Education Session 123 - Hematopoietic Stem Cell Gene Therapy in Large Animal Models

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Presenter Disclosure:
Hans-Peter Kiem, MD

Nothing to disclose
Overview

• Introduction - Hematopoietic stem cell gene transfer

• Large animal studies
  – Gene marking
  – Integration site analyses
  – Stem cell expansion and in vivo selection
  – Disease models
Hematopoiesis

Hematopoietic Stem Cell (HSC)

Lymphoid ‘Stem Cell’

Myeloid ‘Stem Cell’

Pre-T

Pre-B

BFU-Meg

CFU-GM

BFU-E

CFU-M

CFU-G

CFU-E

RBCs

T cell

B cell

Megakaryocyte

Platelets

Mast Cell

Macrophage

Neutrophil

Reti

Lymphoid cells:

Pre-T

Pre-B

B cell

T cell

Myeloid cells:

BFU-Meg

CFU-GM

CFU-E

CFU-M

CFU-G

RBCs

Monocyte

Neutrophil

Macrophage
Retrovirus Vectors

Gammaretrovirus

Lentivirus

Foamy virus

MLV, GALV

HIV, FIV, Visna

HFV, SFV
Diseases Targeted by Stem Cell Gene Therapy

- Immunodeficiencies
  - SCID, ADA

- Hemoglobinopathies
  - Sickle Cell Disease, Thalassemia

- Other Blood diseases
  - Leukocyte adherence deficiency, Chronic Granulomatous Disease, Wiskott Aldrich Syndrome, Fanconi Anemia

- HIV

- Chemoresistance Gene Therapy
  - Introduction of resistance genes, mostly MGMT
What Is the Appropriate Assay?  
Advantages of Large Animal Studies

• Compared to humans:
  – similar stem cell kinetics, cytokine responsiveness, retroviral receptors
  – same limitations - limited number of cells available for transduction and transplantation
  – CD34 selections
  – Similar conditioning regimens with similar TBI or chemotherapy doses
  – Generally good clinical translation
Competitive Repopulation Assay

Stem cell harvest and CD34 enrichment

Myeloablative irradiation

Infusion of pooled cells

Transduction

Vector A
- GFP
- Fibronectin Fragment
- Growth factors
- Envelopes
- Packaging cells
- Vector systems
- Stem cell sources

Vector B
- YFP

Conditions tested:

split cells in two aliquots
GFP/YFP Analysis In Vivo

- gran
- mono
- lymph
Efficient Gammaretroviral Transduction of G-CSF mobilized PBSC using Phoenix-GALV Pseudotyped Vectors

% GFP/YFP+ cells

Days after transplantation

Beard et al. Mol. Ther 2006
Efficient Lentiviral Transduction of Dog Long-Term Repopulating Cells

% GFP+ cells

Days after transplantation

Horn et al. Blood 2004
High Level Lentiviral Marking in the Pigtailed Macaques

J02370

Percent GFP Positive

Granulocytes

Lymphocytes

Days after transplantation

MOI 10

MOI 10

Trobridge et al. Blood 2008
Efficient Gene Transfer Using a Foamy Viral Vector in Dogs
Granulocytes and Lymphocytes

Days after transplantation

% GFP+ cells

Lymphocytes
Granulocytes

Days after transplantation

Kiern et al. Blood 2006
FV Do Not Integrate Preferentially Near Proto-Oncogenes in Dogs

Beard et al., Hum Gene Ther 18: 423-434, 2007
Stem Cell Selection / Expansion

• Therapeutic transgene confers survival advantage (e.g. clinical trials in SCID, Fanconi?)

• Induction of proliferation
  - Modified cytokine receptors (i.e. mpl) (see poster session 251 on Thursday, Emery, Blau)
  - Self renewal genes (HOXB4, NOTCH, WNT)

Cytotoxic drug/ drug resistance gene

See Korashon Watts on Friday 2:45 PM Session 325
MGMT-Mediated /HSC Selection and Chemoprotection

Also see Jen Adair’s Presentation on Friday at 1:15 pm
Experimental Design

MSCV → MGMT P140K → IRES GFP → LTR

Transduce CD34^+ cells
Infusion 920 cGy
MGMT-mediated Chemoprotection

O$_6$-BG/BCNU
0.4 mg/kg

Days after transplantation Neff et al. JCI 2003
P140K-Mediated *In vivo* Selection

