6 June 2008, Melbourne, Australia: Dr John A Zaia presented, over the weekend, an update on the human pilot HIV lymphoma stem cell study at the 11th Annual Meeting of the American Society of Gene Therapy held May 28-June 1, 2008 in Boston, Massachusetts.

His talk was entitled “Gene Therapy Approaches for Treatment of HIV/AIDS: Current Status”.

Dr Zaia is the Chair, Division of Virology, Beckman Research Institute and is a key collaborator for the pilot human HIV study being undertaken at the City of Hope in Duarte, California. This study includes the use of Benitec’s technology as a clinical method to fight HIV-AIDS infection. In this ground-breaking pilot-clinical study patients with AIDS-related lymphoma are being treated using vector expressed RNAi aimed at rendering the cells resistant to the HIV-1 virus infection.

This study entitled “A pilot study of safety and feasibility of stem cell therapy for AIDS Lymphoma using stem cells treated with a Lentivirus vector encoding multiple anti-HIV RNA’s” commenced in Q3 2007.

In his update Dr Zaia presented 60 day data available on the first two patients in the current study. This is still early data however patients are doing well after transplant and pleasingly the gene markers are detectable in these patients. Safe engraftment was seen at 10 days.

This is early data. However if it can be confirmed, then treatment with gene therapy early after HIV infection may be justified, and, if such treatment delayed the need for antiviral chemotherapy, gene transfer would likely become an important strategy for treatment of HIV infection.

A copy of Dr Zaia’s presentation is attached.

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About Benitec

Benitec is an Australian biotechnology company focused on licensing its extensive intellectual property portfolio and developing therapeutics to treat serious diseases using its proprietary ddRNAi technology. For additional information, please visit www.benitec.com.
Significant questions

• What is the best strategy for applying gene transfer for control of HIV/AIDS?

• Is there an antiviral gene (or genes) that can inhibit HIV for long periods of time in vivo and alter the pathogenesis of HIV infection, the progression to AIDS, or the time to initial treatment?

• How promising are lentivirus vectors for gene delivery?

• What is the best method of delivery of this gene?
  • T cell transduction and expansion
  • Blood stem cell transduction and transplantation
Significant questions

• What is the best strategy for applying gene transfer for control of HIV/AIDS?

• Is there an antiviral gene (or genes) that can inhibit HIV for long periods of time in vivo and alter the pathogenesis of HIV infection, the progression to AIDS, or the time to initial treatment?

• Will lentivirus vectors be adequate for gene delivery?

• What is the best method of delivery of this gene?
  • T cell transduction and expansion
  • Blood stem cell transplantation
Natural History of AIDS: Rationale for gene rx

- HIV Load
- Infection
- TIME
- CD4
- Gene Therapy
- ART
Clinical Strategy

Endpoints:
- Alter HIV Infection
- Prevent CD4 decline
- Lengthen Time to HIV chemotherapy

Gene Therapy

HIV Load

CD4

TIME

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**AZT / U1S1**

Very strong synergism

From S. Li et al.  Abstract 410 ASGT 2008
Median-effect plot

Crix / U1S1
Additive Effect

From S. Li et al. Abstract 410 ASGT 2008
Significant issues

- What is the best strategy for applying gene transfer for control of HIV/AIDS?

- Is there an antiviral gene (or genes) that can inhibit HIV for long periods of time in vivo and alter the pathogenesis of HIV infection, the progression to AIDS, or the time to initial treatment?

- Will lentivirus vectors be adequate for gene delivery?

- What is the best method of delivery of this gene?
  - T cell transduction and expansion
  - Blood stem cell transplantation
Complex relationship. HIV (top, purple) relies on more than 200 human proteins to infect immune cells, enter the nucleus, integrate itself into the chromosomes, and then make copies of itself.
HIV Targets for Gene Therapy

**Protein Based:**
Transdominant Mutants:
- Rev; Tat; Gag; Env
- Ig Monobodies to Tat, Env, Rev, Gag, IN, RT
- KDEL-signal retention in ER
- Toxins: bacterial, viral, IFN
- CD8 TCR zeta-Ig hybrid

**RNA Based:**
- Antisense to LTR, TAR, tat, env, rev, gag
- Decoy RNA using TAR, RRE
- Ribozymes: hammerhead, hairpin
- RNAi
- miRNA

Significant issues

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• Will lentivirus vectors be adequate for gene delivery?

