Zinc Finger Nuclease Gene Editing for Functional Cure in a Nonhuman Primate Model of HIV/AIDS

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Study Objectives

- Establish a model of HIV infection on cART in pigtailed macaques
- Edit the CCR5 gene in macaque hematopoietic stem cells (HSC’s)
- Transplant autologous CCR5-deleted hematopoietic stem cells
  - SHIV-naïve animals
  - SHIV-infected, cART-treated animals
Challenge Virus and cART Regimen

Shiv-1157ipd3N4

- SIV-mac239 backbone
- HIV-HXBc2 accessory genes tat, rev, and vpu
- Env isolated from HIV-1 Clade C primary isolate
- 4 NFkB binding sites in 5' and 3' LTR
- Selected for high virulence
  - Passaged through five rhesus macaques to generate a monkey adapted virus
  - Isolated from monkeys with late-stage AIDS

<table>
<thead>
<tr>
<th>Compound</th>
<th>Target</th>
<th>Administration</th>
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<tbody>
<tr>
<td>PMPA</td>
<td>Reverse Transcriptase (NtRTI)</td>
<td>1X/day Subcutaneous</td>
</tr>
<tr>
<td>FTC</td>
<td>Reverse Transcriptase (NRTI)</td>
<td>1X/day Subcutaneous</td>
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<tr>
<td>Raltegravir</td>
<td>Integrase</td>
<td>2X/day Oral with Food</td>
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Significant SHIV-dependent depletion of gut-associated lymphoid tissue

Clinically relevant patterns of tissue viremia and cART pharmacokinetics

Stable virus suppression by cART in peripheral blood and tissues

Seeding of replication competent, cART-suppressed viral reservoirs
Autologous Transplant of CD34⁺ Hematopoietic Stem Cells in the Pigtailed Macaque

- Enrich CD34⁺ cells from primed bone marrow
- Culture ex vivo (TPO, SCF, FLT-3 Ligand)
- Infuse into conditioned animal

Animal receives myeloablative conditioning regimen consisting of 1,020 cGy total body irradiation
Autologous Stem Cell Transplant Alone is Not Curative in SHIV+, cART-Suppressed Animals

-10 0 10 20 30 40 50 60 70 80 90 100
Weeks Post Infection

$1.0 \times 10^8$

Begin anti-retrovirals

$1.0 \times 10^7$

$1.0 \times 10^6$

$1.0 \times 10^5$

$1.0 \times 10^4$

$1.0 \times 10^3$

$1.0 \times 10^2$

$1.0 \times 10^1$

$1.0 \times 10^0$

Plasma Viral Load (Viral RNA Copies/mL)

-10

0

10

20

30

40

Weeks Post Infection

Control Transplanted

Untransplanted
Autologous Transplant of CD34+ Hematopoietic Stem Cells in the Pigtailed Macaque

- Enrich CD34+ cells from primed bone marrow
- Culture ex vivo (TPO, SCF, FLT-3 Ligand)
- Infuse into conditioned animal
- Animal receives myeloablative conditioning regimen consisting of 1,020 cGy total body irradiation
ZFN Editing of CCR5 in Pigtailed Macaque CD34+ Cells for Autologous Transplant

Peterson et al, Submitted

Enrich CD34+ cells from primed bone marrow

Culture 24hr, 37° ex vivo (TPO, SCF, FLT-3 Ligand)

Electroporate cells with ZFN mRNA, recover overnight at 30°C

Infuse into conditioned animal

Animal receives myeloablative conditioning regimen consisting of 1,020 cGy total body irradiation
Benchmarks for Efficacious ΔCCR5 Gene Therapy

- Normal hematopoietic recovery following ΔCCR5 transplant
- Multilineage engraftment of gene-edited cells following CD34⁺ infusion *in vivo*
- Gene editing in *ex vivo* cultured macaque CD34⁺ cells
- Generation of cells with biallelic editing of CCR5
- Long term engraftment of edited cells, and positive selection following SHIV challenge
Benchmarks for Efficacious ΔCCR5 Gene Therapy

- Normal hematopoietic recovery following ΔCCR5 transplant: YES
- Multilineage engraftment of gene-edited cells following CD34+ infusion in vivo: YES
- Gene editing in ex vivo cultured macaque CD34+ cells
- Generation of cells with biallelic editing of CCR5
- Long term engraftment of edited cells, and positive selection following SHIV challenge
Macaque CCR5 ZFNs Generate Up to 60% Bulk Gene Editing and 10% Biallelic Editing ex vivo

A. Percent Disruption at CCR5 (MiSeq)

B. Percent Assayed Colonies

Peterson et al.
CCR5-Edited Stem Cells Engraft in Peripheral Blood, and Persist Following SHIV Challenge

Peterson et al, Submitted
Modeling the Berlin Patient in *M. Nemestrina*

- **Baseline Sample Collection**
- **ΔCCR5 Transplant**
- **SHIV Infection**
- **Withdraw Antiretroviral Therapy**

SHIV Status?
Macaque CCR5 ZFN Transplants are Feasible in a Preclinical Model of Suppressed HIV Infection

A. Percent Disruption at CCR5 (MiSeq)

- Bulk Disruption
- Uninfected
- Infected, cART-suppressed

B. Percent Assayed Colonies

- Mono-/Biallelic
- Uninfected
- Infected, cART-suppressed

SHIV Status at Transplant
Conclusions and Future Directions

- Efficient Targeting of CCR5 with ZFNs in Macaque HSCs; up to 10% biallelic editing
  - Maximize ZFN delivery to true HSCs
  - Insert a selectable marking following gene disruption to select against CCR5\(^{wt/wt}\) cells
- CCR5 Gene Editing is Equally Feasible in SHIV\(^+\), cART-suppressed animals
  - Define the threshold effect of ΔCCR5 therapy
  - Combinatorial therapies
Thank You...

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ΔCCR5 Transplant Animals Display Normal Kinetics of Hematopoietic Recovery

A11217  A11210
Z12161  Z12220  Healthy Minimum Value

Peterson et al, Submitted
Edited CCR5 Loci Are Found in All Hematopoietic Lineages

Peterson et al, Submitted

Lymphoid/Myeloid Sorts

% Disruption at CCR5 (MiSeq)

- Total PBMC
- CD4+
- CD8+
- CD20+
- CD14+

A11217
A11210
Z12161
Z12220