Curing HIV

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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

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
Curing HIV: What is the HIV Reservoir?

It’s 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

Curing HIV: What is the HIV Reservoir?

HIV Provirus Status in the Reservoir

- Large Deletions: 50%
- Hypermutated virus: 28%
- Other mutations: 10%
- Inducible: 12%

Inducible, replication competent HIV
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.

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-retroviral therapy (e.g. elite controllers).
Sterilizing cure is possible!

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

Mother: HIV+, CD4 644, HIVRNA 2423
Not on therapy

35 week gestation
Vaginal delivery. No ARVs during delivery

31 hrs later at U of M
HIV DNA + times 2
HIV RNA 19,812
Started on AZT/3TC/NVP

At 7 days ARVs changed to
AZT/3TC/Kaletra

Day 20, HIVRNA < 48

18 months
Off ARVs
LTFU

23 months
HIV RNA neg
HIV ELISA neg
SC RNA = 1
HIV DNA = 37
RC virus cult neg

26 months
HIV RNA neg
HIV ELISA neg
HIV DNA = 4
2LTR neg

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


- 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
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)

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

Creating, Expanding and Infusing HIV resistant (CCR5-) T-cells

- Leukopheresis
- Select and expand CD4+/CCR5+ (★) T-cells
- Introduce CCR5 ZnF
- Expand and infuse

HSC Tx with CCR5- cells

- Leukopheresis
- Select and expand HSC CD34+/CCR5+ (★)

Conditioning Regimen (chemo)

Allogeneic

Autologous
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
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).
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.
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
HIV Cure Strategies: Combination Treatments

HIV Reservoir $\sim 1 \times 10^6$ cells

Depleting treatment/ add HIV Resistant cells

Fully suppressive ART

Cure?

Much less than 60 years
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.