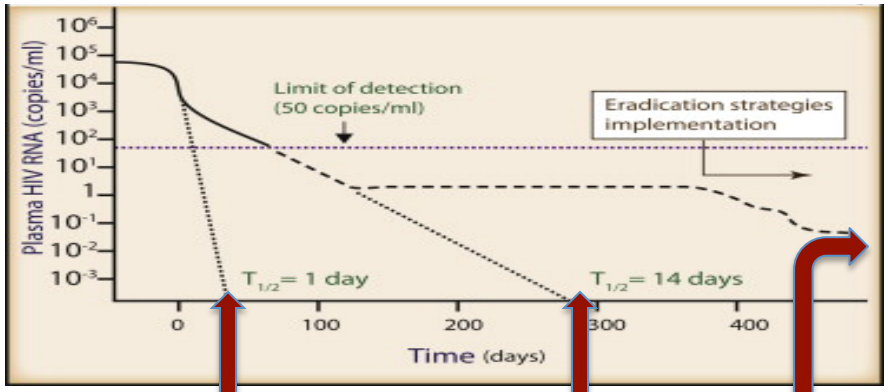


(Richman, Science, 2009)

Curing HIV

The Effect of HAART and Initial Predictions

- Before evidence for the existence of long-lived latently infected cells it was predicted that complete suppression of HIV replication would lead to cure in 2-3 years
- Knowing the presence of latently infected central memory T-cells and based on longitudinal analysis of patients on HAART it is now estimated that eradication of the latent reservoir would take at least ~60 years.
  - This assumes that there is no on-going replication that is constantly renewing the reservoir



Time to cure if all HIV+ cells died in <u>days</u> = days-to-weeks

Time to cure if all HIV+ cells died in <u>weeks</u> = months-to-years

Time to cure if all HIV+ cells died in <u>years</u> = decades

(Perelson, 1997, Silicano 2003, Strain 2003)

Curing HIV: Types of Cure

- Sterilizing cure: complete eradication of all replication-competent forms of HIV. The reservoir is gone.
- Functional cure: the reservoir remains but there is permanent control of viral replication without anti-retro-viral therapy (e.g. elite controllers).

Curing HIV: Timothy Brown

The NEW ENGLAND JOURNAL of MEDICINE

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.

Sterilizing cure is possible!

But this case required:
- Intense TBI/chemo (twice)
- CCR5 minus donor
- Graft Vs Host (HIV reservoir)