Donor Chimerism

Days After Transplantation

% GFP+ / Donor Chimerism

Chemotherapy

Granulocytes

Lymphocytes
In Vivo Selection and Chemoprotection With Temozolomide after Autologous Transplantation

Granulocytes and Lymphocytes

Cells/μl x 10^3%GFP+ cells

Days after transplantation

Neff et al. Blood 2005
Naturally Occurring Pyruvate Kinase (PK) Deficiency in the Dog

- Severe hemolytic anemia
  - Hematocrit range from 18-27% (37-55%)
- Lack expression of R-type PK isoenzyme in maturing erythrocytes
- ‘Fragile’ red blood cells
- Progressive myelofibrosis and liver fibrosis

See Brian Beard’s Presentation Friday 1:45 PM #344

Grant Trobridge (now WSU)
Naturally Occurring Pyruvate Kinase (PK) Deficiency in the Dog

- Cured with allogeneic transplantation
- Explore Autologous gene-modified cells
- Challenging model will likely require high % of corrected cells
- Previously established efficient in vivo selection in dog using MGMTP140K

Goals for Gene-Modified Autologous Transplantation

- Develop a foamy virus (FV) vector-mediated HSC gene therapy approach for canine PK deficiency
- Use MGMTP140K to increase marking levels to therapeutic levels
In vivo Selection Using P140K
Therapeutic Effect from 2\textsuperscript{nd} Gene

- Patient population where ablative conditioning is not feasible and/or high levels of gene marking are required
  - Requires an effective post-transplant \textit{in vivo} selection approach
**Experimental Design**

- **Autologous leukapheresis**
- **Chemotherapy**
- **Infusion**
- **CD34 enrichment**
- **Foamy retrovirus transduction**

- **Conditioning (TBI) & CSP**
- **FV-cPK-R, P140K, GFP**
Elevated Hematocrit in PK-Affected Dog following Autologous Gene-Modified Transplantation

- Post-transplant *in vivo* selection of gene-modified cells has stabilized hematocrit

![Graph showing hematocrit and GFP+ granulocytes over time after transplantation.](Image)

- Hematocrit & Reticulocyte
  - Days After Transplantation
  - % GFP by FACS
  - Hematocrit
  - Reticulocyte
  - GFP+ Granulocytes
  - O6BG/BCNU
  - Transfusions
Reduced NRBCs in PK-Affected Dog following Autologous Gene-Modified Transplantation

- Post-transplant *in vivo* selection of gene-modified cells has reduced nucleated red blood cells

Days After Transplantation

NRBCs

0 100 200 300 400 500 600

% GFP by FACS

0 10 20 30 40

Transfusions

O6BG/BCNU

GFP+ Granulocytes

NRBCs PK-Deficient

NRBCs

0 100 200 300 400

Reduced NRBCs in PK-Affected Dog following Autologous Gene-Modified Transplantation

Reduced LDH Levels in PK-Affected Dog following Autologous Gene-Modified Transplantation

LDH [U/L]

- Normal Dogs: G368, G953, G550
- PK-Deficient Dogs: H145, H406, H408

*PK-Deficient Dog After Gene-Modified Transplant*
Characteristics of Canine XSCID

• Failure to thrive (growth retardation).
• Fatal between 8 to 16 weeks of age.
• Low to absent peripheral T cells.
• Normal to elevated peripheral B cells.
• Serum IgM levels may be normal but low to absent serum IgG and IgA.
• Lack of a specific IgG antibody response.
• Inability of T cells to proliferate.
• Dysplastic thymus.
• Absence of lymphoid tissue.

