Broadening the Applicability of Antigen-specific T-cell Immunotherapy for HIV-1 Infection

Michael Keller, MD
Program for Cellular Enhancement & Technologies for Immunotherapy

Director: Catherine M. Bollard, MD
Prevalence of HIV

1.2 million people living in the United States with HIV

Estimated 36.9 million people infected worldwide

Per 100,000

- 40.1 - 129.3
- 129.4 - 202.4
- 202.5 - 319.3
- 319.4 - 428.0
- 428.0 - 3365.2

Data source: National HIV Surveillance System. Rates are not adjusted for reporting delays. Inset map not to scale.

CDC 2015
Prevalence of HIV in DC

- Epidemic is considered >1% of population
- Incidence of HIV in DC = 2.5% of adult residents
  - Over 16,000 people infected with HIV
- Compared to African countries

Per 100,000

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<thead>
<tr>
<th>Range</th>
<th>Color</th>
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</thead>
<tbody>
<tr>
<td>40.1 - 129.3</td>
<td>Light blue</td>
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<tr>
<td>129.4 - 202.4</td>
<td>Blue</td>
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<tr>
<td>202.5 - 319.3</td>
<td>Red</td>
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<tr>
<td>319.4 - 428.0</td>
<td>Orange</td>
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<tr>
<td>428.0 - 3365.2</td>
<td>Dark red</td>
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World Bank

CDC 2015
Antiretroviral Therapy

• Successful in suppressing HIV and stopping disease progression
• Cost - $24K annually
• Not curative
• Social stigma
Viral control depends on Robust T-cell immunity

• T-cell deficiency impacts susceptibility to severe viral infections

• HSCT
• Primary Immunodeficiency
• Iatrogenic immune suppression due to autoimmunity, cancer.
Adoptive T-cell Immunotherapy restores antiviral immunity

- Transfer of virus-specific T-cells (VST) from a donor to recipient
  - Utilizes selection or ex vivo expansion
  - Highly successful in post-HSCT period
    - >400 patients treated internationally
    - Minimal risk of GVHD
A: Virus-exposed donors

- PBMC
- Hexon
- Penton
- EBNA-1
- LMP-2
- IE-1
- pp65
- G-Rex
- IL-4
- IL-7
- VST in 10-12 days

B: Cord blood / Virus-naïve donors

- CBMCs or Naïve T-cells
- Dendritic Cells
- IE-1
- pp65
- Hexon
- Penton
- EBNA-1
- LMP-2
- IL-4
- IL-7
- IL-12
- IL-15
- EBV-LCL
- G-Rex
- VST in >28 days
LMP/EBNA specific T cells Effectively Treats EBV-PTLD Post Allo BMT

Multivirus T cells (Targeting LMP2 and EBNA1)

Rituximab

<table>
<thead>
<tr>
<th>day</th>
<th>DNA Copies/mL Blood</th>
<th>Spots per 200,000 cells</th>
</tr>
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<tbody>
<tr>
<td>-16</td>
<td>0</td>
<td>0</td>
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<tr>
<td>-13</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>-10</td>
<td>100</td>
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<td>-3</td>
<td>150</td>
<td>150</td>
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<tr>
<td>0</td>
<td>200</td>
<td>200</td>
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<tr>
<td>4</td>
<td>250,000</td>
<td>250</td>
</tr>
<tr>
<td>7</td>
<td>200,000</td>
<td>200</td>
</tr>
<tr>
<td>14</td>
<td>150,000</td>
<td>150</td>
</tr>
<tr>
<td>21</td>
<td>100,000</td>
<td>100</td>
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</table>
Expansion of Sources and Targets has increased applicability of VSTs

• Expanded Donors
  • Cord Blood VST (CMV/EBV/Adv): ACTCAT1/2 trials
  • CMV-seronegative adults: MUSTAT trial (Children’s Natl)

• Expanded targets
  • ARMS protocol: CMV/EBV/Adv/HHV6/BK VST
  • Upcoming targets: Human parainfluenza-3, HPV