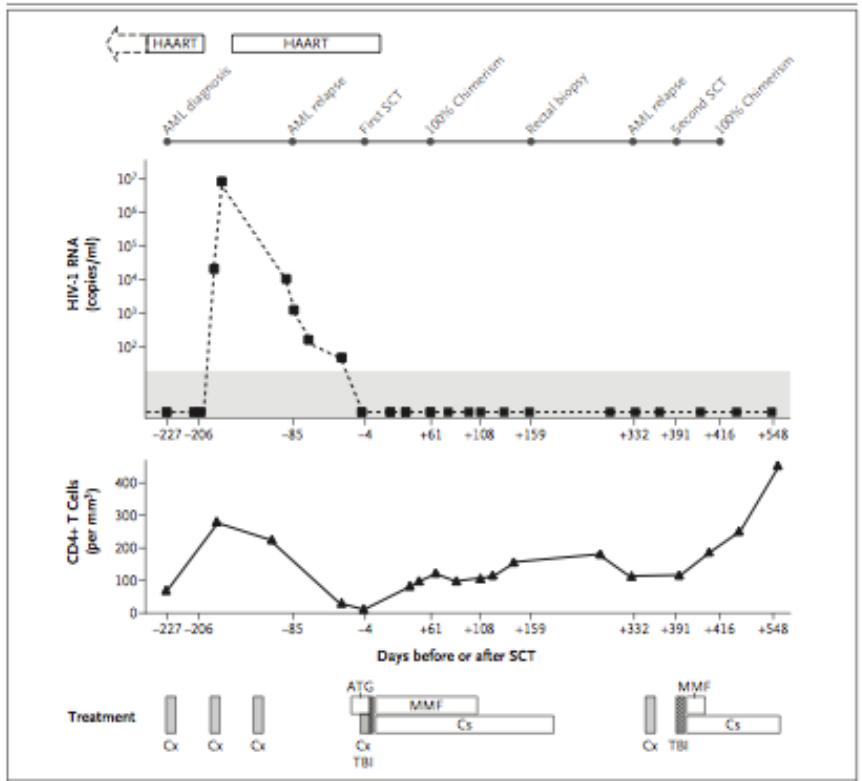
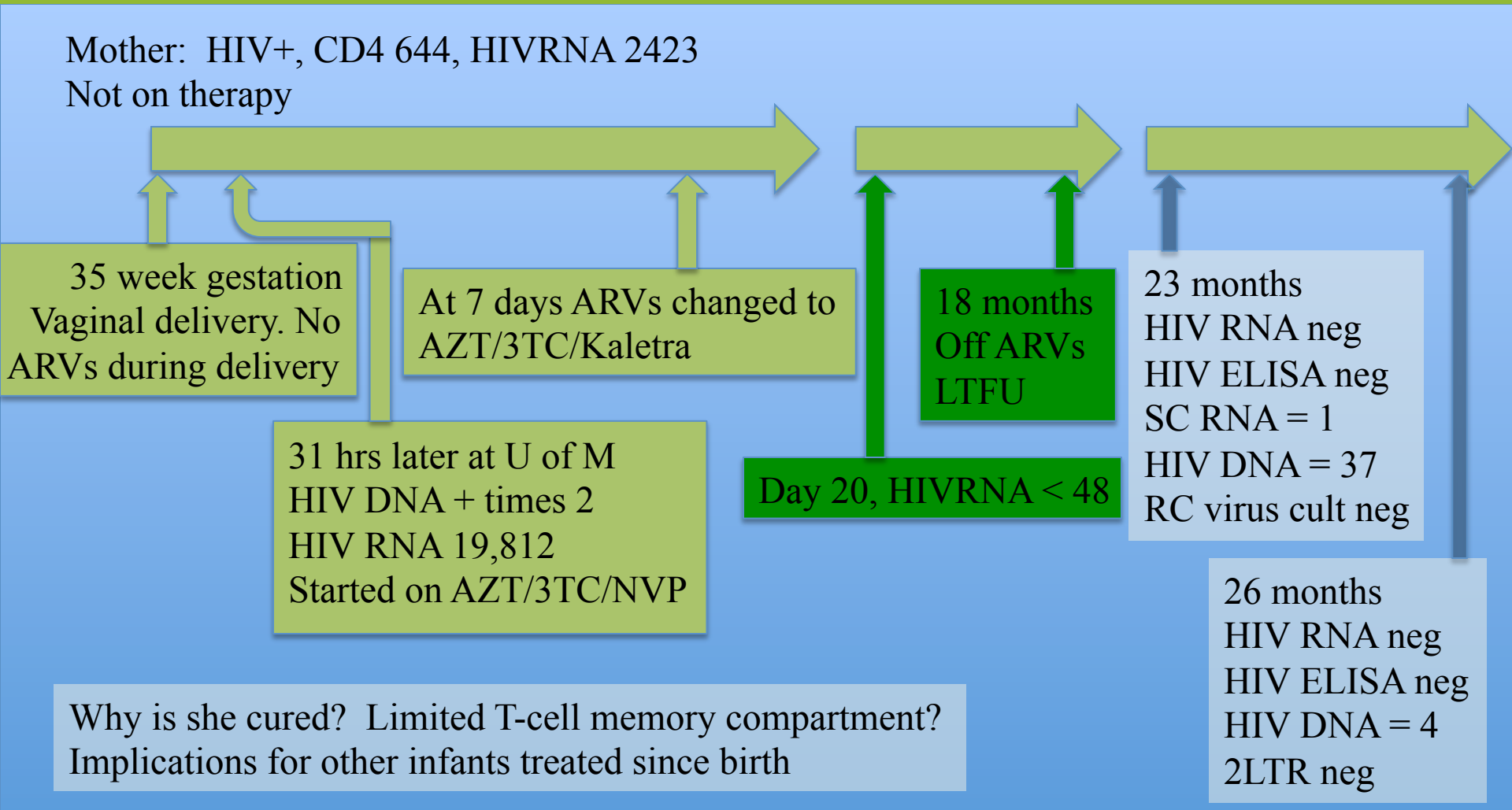


Figure 3. Clinical Course and HIV-1 Viremia.

The clinical course and treatment of acute myeloid leukemia (AML) as well as HIV and the measurement of HIV-1 viremia by means of RNA polymerase-chain-reaction assays are shown from the point of AML diagnosis to day 548 after stem-cell transplantation (SCT). HIV-1 RNA was not detected in peripheral blood or bone marrow from the point at which highly active antiretroviral therapy (HAART) was discontinued, 1 day before SCT, until the end of follow-up, 548 days after SCT. (The shaded area of this graph indicates the limit of detection of the HIV–RNA assay.) The CD4+ T-cell count in the peripheral blood is shown in reference to the immunosuppressive treatments. ATG denotes antithymocyte globulin, Cs cyclosporine, Cx chemotherapy, MMF mycophenolate mofetil, and TBI total-body irradiation.

