HIV Eradication and the Quest for Functional Cure

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Disclosures

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  – Koronis
  – InnoVirVax
  – Merck
  – Tobira
  – ViiV
Success of current ART

- Substantial reduction in AIDS-related mortality


Adult life expectancy in rural South Africa

Bar et al Science 2013
Why search for a cure?

- **Need for life-long ART**
  - Side-effects and long-term toxicities
  - Burden of life-long adherence
  - Cost
  - Sustainability

- **Adverse effects of HIV-1 persistence**
  - Inappropriate immune activation
  - Cardiovascular, CNS, other end-organ damage

- **Potential risk for transmission**

- **Ongoing stigma of HIV infection**

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Persistent low-level viremia in patients on suppressive ART

Palmer et al PNAS 2008
Persistence of latently infected CD4+ cells

Siliciano et al Nat Med 2003

Persistent immune activation

Hunt et al J Infect Dis 2003
Immune activation is associated with increased risk of death and CVD

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>All-Cause Mortality (N=85)</th>
<th>Fatal or Non-fatal CVD (N=136)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>P-value</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>3.5</td>
<td>0.004</td>
</tr>
<tr>
<td>IL-6</td>
<td>12.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Amyloid A</td>
<td>2.3</td>
<td>0.08</td>
</tr>
<tr>
<td>Amyloid P</td>
<td>1.1</td>
<td>0.90</td>
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<tr>
<td>D-dimer</td>
<td>13.3</td>
<td>&lt;0.0001</td>
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<tr>
<td>F1.2</td>
<td>1.4</td>
<td>0.45</td>
</tr>
</tbody>
</table>

Why look for novel approaches?

- A survival deficit persists despite ART
Opportunity

- Better understanding of HIV persistence
- Identification of novel targets for drug development
- Novel approaches have led to testable hypotheses
- Potential utility of animal models

Apparent functional cure following stem cell transplant from a $ccr5^{Δ32/Δ32}$ donor

Questions

- What factors contributed to “functional cure” in this case?
  - Myeloablative chemotherapy
  - Replacement of CCR5+ with ccr5Δ32 HSC?
  - Graft versus host disease?
  - Immunosuppressive therapy?
  - Other?

- Can this outcome be replicated?

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Long-Term Reduction in Peripheral Blood HIV-1 Reservoirs Following Reduced-Intensity Allogeneic Stem Cell Transplantation in Two HIV-Infected Individuals

Study Aims

- Examine long-term changes in the peripheral HIV-1 reservoir following allogeneic HSCT in the setting of cART
- Explore HIV-1 coreceptor usage, PBMC coreceptor expression and HIV-specific antibody responses pre- and post-HSCT

Patients

- 2 HIV-1 infected patients on combination ART who underwent reduced-intensity conditioning (RIC) allogeneic HSCT
  - RIC = non-myeloablative chemotherapy, no total body irradiation or anti-thymocyte globulin
Methods

- Quantified proviral HIV-1 DNA from peripheral blood mononuclear cells (PBMCs) and purified CD4+ T cells by real-time PCR
- Quantified 2-LTR circles from PBMC episomal DNA
- Quantified plasma viremia by a single-copy assay
- Viral outgrowth assays using ~10^7 patient-derived CD4+ T cells and CD8 T cell-depleted lymphoblasts from an HIV-negative donor
- CCR5 genotyping/flow cytometric quantification of CCR5 expression on CD3+ T lymphocytes
- Genotypic and phenotypic determination of HIV-1 coreceptor usage
- Quantified HIV-1-specific Ab levels & avidity

Henrich et al J Infect Dis 2013

Patient A

- Male with perinatally acquired HIV-1 on long-term cART*
  - 2006: Stage IV Hodgkin disease → standard treatment
  - Disease recurrence → salvage therapy
  - 2007: Autologous HSCT
  - 2008: Relapse → RIC partially mismatched unrelated-donor HSCT

* cART: TDF/FTC/EFV 3-4 years pre-HSCT with undetectable VL

Henrich et al J Infect Dis 2013
**Patient B**

- Male with sexually acquired HIV-1 in mid-1980’s
  - 2003: Large B-cell lymphoma → chemotherapy, cART*
  - 2006: New stage IV Hodgkin lymphoma
  - Disease recurrence → salvage therapy
  - 2007: Autologous HSCT
  - 2010: MDS (Tx-related) → RIC matched related-donor HSCT