• Protective regardless of source of VSTs (naïve or memory-derived)

Leen et al, Nature Medicine 2006
Bollard et al, JCO 2014
Hanley et al, Science TM 2015
Developing T Cell Therapeutics for HIV: Previous trials
Antigen specific T cell therapy

- Safe in both HIV+ and other settings
- Less off-target effects
- Proliferate and migrate in vivo
- Potentially long-lasting immunity due to memory
## Antigen-specific T cell studies for HIV

<table>
<thead>
<tr>
<th>Study</th>
<th>Method</th>
<th>Dose</th>
<th>n</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lieberman et al., Blood 1997</td>
<td>HLA-A02 restricted clonal T-cells</td>
<td>1x10⁸ x 1 dose</td>
<td>6</td>
<td>• Transient increase in CD4 count and decrease in viral load</td>
</tr>
<tr>
<td>Tan et al., Blood 1999</td>
<td>Autologous expanded HLA-A02-restricted CD8+ clones (Gag, Pol-specific)</td>
<td>3x10⁹ x 1 dose</td>
<td>1</td>
<td>• No change in CD4 count or viral load</td>
</tr>
<tr>
<td>Brodie et al., Nat Med 1999</td>
<td>CD8+ Gag-specific T-cell clones</td>
<td>3 doses, up to 3x10⁹</td>
<td>3</td>
<td>• Decrease in productively infected CD4+ T-cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• No impact on viral load.</td>
</tr>
<tr>
<td>Chapuis et al., Blood 2011</td>
<td>HIV-specific CD8+ T-cells specific for HIV clade B peptides; central memory enriched</td>
<td>3.3x10⁹/m² x 1 dose</td>
<td>7</td>
<td>• Clones persisted for up to 84 days</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>• found to traffic to rectal mucosa in 4/7 patients for &gt;100 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• HIV replication not accessed</td>
</tr>
</tbody>
</table>
CARs for HIV

- Phase II trial - CD4ζeta CAR - extracellular domain, binds to HIV Env glycoprotein.
- T-cells expressing CD4ζ become activated upon binding HIV gp120 envelope protein on infected cells.
- 24 HIV+ individuals → single infusion +/- IL-2.
- CD4+ and CD8+T cells trafficked to rectal tissues → > 0.5 log decrease in rectal tissue-associated HIV but not plasma.
- T cells detected at 1 year.

Cell Genesys Inc in collaboration with Hoechst Marion Roussel.
UCLA, UCSF, Uni Colorado
CARs for HIV

- UCSF and U Penn
- Phase II randomized study CD4zeta-modified CD4+ and CD8+ T cells.
- 40 HIV+ subjects on HAART received T cell infusions (20 gene-modified, 20 unmodified).
- All subjects received $1 \times 10^{10}$ T cells x3
- Decrease in HIV burden of patients infused with unmodified T cells
- CD4zeta-modified CAR T cells in 98% of samples up to 11 years

## Summary - Previous T cell therapies for HIV

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Approach</th>
<th>Limitations</th>
</tr>
</thead>
</table>
| CD8 clones\(^1\)                | Select high reactivity clones and expand                                 | • Short persistence  
• No CD4 help  
• Limited specificity                                                                                                                            |
| High-affinity T cell receptors (SLY)\(^2\) | Find conserved epitopes and express artificial receptors in T cells       | • HLA-A*0201 restricted  
• Have to generate new receptor for every HLA type  
• Safety concerns of Artificial TCRs                                                                                                               |
| CD4 CAR T cells                  | Targets HIV infected CD4 T cells - contains the extra cellular domain of human CD4; binds to HIV env | Long term persistence in some  
Decrease in HIV burden  
None cured  
?toxicity concerns                                                                                                                                      |

\(^1\) Adapted from van der Burg et al. 2006  
\(^2\) Adapted from Hay et al. 2016

*Patel, Jones and Bollard, Cytotherapy 2016*
Developing T Cell Therapeutics for HIV Targeting Multiple Antigens
Objective