Mississippi Miracle



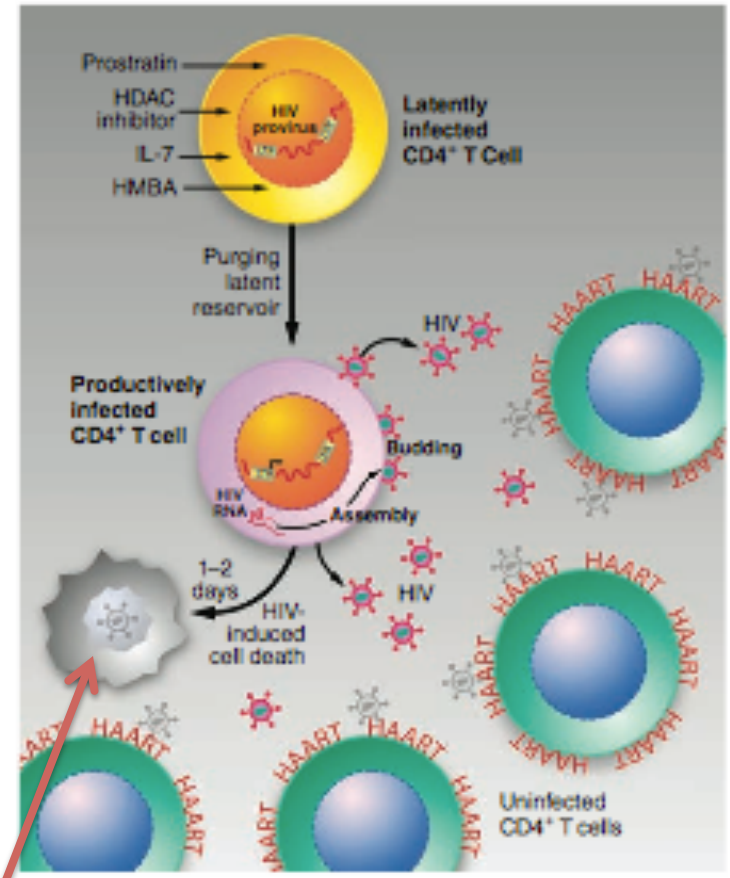
Why is she cured? Limited T-cell memory compartment?
Implications for other infants treated since birth

NW AETC | echo

HIV Cure Strategies: Purging the Reservoir

Activate the reservoir (latently infected resting cells) with:

1) HDAC inhibitors to open chromatin
2) IL-7 signaling through JAK/ STAT pathway (ERAMUNE)
3) Prostratin signaling through protein kinase C (increase NFkB)
4) DNA methylation inhibitors
5) Other T-cells are protected from infection by HAART
6) Activated cells die??



(Richman, Science, 2009, Wang JCI 2005, Levy JCI 2009, Sereti Blood 2009, Reuse PloS One 2009, Archin Nature 2012, Blazkova PLoS Path 2009))

HIV Cure Strategies: Purging the Reservoir

Vorinostat (SAHA) induced HIV replication (Archin, Nature, 2012)

- 8 patients on HAART given 400 mg of vorinostat
- Resting CD4+ T-cells removed from patients and tested for HIV replication by measurement of intracellular unspliced *gag* RNA

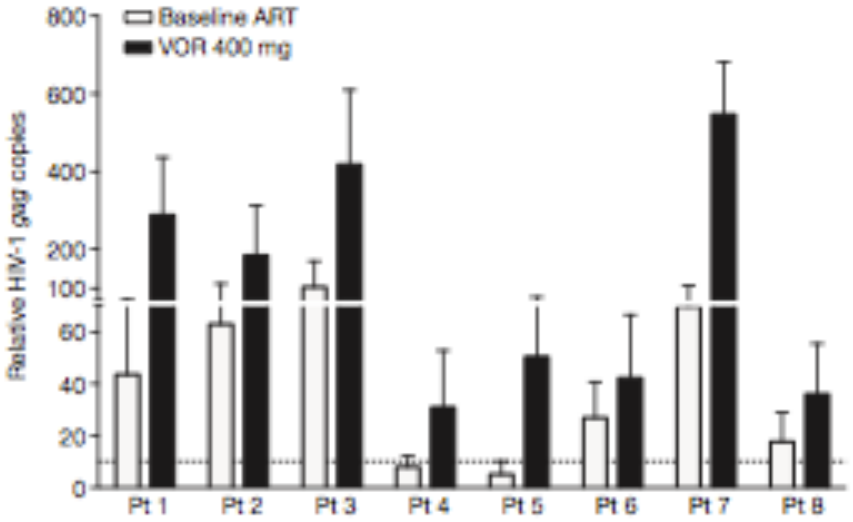


Figure 3 | VOR upregulates HIV RNA expression. The relative HIV-1 RNA copy number (mean +1 s.d.) measured in the resting $CD4^+$ T cells of eight HIV-positive patients with plasma HIV RNA BDL is shown on background ART and on ART following a single 400 mg oral dose of VOR. For each subject, the differences are significant ($P < 0.01$).