  *cART: TDF/FTC/RAL peri-transplant with undetectable VL

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**Patient A**

[Graph showing viral load and CD4 T-cell counts]

Viral outgrowth assay negative day +1266

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### Patient B

- Viral outgrowth assay negative day +652

### CCR5 status pre- and post-HSCT

- Both patients heterozygous for ccr5Δ32 mutation
- PBMC homozygous wild-type for CCR5 after engraftment
- Percentage of CCR5-expressing lymphocytes nearly doubled after full donor engraftment in Patient A (sufficient sample)
- Full-length HIV-1 env amplified from proviral DNA at pre- and 1st post-HSCT PBMC samples (later timepoints negative)
  - V3-loop genotyping predicted CCR5 usage pre- and post-HSCT
  - R5 phenotype confirmed by tropism assay of pseudotyped viruses expressing PBMC-derived env
### CCR5 genotype pre- and post-HSCT

<table>
<thead>
<tr>
<th>Patient A</th>
<th>Patient B</th>
<th>Controls</th>
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<tbody>
<tr>
<td>Before HSCT Receipt</td>
<td>Before HSCT Receipt</td>
<td>DNA Ladder</td>
</tr>
<tr>
<td>Day 0</td>
<td>Day 6</td>
<td>CCR5 32R</td>
</tr>
<tr>
<td>Day 0</td>
<td>Day 6</td>
<td>CCR5 32R</td>
</tr>
<tr>
<td>Day 0</td>
<td>Day 6</td>
<td>CCR5 32R</td>
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</table>

Henrich et al. J Infect Dis 2013

### Serum HIV antibody titers

<table>
<thead>
<tr>
<th>VITROS Enzyme Immunoassay</th>
<th>LS-VITROS Enzyme Immunoassay</th>
<th>Limiting Antigen-Avidity Assay</th>
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<tbody>
<tr>
<td><img src="image1" alt="VITROS Enzyme Immunoassay Graph" /></td>
<td><img src="image2" alt="LS-VITROS Enzyme Immunoassay Graph" /></td>
<td><img src="image3" alt="Limiting Antigen-Avidity Assay Graph" /></td>
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</table>

Henrich et al. J Infect Dis 2013
Summary and Conclusions

- Allogeneic HSCT with RIC in the setting of suppressive ART led to a substantial and sustained reduction in the HIV-1 reservoir in PBMC
  - Reduction in proviral HIV-1 DNA correlated temporally with full donor engraftment
- Engraftment of susceptible donor cells without infection adds supportive evidence that HIV-1 replication is fully suppressed by effective cART
- Declining HIV-specific Ab levels/avidity provide further evidence for minimal persistence of HIV-1 antigen
- Tissue sampling and analytic treatment interruption are necessary to fully assess the extent of HIV-1 reservoir reduction after allogeneic HSCT

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Mechanisms of HIV-1 latency and activation

Trono et al Science 2010
Epigenetic silencing of HIV transcription

HIV provirus
Integration into activated gene
Transcribed provirus
Recruitment of HDACs
Attenuated provirus
Recruitment of H3KMTs
Silenced provirus
DNA methylation
Latent provirus

Courtesy of D. Margolis

HIV lives within chromatin: restriction at initiation

"Closed" Nucleosome
Transcription Repressed

"Open" Nucleosome
Transcription Enabled

deacetylated
Acetylation

Courtesy of D. Margolis
Possible approaches to HIV-1 “cure”

- **Drugs to activate HIV-1 in latently infected cells**
  - SAHA, romidepsin, panobinostat, others (HDACi)
- **Drugs to transcriptionally silence HIV-1 in productively infected cells**
  - Ruxolitinib (JAK/STAT inhibitor)
- **Immune-based therapies to boost HIV-specific immunity or blunt immune activation**
  - Cytokines, mAbs, therapeutic vaccines
- **Genetically modified CD4+ T-cells or bone marrow-derived stem cells**
  - siRNA, Zn-finger nucleases, CCR5-deleted cells

Effect of vorinostat on HIV expression

*Archin et al. Nature 2012*
Change in cell-associated unspliced HIV RNA in response to vorinostat