• Determine whether we can generate HIV-specific T cells (HXTCs), derived from both HIV\(^+\) and HIV-negative donors

- Multi-antigen HIV-specific T cells
  - Prevent immune escape
- Include both CD8\(^+\) and CD4\(^+\) T cell subsets
  - Improve persistence of infused T cells
HIV Antigens

Choosing HIV Antigens:
- More conserved than envelope proteins
- Target early and late infection stages
  - Nef (early) & Gag, Pol (late)
- Dominant Gag-specific T cell responses found in elite controllers

1Rolland et al, PLoS Path 2007
Generating HIV- T cells (HXTCs) from HIV+ Individuals

• 7 HIV+ subjects with either acute (2) or chronic infection (5)

• All on ART with viral suppression ≥3 years

Approach for HIV+ derived HXTCs

*Cells are grown in amprenavir→raltegravir and indinavir

The PepMixes consist of 150 15-mer peptides. Based on a proprietary algorithm held by JPT to provide the broadest coverage across all clades of HIV.
HXTCs Display Multi-HIV Antigen Specific Activity


Spot Forming Cells (SFC) Secreting IFNγamma
HXTCs Produce Polyfunctional Response to HIV Antigens

HXTC Suppress HIV in vitro

HIV Strain: JR-CSF

Phase I Study

- Collaboration with UNC (NCT02208167)
- Phase I single-site study of HXTC Therapy
  - Evaluate the safety, immunologic, and virologic response
  - HIV+ participants on ART with chronic or acute HIV
  - Patients receive 2 HXTC infusions (2x10^7 cells/m^2 each) given 2 weeks apart
Preliminary Clinical Data

• Autologous HXTCs generated from 3 HIV⁺ subjects
• 2 subjects received 2 doses of HXTCs
HXTCs Expand In Vivo

HXTC 02 Follow-Up Samples

IFNγ SFC/1x10^5 cells

- cells alone
- actin
- gag
- pol
- nef
HXTCs Suppress HIV Replication Following Infusion (n=3)

**HXTCs**
- Suppress HIV replication following infusion.

**Graphs**
- Jr-CSF infusion: p24 levels decrease over weeks post-infusion.
- Autologous Reservoir Virus infusion: p24 levels remain higher but show a trend towards decrease over weeks post-infusion.

**Legend**
- No CD8s
- 0, 1, 2, 3, 12 weeks post-infusion
- * indicates significant difference
No Viral Recovery possible Post-HXTC Infusion

Enrolled patients still on ART – therefore without doing a controlled treatment interruption unable to understand role of HXTCs as a cure strategy
Summary to date

- Autologous HXTCs may aid HIV control

- *Subjects currently remain on ART*
  - Unclear if autologous HXTCs have a role as a cure strategy

- Unclear if HXTCs can reach latent viral reservoir
Latency is controlled by epigenetic regulation

Deacetylase/Methyltransferase

Nuc

3-Me

HDAC

HMT

Acetyltransferase

HAT

Ac

Nuc

“open” chromatin structure
Transcriptional activation

“closed” chromatin structure
Transcriptional repression

—Ac

Coiras et al. Nat Rev Microbiology 20
HIV “purging” strategies


Latently infected CD4 T cell → Productively infected CD4 T cell

HDACi, IL-15

Efficacy in vivo still unknown

Viral cytopathic effect

Killing by stimulated CTL
Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy

N. M. Archin¹, A. L. Liberty¹, A. D. Kashuba¹, S. K. Choudhary¹, J. D. Kuruc¹, A. M. Crooks¹, D. C. Parker¹, E. M. Anderson², M. F. Kearney², M. C. Strain³, D. D. Richman³, M. G. Hudgens¹, R. J. Bosch⁴, J. M. Coffin², J. J. Eron¹, D. J. Hazuda⁵ & D. M. Margolis¹
HXTCs suppress viral recovery after SAHA-induced reactivation

Sung, Lam et al. JID 2015
Vorinostat impairs CD8 T cells only after 48 hrs of exposure