(Archin, Nature, 2012)

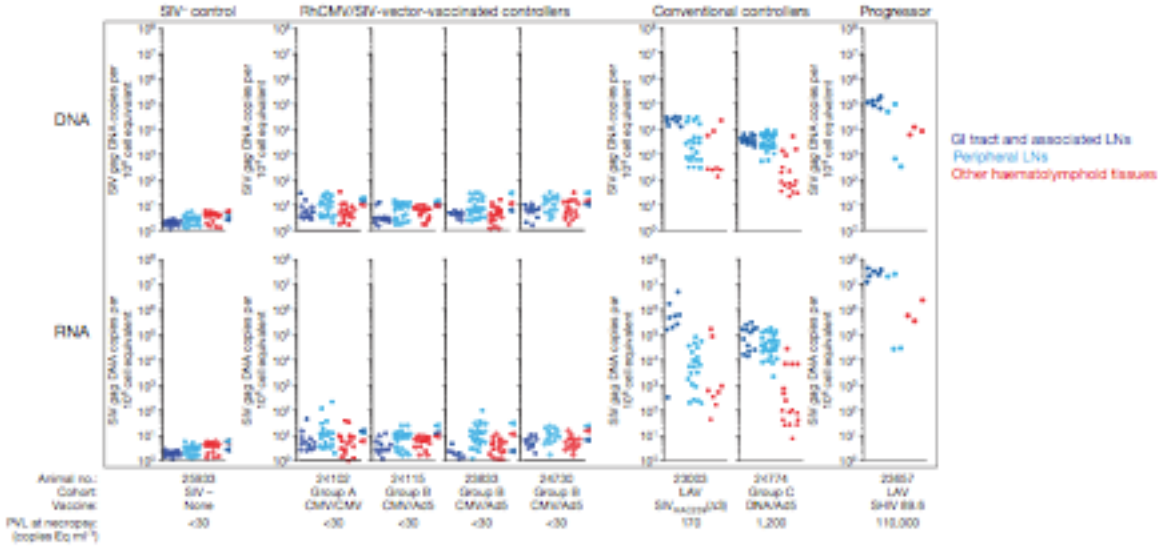
HIV Cure Strategies: Therapeutic Vaccination

- Concept is to use an HIV vaccine to induce immune control of HIV in already infected patients (i.e. as in elite controllers)
- Humans studies (e.g. ALVAC-HIV) have been disappointing (actually made things worse!)
- One interesting study in rhesus macaques
  - Used RhCMV vector vaccine with or without an Ad5 vaccine in SIV infected animals
  - 12/24 animals developed complete control (maybe even eradication)

(Autran, AIDS, 2008 and Hansen, Nature, 2011)

HIV Cure Strategies: Therapeutic Vaccination

Blood and Tissue Levels of SIV RNA and DNA at Necroscopy in Vaccinated and Unvaccinated Animals



Hansen, Nature, 2011

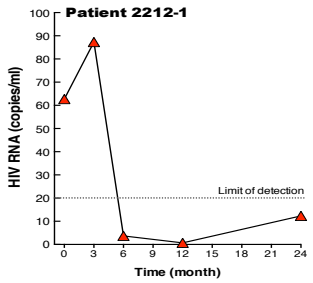
HIV Cure Strategies: Transplantation

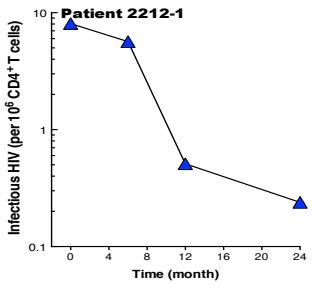
- Autologous transplant
  - Is it possible to eradicate or deplete the reservoir?
  - TBI +/- chemo to ablate lymphocytes (limited by toxicity to lung and liver)
  - Rescue with patient's own cells: avoid GVHD but may give back HIV+ cells
- Allogeneic transplant
  - Can use ablative or non-ablative (mini) conditioning regimens
  - Limited by histocompatibility
  - Rescue with donor cells: GVHD will occur
    - GVHD may be useful to purge the HIV reservoir
    - GVHD itself has significant morbidity and mortality