Peter Felsburg
Canine XSCID

Basset (1985) - Gus

Corgi (1995) - Brusier
Dog $\gamma_c$ Mutations

Basset – 4 bp deletion at positions 30-33.
Corgi – single base substitution at position 583.
Both result in premature stop codon.
Number of Gene-Corrected T Cells

- Normal Range

Months Post Treatment

Cells/µL

- 1547
- 1655
- 1830
- 1836
Proportion of Gene-Corrected B Cells and Granulocytes

Normal IgG-specific antibody response
AIDS Gene Therapy Approach:

Transplant HIV-resistant HSCs to protect the hematopoietic system from HIV-1 infection to prevent or cure AIDS

Also see Patrick Younan’s Poster #485 on Friday!
### Global Summary of the AIDS Epidemic 2009

#### Number of people living with HIV

<table>
<thead>
<tr>
<th>Category</th>
<th>Total</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>30.8 million</td>
<td>[29.2 million–32.6 million]</td>
</tr>
<tr>
<td>Women</td>
<td>15.9 million</td>
<td>[14.8 million–17.2 million]</td>
</tr>
<tr>
<td>Children (&lt;15 years)</td>
<td>2.5 million</td>
<td>[1.6 million–3.4 million]</td>
</tr>
</tbody>
</table>

#### People newly infected with HIV in 2009

<table>
<thead>
<tr>
<th>Category</th>
<th>Total</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>2.2 million</td>
<td>[2.0 million–2.4 million]</td>
</tr>
<tr>
<td>Children (&lt;15 years)</td>
<td>370 000 [230 000–510 000]</td>
<td></td>
</tr>
</tbody>
</table>

#### AIDS deaths in 2009

<table>
<thead>
<tr>
<th>Category</th>
<th>Total</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>1.6 million</td>
<td>[1.4 million–1.8 million]</td>
</tr>
<tr>
<td>Children (&lt;15 years)</td>
<td>260 000 [150 000–360 000]</td>
<td></td>
</tr>
</tbody>
</table>
### Regional HIV and AIDS statistics and features | 2009

<table>
<thead>
<tr>
<th>Region</th>
<th>Adults and children living with HIV</th>
<th>Adults and children newly infected with HIV</th>
<th>Adult prevalence (15–49) [%]</th>
<th>Adult &amp; child deaths due to AIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-Saharan Africa</td>
<td>22.5 million [20.9 million – 24.2 million]</td>
<td>1.8 million [1.6 million – 2.0 million]</td>
<td>5.0% [4.7% – 5.2%]</td>
<td>1.3 million [1.1 million – 1.5 million]</td>
</tr>
<tr>
<td>Middle East and North Africa</td>
<td>770 000 [560 000 – 1.0 million]</td>
<td>75 000 [61 000 – 92 000]</td>
<td>0.2% [0.2% – 0.3%]</td>
<td>24 000 [20 000 – 27 000]</td>
</tr>
<tr>
<td>South and South-East Asia</td>
<td>4.1 million [3.7 million – 4.6 million]</td>
<td>270 000 [240 000 – 320 000]</td>
<td>0.3% [0.3% – 0.3%]</td>
<td>260 000 [230 000 – 300 000]</td>
</tr>
<tr>
<td>East Asia</td>
<td>240 000 [220 000 – 270 000]</td>
<td>17 000 [13 000 – 21 000]</td>
<td>1.0% [0.9% – 1.1%]</td>
<td>12 000 [8500 – 15 000]</td>
</tr>
<tr>
<td>Central and South America</td>
<td>1.4 million [1.2 million – 1.6 million]</td>
<td>92 000 [70 000 – 120 000]</td>
<td>0.5% [0.4% – 0.6%]</td>
<td>58 000 [43 000 – 70 000]</td>
</tr>
<tr>
<td>Caribbean</td>
<td>820 000 [720 000 – 910 000]</td>
<td>31 000 [23 000 – 40 000]</td>
<td>0.2% [0.2% – 0.2%]</td>
<td>8500 [6800 – 19 000]</td>
</tr>
<tr>
<td>Eastern Europe and Central Asia</td>
<td>1.4 million [1.3 million – 1.6 million]</td>
<td>130 000 [110 000 – 160 000]</td>
<td>0.8% [0.7% – 0.9%]</td>
<td>76 000 [60 000 – 95 000]</td>
</tr>
<tr>
<td>Western and Central Europe</td>
<td>1.5 million [1.2 million – 2.0 million]</td>
<td>70 000 [44 000 – 130 000]</td>
<td>0.5% [0.4% – 0.7%]</td>
<td>26 000 [22 000 – 44 000]</td>
</tr>
<tr>
<td>North America</td>
<td>57 000 [50 000 – 64 000]</td>
<td>4500 [3400 – 6000]</td>
<td>0.3% [0.2% – 0.3%]</td>
<td>1400 [&lt;1000 – 2400]</td>
</tr>
<tr>
<td>Oceania</td>
<td>33.3 million [31.4 million – 35.3 million]</td>
<td>2.6 million [2.3 million – 2.8 million]</td>
<td>0.8% [0.7% - 0.8%]</td>
<td>1.8 million [1.6 million – 2.1 million]</td>
</tr>
</tbody>
</table>

