Vector Immunity as Barrier to Stem Cell Engraftment & Survival

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CCR5Δ32 donor

Patients’ own cells are treated with ZFNs

HIV “selection” for CCR5(-) cells?

Berlin patient

AIDS lymphoma patient

Courtesy of P. Cannon
Getting ZFNs to work in HSC
(goal: transient expression of the ZFN in the HSC to cause permanent gene disruption)

Nucleofection - DNA
Nucleofection - mRNA
Adenovirus
Adeno-associated virus
Non-integrating lentivirus

HSC source: CD34+ cells from cord blood

% CCR5 disruption

0 5 10 15 20 25

Courtesy of P. Cannon/M. Holmes
AIDS Lymphoma Clinical Platform

HSC mobilization
- G-CSF (10 μg/kg)
  - 1 2 3 4 5 6 7 8......

Aphereses and HSC purification
- #1 #2 #3 #4

Transient ZFN Rx

Untreated ‘back-up’ cells

Conditioning regimen, chemotherapy
- BCNU BCNU BCNU VP16

Days pre-transplant
-7 -6 -5 -4 -3 -2 0 12+
Gene Rx

Multipotent Progenitor Cell -> CFU-Blast

CFU-Blasst

NK-Pre

Pre-B

Pre-T

B cell

T cell

PMNL

NK cell

B cell

Plasma Cell

Retic

RBC

CFU-GM

CFU-G

CFU-M

BFU-Meg

BFU-E

CFU-GEMM

Gene Rx
NSG: NOD/SCID/IL-2Ry$^{\text{null}}$

Successful engraftment of human cells?

Adapted from Holt et al Nat Biotech 2010; 28: 839-47
ZFN-engineered HSC support secondary transplantations

Courtesy of P. Cannon/M
HIV Challenge Model

Mock or ZFN CD34+ cells

2 mths

HIV-1\textsubscript{BaL}

3+ mths

Adapted from Holt et al Nat Biotech 2010; 28: 839-47
ZFN-treated mice control HIV-1

HIV-1 in blood

HIV-1 in gut mucosa

Adapted from Holt et al Nat Biotech 2010; 28: 839-47
HIV infection appears to select for CCR5(-) cells

Adapted from Holt et al Nat Biotech 2010; 28: 839-47
Vector Issues

• Immediate immune reaction: e.g. TLR; danger signal-responses; AdV direct injection
• Delayed response to vector or to neoantigen: e.g. neo selection; Factor XI/AAV
• In vitro transduction might mitigate effect of immune recognition of residual vector; e.g. lentivirus transduction of HSCs leads to long term engraftment and expression
• Non-integrating modification might mitigate immune recognition of continual expressed transgene; e.g. AdV-ZFN
Experiment

• Does anti-AdV alter engraftment in the NSR model?
• Passive transfer of human Ig = neutralizing titer
• Comparison of engraftment potential as measured by % CD45+ cells in marrow/spleen
Ad Neutralization pilot study

From M. Holmes
Anti-AdV Effect on Engraftment

**NSG neonate**

- Injected with **CD34+ cells** after 150 cGy

**2 mths**

**Successful engraftment of human cells?**

Gated on human CD45

- **Bone marrow**
- **Thymus**
- **Spleen**
- **GALT**
AIDS Lymphoma Patient Screening

[Graph showing a scatter plot with various data points and lines, labeled with different categories such as 'pooled human serum (+ control)', 'FBS (- control)', '201', '203', '204', '208', '209', '301', '302', '303', '304']


<table>
<thead>
<tr>
<th>Sample</th>
<th>Normalized EC 50</th>
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<tbody>
<tr>
<td>Patient 1</td>
<td>0.4</td>
</tr>
<tr>
<td>Patient 2</td>
<td>1.4</td>
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<tr>
<td>Patient 3</td>
<td>1.7</td>
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<td>Patient 4</td>
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<td>Patient 5</td>
<td>0.8</td>
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<td>Patient 6</td>
<td>0.0</td>
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<tr>
<td>Patient 7</td>
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<tr>
<td>Patient 8</td>
<td>0.0</td>
</tr>
<tr>
<td>Patient 9</td>
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<td>Patient 11</td>
<td>32.8</td>
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<td>37.8</td>
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<td>8.4</td>
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<td>Patient 16</td>
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<td>Patient 19</td>
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<tr>
<td>50 ul IgG</td>
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<tr>
<td>100 ul IgG</td>
<td>15.7</td>
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<tr>
<td>Avg. Positive Control</td>
<td>94.7</td>
</tr>
</tbody>
</table>

**Table 2.** The EC50 values for the 19 plasma samples taken from AIDS Lymphoma patients just prior to HSPC infusion are listed. For comparison, the serum samples from mice receiving either 50 or 100 ul of purified human IgG are also listed. The average positive control value is derived from taking the average EC50 for pooled human serum from 3 independent studies.
Summary

• The NSG model is immunologically incomplete and any specific predictive value for vector immunity and engraftment is unknown

• Engraftment into an immunodeficient target population would mitigate any host anti-vector effect

• For blood stem cell transplantation, the option for backup, using untransduced stem cell, exists as a fall-back strategy