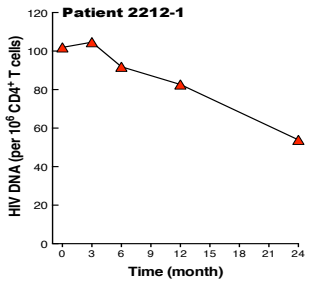
HIV Cure Strategies: Transplantation

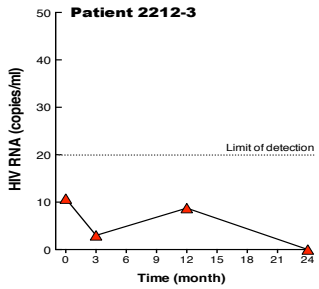
Autologous transplant: FHCRC Experience

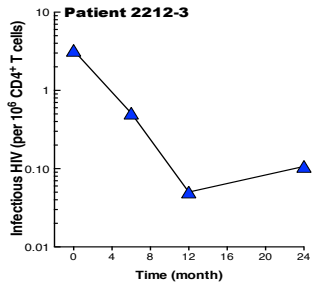
- 3 patients with HIV and lymphoma, 1 died after relapse, 2 evaluable; 1 conditioned with BEAM, the other with TBI +VP-16+CY. Both on ART with ND virus throughout Tx

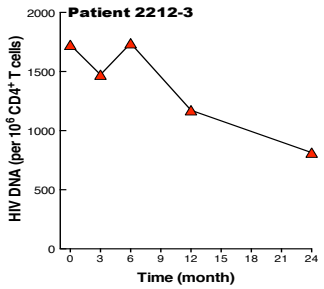












~ 1 log reduction

Significant reduction in reservoir, not eradication

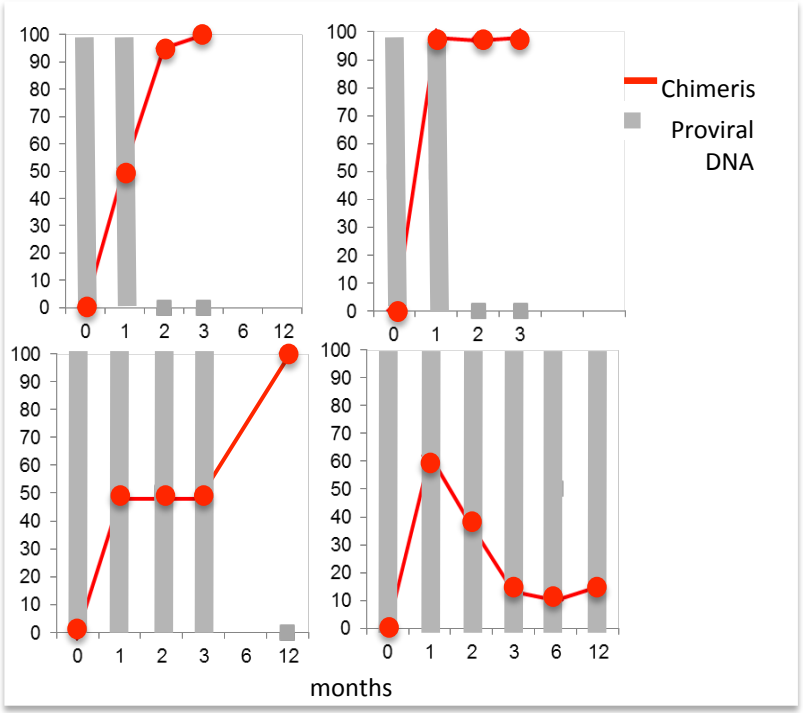
Depletion may lead the way for cure when combined with other treatments.

Ann Woolfrey, FHCRC and TW Chun, NIH

HIV Cure Strategies: Transplantation

Allogeneic transplant: FHCRC Experience

- 4 patients received Non-ablative transplant (mini).
- In ¾ patients as donor cells replaced host cells, HIV proviral DNA declined
- Graft Vs HIV reservoir?