Sung, Lam et al.
JID 2015
Caveats and Future Directions

- Does vorinostat actually increase HXTC mediated viral suppression?
  - Measure increased antigen expression, if any

- Do HXTCs maintain anti-viral function in presence of other latency reversal drugs?
  - Jones et al. suggested impairment with other HDACi
Overall Summary

1. HIV+

2. Generate HXTCs
   - Multi-antigen specificity
   - Polyclonal

3. HDACi + infusion

Latent reservoirs
HIV-Specific T Cells: A Cure Strategy Post-Allogeneic HSCT
J1331 Clinical Trial

• Hypothesis: The combination of the allogeneic effect and continuous ART can reduce or completely eradicate HIV reservoirs

• Study Population:
  ▫ HIV+, BMT for cancer
JH HIV Allo-BMT: 7 allo-BMT patients

<table>
<thead>
<tr>
<th>N=7</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>6</th>
<th>7</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>34</td>
<td>53</td>
<td>38</td>
<td>50</td>
<td>51</td>
<td>46</td>
<td>50</td>
</tr>
<tr>
<td>Cancer</td>
<td>HL</td>
<td>DLBCL</td>
<td>AML</td>
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<td>AML</td>
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<td>Survival, oncology outcomes</td>
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<td>Died at week 64, GVHD</td>
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<td>Alive, 46 weeks</td>
<td>Alive, 36 weeks</td>
<td>Alive, 24 weeks</td>
</tr>
<tr>
<td>% donor</td>
<td>100%</td>
<td>80%</td>
<td>100%</td>
<td>77%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
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JH HIV Allo-BMT: 7 allo-BMT patients

- The HIV reservoir not detected post transplant in any patient with 100% donor chimerism
- When only donor cells were detected in blood, no HIV reservoir detected by Quantitative Viral Outgrowth Assay
HIV Meningoencephalitis following ART non-compliance at 5 months

- Week 20: fevers and a change in mental status
  - LP: 28 WBC, Protein 150, glucose 50
  - Consistent with Meningoencephalitis
  - HIV plasma viral load = 25,518 copies/ml
  - The CSF viral load = 17,000 copies/ml
- Recovered with parenteral HIV therapy
Boston Patients—BMT with Antiretroviral Therapy ➔ Interruption ➔ Aggressive Viral Rebound
Summary: Allo-BMT with antiretroviral therapy

- Clears measurable viral reservoir when full donor chimerism achieved
- Puts patients at risk for a syndrome of aggressive viral rebound when antiretroviral therapy is interrupted
The Berlin Patient

- SCT: A curative approach to HIV?
  - Received SCT from a homozygote CCR5-delta32 donor
  - ‘HIV cured’: Unable to detect HIV in blood or biopsies after discontinuation of ART
CCR5-delta32

- **Homozygotes: 1% caucasians**
  - No CCR5 expression
  - Largely resistant to infection
- **Heterozygotes: 10%**
  - Reduced CCR5 expression
  - Slowed viral progression (before antiretroviral therapy)
Not repeated because identifying perfect HLA matches and CCR5-delta32 homozygous donors is difficult
Autologous CCR5 Gene Editing

- NCT00842634, Tebas, et al. NEJM 2014
- ZFN-modified (CCR5 targeting) autologous CD4+ T cells.
- 12 participants on HAART open label, nonrandomized, uncontrolled study.
- Single dose of 10x10e8 ZFN-modified CD4+ T cells.
- ZFN-modified CD4+ T cells detected up to 42 months.
- HIV DNA decreased in most patients.
Replicating the “Berlin Patient”

- Genetic engineering—works well in vitro.. but no one has been successful in achieving immune reconstitution with most cells protected from HIV in humans
- Current phase I protocol for CCR5 gene editing of HSCs for allogeneic BMT (City of Hope)
  - Lentiviral siRNA approach

*Peterson....Kiern Blood. 2016 Mar 15. Blood*
Replicating the “Berlin Patient”