The ranges around the estimates in this table define the boundaries within which the actual numbers lie, based on the best available information.
The need for alternative strategies for treating HIV+/AIDS-patient

Limitations of ART

• Toxicity issues.
• Immune reconstitution is not achieved.
• Well established that ART will not lead to the eradication of viral reservoirs.
• Transmission while on ART is still possible.
• Life-long, daily requirement to take medication, costs, side effects and risk of developing viral resistance if medication is not taken.
• There is no guarantee that we can stay ‘ahead’ of viral evolution (HIV has shown that it can evolve drug resistance even with continued antiretroviral treatment).

Ideal treatment

• Eliminate viral reservoir.
• Prevent/reduce viral replication in the absence of ART.
• Reduce/eliminate chronic inflammation.
• Prevent infections from spreading to new target cells.

Reconstitute a fully functional Immune System
HAART-free 3.5 years after receiving transplant.

Hutter et al. NEJM 2009
Target: host CCR5 mRNA

m7G  ___________ pA

12  479  741  1024

identical between macaque/human

shRNA Targets for Inhibition of HIV-1 Replication

Percent knockdown

- Rhesus CCR5
- Human CCR5

Control  741  1024
Targets for Inhibition of HIV-1

C46 HIV Fusion Inhibitor

- C46 peptide derived from C-terminal of gp41
- inhibits R5 and X4 strains
- inhibits HIV-1 and SHIV
Site-specific Gene Correction

DNA repair mechanisms

Zinc-finger nucleases

<table>
<thead>
<tr>
<th>DNA double strand break (DSB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAGLIDADG homing endonucleases</td>
</tr>
</tbody>
</table>

Non-homologous End Joining (NHEJ)

Homologous Recombination (HR)

Presentation by Nina Munoz on Saturday Session 414 on Gene Targeting
## Nonhuman Primate Mode

### HIV/AIDS

<table>
<thead>
<tr>
<th></th>
<th>HIV-1 chimp.</th>
<th>SIVmac/HIV-2 macaques</th>
<th>SHIV macaques</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 env/tat/rev/vpu</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>CD4 T cell loss</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Time to CD4 &lt;500/μl</td>
<td>NA</td>
<td>months/years weeks/months</td>
<td></td>
</tr>
<tr>
<td>Immunodeficiency</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Encephalitis</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

AIDS Gene Therapy in the Macaque

mobilization

transduce with anti-HIV vector

CD34+ cells

conditioning

infuse transduced cells

i) in vivo selection (MGMT)

ii) ex-vivo challenge of PBMCs

iii) challenge with SHIV
MGMT-Mediated \textit{In vivo} Selection of Granulocytes and Lymphocytes

- Myeloablative (HIV-derived lentivirus)

Beard et al. JCI 2010
Ex vivo Protection of Macaque Hematopoietic Repopulating Cell-Derived Lymphocytes

Trobridge et al. PlosOne 2009
Conclusions – Large Animal Studies

• Similar conditions to human studies
  – Growth factors
  – Vectors
  – CD34 cells or other subpopulations
  – Scale up
  – Conditioning regimens and engraftment
  – Long-term follow up – vector integration and expression
  – Specific disease corrections eg PK and SCID-X1
  – HIV/AIDS studies
  – Generally good translation to human studies
Acknowledgments

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- Primate Center

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- John Rossi
- John Zaia
- Philip Gregory
- Mike Holmes
- Peter Felsburg

Former Members

- Grant Trobridge

NIH – NCI, NHLBI, NIDDK and NIAID
Homologous Recombination

A. Site specific nuclease (green) capable of inducing double strand break and a DNA template with the desired sequence (purple/red section of short duplex

B. The nuclease binds to and cuts the genomic DNA at specific site.

C. Cellular homologous repair mechanisms are activated.

D. A scarless modification of the targeted region results