Woolfrey, Blood, 2008 and TW Chun, NIH

HIV Cure Strategies: Gene Therapy

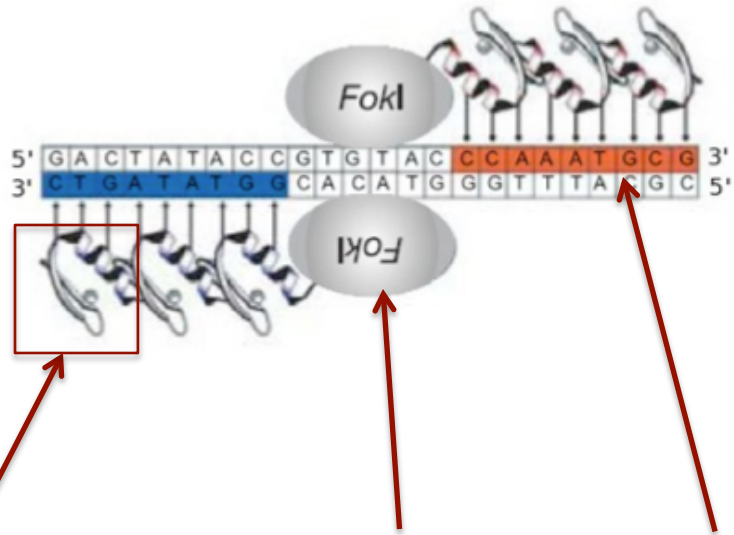
- Engineer cells to eliminate genes rendering them resistant to HIV infection (e.g. CCR5 knock-out)
- Target integrated HIV provirus with gene-cleaving enzymes (challenge would be delivery to *every* HIV+ cell)
- Engineer cells by adding genes rendering them resistant to HIV infection
- Combine gene therapy with transplantation to both deplete the reservoir and replace the immune system with HIV resistant cells