- Cord blood—several hundred cord blood units that are HIV resistant have been identified
- Several cord blood transplants done for HIV\textsuperscript{*} patients, no long term cure to date.
New HSCT Protocols Permit Greater HLA mismatch

• HaploBMT protocol with post-HSCT cytoxan
  • Increasing HLA mismatch does not worsen outcome

Kasamon et al BBMT 2010
Some Patients Don’t Have Matched Unrelated Donors or Related Haplo Donors

• At Hopkins, about 5% of patients fall into this category
• →trial with non-myeloablative prep and post-transplant cy that accepted unrelated donors mismatched at up to 5 of 10 loci.
JHH preliminary results (ASH 2015)

• No prohibitive toxicities or TRM despite up to 5 mismatched loci (n=16)
• No acute grade 3-4 GVHD

→ Unrelated partially mismatched allo-BMT donors should be considered acceptable
### JHH HIV+ allo-HSCT

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</tr>
<tr>
<td>Donor</td>
<td>MUD</td>
<td>MUD</td>
<td>Matched sib</td>
<td>Matched sib</td>
<td>Haplo</td>
<td>Haplo</td>
<td>Mismatched unrelated</td>
</tr>
</tbody>
</table>
DKMS

- ~2 million donors typed at CCR5
- ~20,000 CCR5delta32 resistant donors
Hypothesis

If we prioritize CCR5 delta32 HIV resistant donors over best HLA match, it should be possible to identify HIV resistant donors for a substantial fraction of patients.

This should protect patients from the aggressive HIV rebound syndrome and may cure some patients of HIV.
Adoptive T Cell Therapy for HIV After SCT

- Adoptive T cell therapy can be used to restore antiviral immunity post-transplant (SCT)
  - Donor-derived HIV-specific T cells could be used to target HIV reservoirs after SCT
Developing a dHXTTC Product for use after SCT

• Determine if HIV-specific T cells can be generated from HIV seronegative donors (dHXTTCs) and suppress HIV replication in vitro

• Ultimate goal: Infuse dHXTTCs from eligible HIV-seronegative CCR5Δ32 HSCT donors to effect a cure post-transplant
Generation of dHXTCs

**STEP 1**
Donor T Cells

**STEP 2**
Autologous DCs & PHA blasts used as APCS
+ cytokines that stimulate proliferation

**STEP 3**
HIV-specific T Cells

Choosing HIV Antigens:

- Less mutable/more conserved than envelope proteins\(^1\)
- Target early and late infection stages
  - Nef (early) & Gag (late)
- Dominant Gag-specific T cell responses found in elite controllers\(^2\)

\(^1\)Rolland et al, PLoS Path 2007
dHXTCs Produce Polyfunctional Response to HIV Stimulation

Seronegative donor 6

Seronegative donor 7

Patel et al, BBMT 2016
dHXTCs Suppress HIV in vitro

HIV Strain: SF162

Patel et al, BBMT 2016
HXTCs can be generated from Naïve Cord blood T-cells

S Patel, ASGCT 2016
Summary of Virus-naïve dHXTCs

- Seronegative-derived dHXTCs have the ability to:
  - Recognize multiple HIV antigens: Gag/Nef
  - Produce a polyfunctional immune response
    - IFN$_{\gamma}$, IL-2, TNF$_{\alpha}$, IL-6, IL-8, perforin
  - Recognize Class I and II restricted HIV peptides
  - Suppress HIV replication in vitro
Future Directions

• Utilize donor-derived HXTCs as a curative strategy post allo SCT

• On dHXTCs:
  • Knockdown CXCR4 and CCR5 HIV co-receptors using gene editing approaches: CRISPR/Cas9, ZFN, or TALEN

  ▫ CCR5-delta32 mutation donor bank for SCT and for dHXTC generation
Concluding Remarks

HIV specific T cells in the autologous setting may need to be combined with latency reversing agents.

HIV specific T cells in the post allo BMT setting may offer a curative strategy if rendered resistant to HIV infection.
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