Mean fold increase in US HIV RNA
Pre to on treatment = 2.65 (95% CI 1.76, 3.52, p=0.023)
Pre to off treatment = 3.00 (95% CI 2.16, 3.84, p=0.018)

GEE analysis grouping all “on drug” and “post drug” data points and adjusting for CV of the assay

Lewin et al 20th CROI, March, 2013

Therapeutic vaccination and HIV cure

Shan et al Immunity 2012
ACTG 5197: Study Design

- Ad5 HIV-1 gag therapeutic vaccine
- 110 participants
- Objectives:
  - Effects on viral rebound during the ATI
  - Immunogenicity

Study Week

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Vaccination

Virologic analysis

Vaccination

Analytical Treatment Interruption (ATI)

Schooley et al J Infect Dis 2010

ACTG A5197: Viral rebound kinetics

Plasma HIV-1 RNA, Copies on Log Scale

- Vaccine Arm
- Placebo Arm

Schooley et al J Infect Dis 2010
ACTG A5197: Results

- **Co-primary endpoints:**
  - Time-Averaged AUC
    - 0.24 log10 lower VL in vaccine arm ($P = 0.04$)
  - Mean ATI week 12 and 16 viral loads (Set Point)
    - 0.26 log10 lower VL in vaccine arm ($P = 0.07$)

- **Secondary endpoint:**
  - ATI week 16 viral load
    - 0.5 log10 lower VL in vaccine arm ($P = 0.03$)

- **IFN-γ production by CD4+ T-cells in response to Gag** inversely correlated with viral setpoint

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What are the goals?

- Radical (sterilizing) cure?
- Functional cure?
- Reduction (elimination) of residual viremia?
- Reduction (elimination) of the viral reservoir in specific compartments?

Schooley et al J Infect Dis 2010
What are the endpoints?

- Evidence of biological activity on proposed target or based on proposed mechanism?
- Quantitative effect on viral reservoir?
  - Residual plasma viremia?
  - Proviral DNA in PBMC?
  - Proviral DNA in tissue compartments (which one[s])?
  - Cell/tissue-associated viral RNA?
- Absence of viremia during analytic treatment interruption (ATI)?

Pros and cons of ATI?

PROS
- Rigorous test of functional cure
- Suitable for qualitative or quantitative analysis
  - Rebound versus no rebound
  - Time to 1st positive VL?
  - Time to set point?
  - Set point at new steady state?

CONS
- Risk of primary infection syndrome
- Risk of OI, CV events, death (SMART)
- Risk of transmission
What criteria are needed to justify ATI?

- Evidence of biological activity through proposed mechanism or on proposed target?
- Quantitative effect on some measure of viral reservoir (which)?
- Reduction in immune activation?
- No evidence needed?

Whom to study?

- Patients on suppressive ART
  - Initial versus subsequent regimens?
  - Highly treatment-experienced?
- Patients with well-preserved immune function (high CD4 counts) or advanced disease?
- “Elite” controllers?
- Patients with acute HIV infection?
- Patients requiring bone marrow transplantation?
What is the risk-benefit ratio?

- Need to balance risk of significant toxicity, morbidity and mortality of novel strategies against the possible benefit to individual patients and knowledge gained overall
- Challenging to define an acceptable level of risk in the context of generally safe and effective lifelong therapy

How are risks perceived?

- If functional cure could be achieved but required a period of treatment with somewhat toxic drugs, how much toxicity would be acceptable?
  - No more toxic than current ART regimens
  - Would accept a small risk of fatal complications (e.g., similar to pancreatitis with ddI or HSR with abacavir)
  - Would accept a modest risk of mortality similar to coronary artery bypass grafting (1-2%)
  - Would accept a significant risk of mortality similar to bone marrow transplantation (10-20%)
Who adjudicates those risks?

- Patients
- Clinicians
- Investigators
- FDA
- IRB
- Funding agencies
- Biosafety committees/RAC

Basic principles in human subjects research

- Autonomy
- Beneficence
- Equity
Informed consent

- The “cure” word
  - Coercive?
  - Misleading?
  - Appropriate?

Additional considerations

- Will absence of transmission need to be proven as well?
  - Sexual
  - Blood-borne
  - Mother-to-child

- How to assess long-term toxicities

- Risk of relapse
  - Distinguishing relapse from reinfection
Acknowledgments—HSCT study

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