HIV Cure Strategies: Gene Therapy

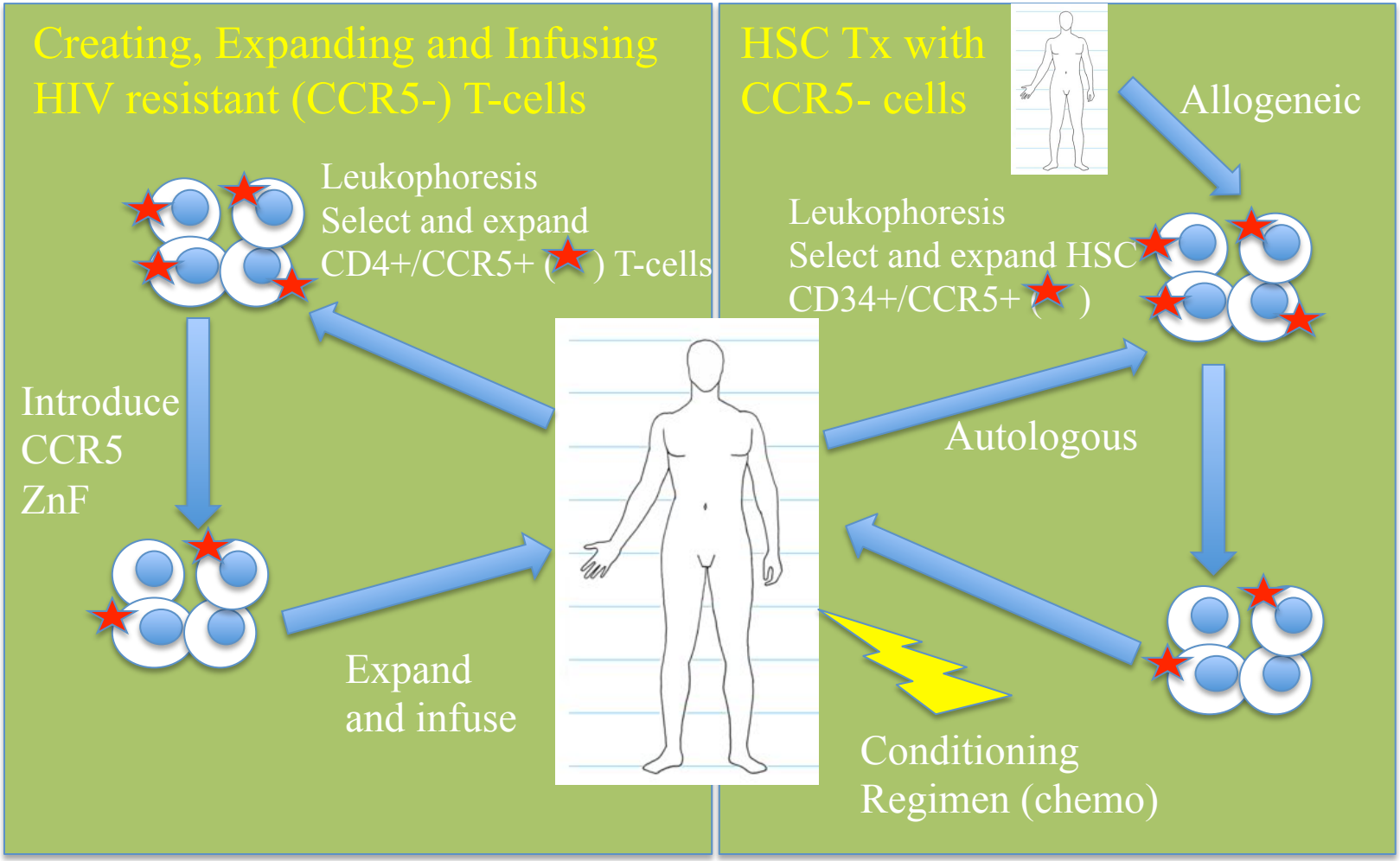
Zn finger endonucleases

- ZnF is a DNA binding protein that links to specific NA sequences
- Allows one to target genes with complimentary sequences
- Linking ZnF to an endonuclease (e.g. Fok1) permits targeted cutting (disruption) of dsDNA
- This typically leaves 5-7 BP gap that is repaired with a high frequency of errors… leading to gene inactivation

- ZnF targeting CCR5 has been developed by Sangamo corp
- Has been used to disrupt CCR5 gene and eliminate CCR5 expression



HIV Cure Strategies: Gene Therapy



HIV Cure Strategies: Gene Therapy

Disruption of CCR5 in ZnF-nuclease-treated CD4 T-cells: Phase I trials, Tebas and June (#165)

- 2 studies: U PENN (Jacobi) and Quest Clin Res/UCLA
- N = 14 patients infused to date, 9 evaluable. In a variety of cohorts stratified by CD4 count and viremia
- Single infusion of 10-20 billion ZnF modified CD4 cells (~ 25% CCR5-)
- Rise in CD4 count of > 100 cells
- Engraftment of CCR5- cells in 8/9 pts
- Day 14: 1.2% to 30% of PB CD4+ cells are CCR5-
- Day 90: median of 5.2% of PB CD4+ cells are CCR5- (indicating expansion)
- CD4+/CCR5- cells detected in gut mucosa

CROI, 2012, Abs # 165

HIV Cure Strategies: Gene Therapy: CROI 2013

Human Study

- Adoptive Immunotherapy with ZN-CCR5 CD4+ T-cells (SB-728-T): 9 HIV+ subjects, infused with 20-30 billion modified cells. Led to a median rise of 103 CD4 cells at 1 year. 2 groups of responders:
  - High responders (N=5),median CD4 increase of 227, T_{CM} % increase of 2.2. Patients had lower levels of inflammation (lower PD-1 expression) post cell-infusion and lower levels of inflammation (CD16+, CD163+) at the time of cell-infusion.
  - Low responders (N=4), median CD4 increase of 44, T_{CM} % increase of 1.1. Had higher level markers of inflammation before and after cell infusion.
  - Suggests that the inflammatory state might be responsible for death of these modified cells (induces apoptosis).

CROI, 2013: Abst #: 126 Zeiden and Sekaly, et al

HIV Cure Strategies: Gene Therapy: CROI 2013

Non-human Primate Study

- Autologous HSCT of Pig-tail Macaques with gene-modified CD34+ stem cells expressing a fusion inhibitor (mC46), followed by SHIV infection. 4 monkeys; 2 controls, 2 mC46 transduced.
  - At 3 weeks mC46 CD4+ cells represented > 90% of CD4 cells
  - These cells persisted over time but their % declined – due to a rise in non-transduced CD4+ cells…suggesting the transduced CD4+ cells were helping the non-transduced CD4+ cells survive (perhaps though better control of HIV infection)
  - mC46 monkeys also had better HIV control, higher levels of anti-SHIV neutralizing Abs and CTL responses as well as SHIV specific CD4+ T-cell responses.

CROI, 2013: Abst #: 127 Younan, H-P Kiem, et al

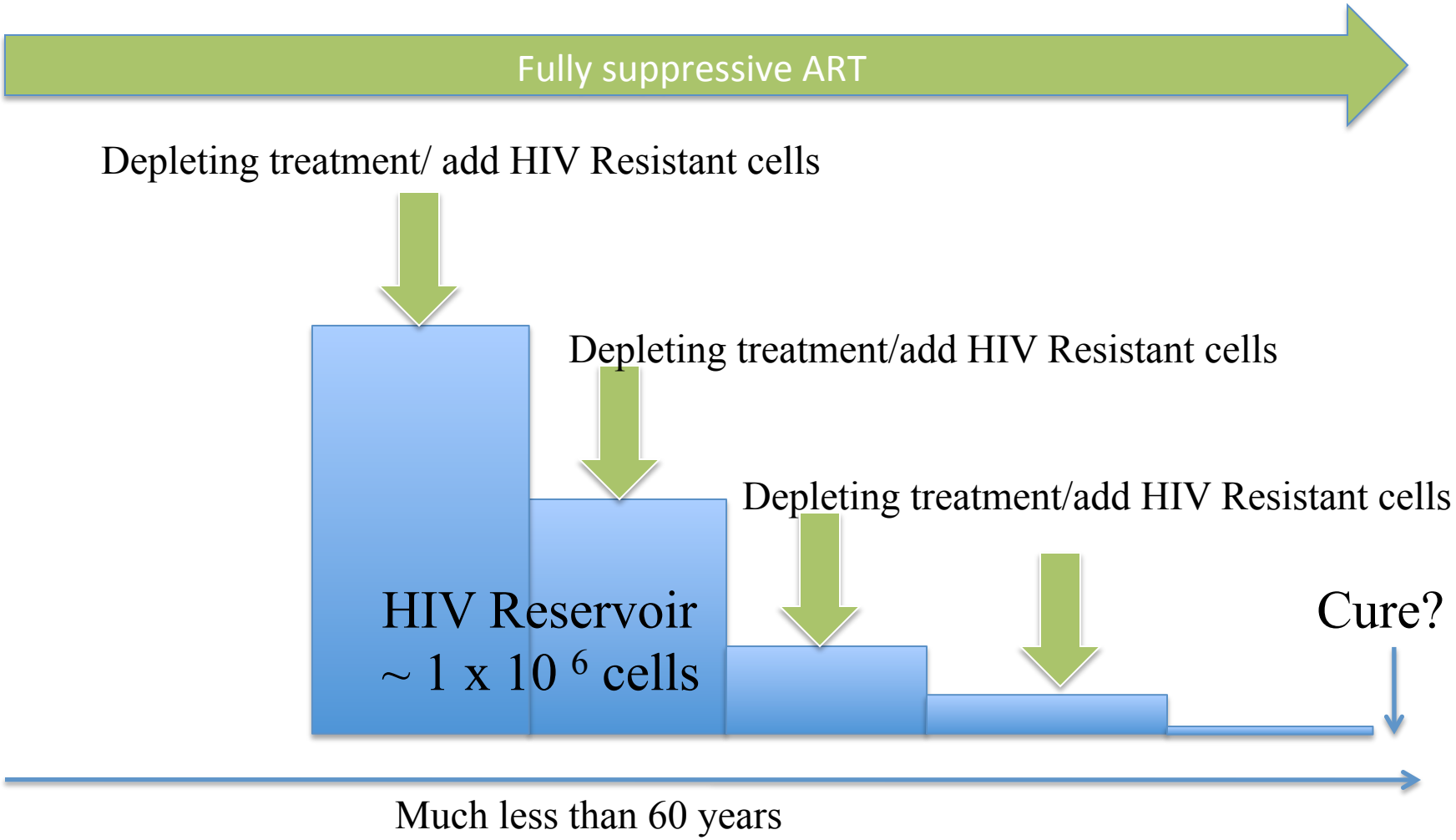
HIV Cure Strategies: Gene Therapy: CROI 2013

In-vitro Study

- C34 peptide from GP41 prevents infection with HIV.
  - Retroviral constructs encoding either CCR5 or CXCR4 fused at the N-termini to C34 peptide from GP41
  - Cell lines transduced with these vectors were resistant to HIV whether they were infected with an X4 or R5 virus and independent of which co-receptor had the C34 peptide (worked in trans). No effect if the C34 peptide was tagged to CD4. Positioning of the C34 peptide on the co-receptor was key and it did not matter which co-receptor: C34-CCR5 blocked R5 and X4 viruses and vice versa.
  - Same result when primary CD4 cells were used instead of cell lines
  - Then used ZnF endonuclease to introduce the C34 gene into the genes for either CCR5 or CXCR4 in primary cells and these too were resistant to HIV

CROI, 2013: Abst #: 129 Leslie, Hoxie, et al

HIV Cure Strategies: Combination Treatments



Curing HIV: Conclusions

- HIV cure is possible! Either by eliminating the reservoir (Berlin patient) or treating with ART before one is developed (Mississippi baby).
- The HIV reservoir is composed long-lived cells ($T_{CM} > T_{TM}$, T_{GD}, HSC, others) containing integrated HIV DNA proviral copies, only a fraction of which can be induced to produce infectious virions.
- Treatments that activate the HIV reservoir exist: the challenge will be to develop these therapies so that they are more effective, non-toxic and not only activate latently infected cells but kill them.
- Strategies to induce a functional cure using vaccines or gene therapy to introduce HIV-resistant cells are promising and under development.
- Combinations of reservoir depleting treatments with or without vaccines and gene modified HIV resistant cells plus ART hold promise for a sterilizing HIV cure that could be scalable.