• What is the best method of delivery of this gene?
  • T cell transduction and expansion
  • Blood stem cell transplantation
From HIV-1 to Lentivirus Vector

• First generation lentivirus—made by substituting VSVg for env

• Second generation lentivirus—made by removal of accessory genes

• Third generation lentivirus—made by substituting CMVp for LTR-U3 deletion; so-called “self-inactivating vector”
VirXsys & City of Hope Lentivirus Vectors

Adapted from Lu et al. J Gene Med 2004

Adapted from Yam et al. Mol Ther 2004
4-plasmid system of lentivirus vector production

DNA Plasmids

- pHIV7-GFP
  - CMV
  - R
  - US
  - ψ
  - RRE
  - Iap
  - CMV
  - eGFP
  - WPRE
  - U3
  - R
  - US

- pC-GP2
  - CMV
  - gag/pol
  - RRE
  - pA

- pC-rev2
  - CMV
  - rev
  - pA

- pVSV-G
  - CMV
  - VSV.G
  - pA

Release of virions

Cell membrane

Cytoplasm

Nucleocapsid
rHIV7-shI-TAR-CCR5RZ

From M. Li et al Mol Ther 2005
Antiviral effect of triple anti-HIV RNAs

From M. Li et al Mol Ther 2005
Downstream sub-batch Lentivirus processing

1. Transfection in 10-layer cell factories
2. Harvest of Post-Transfection Supernatant
3. Clarification of Supernatant
4. Concentration / Diafiltration 500K MWCO
5. Fill and Finish
6. Resuspend Pellet in Final Formulation
7. High Speed Centrifugation
8. 40% 10 fold volume reduction
9. 50% 15-20 fold volume reduction
10. 80-90% 0.45 um
11. 70%
12. 37 C
13. ON 4 degree

From L. Couture
Preclinical testing: rHIV7-shI-TAR-CCR5rz

- RCL free vector
- Absence of cell toxicity:
  - CD34 differentiation in SCIDhu/thy mice
  - Absence of interferon pathway induction
  - Absence of disturbed miRNA array pattern
  - HIV-like integration pattern
- Antiviral effect after cell differentiation to T cells
- Demonstration of intact integration element in transduced cells
Triple Vector Transgenic Stem Cells Expanded in SCID-hu Thymocytes

From Anderson et al. Mol Ther 2007; 15: 1182-88
HIV-1 Challenge of Triple Transgenic Thymocytes

From Anderson et al. Mol Ther 2007; 15: 1182-88
Proviral integration pattern of triple vector

Detection of therapeutic insert in transduced HeLa cells by Southern probe
Integration pattern of triple vector

Schematic representation of full-length and deleted sequences.
vRNA, viral RNA; FL, full-length; gDNA, genomic DNA; ΔU6, U6 deletion.

vRNA

FL-Proviral

ΔU6-Provirus

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Integration pattern of triple vector
Significant issues

• What is the best strategy for applying gene transfer for control of HIV/AIDS?

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• What is the best method of delivery of this gene?
  • T cell transduction and expansion
  • Blood stem cell transplantation
Retroviral T Cell-based Gene Therapy in HIV/AIDS

• Initial clinical trial of gene rx used T cells transduced with RevM10
  C Woffendin et al. PNAS 1996; 93: 2889-2894

• Gene modified T cells can be targeted by transgene-specific immunity

• HIV-specific gene modified CD8 cells can traffic to lymph nodes at sites of HIV replication and retain ag-specific potential

• Using a genetically modified TCR, CD4zeta, T cells survived at 1-3% of blood T cells at 8 wks and 0.1% at 1 year

• Genetically modified syngeneic CD4 and CD8 cells persisted for >1yr
  R. Walker et al Blood 2000; 96:467-474
First lentivirus trial in humans completed at UPENN using a vector developed by VirXsys Inc.

CD4 T cells present rapid in vivo assessment of any relative efficacy of gene therapy, i.e. protection of cells from HIV infection.
T-cell based lentivirus gene therapy

- 5 AIDS patients with ART-failure were treated with a single dose of T cells transduced with VRX496 [Levine et al. J Exp Med: 2006]
  1. Safety was demonstrated
  2. Marking of PBMC ~1:1000-10000 cells lasted up to one yr
  3. Mobilization of vector by wild-type HIV
  4. Unexplained drop in HIV load in some patients

- VirXsys Phase 2 Study (40 patients)
- UPENN STI Study (on-going)
A graph showing the total cell number over the days of culture for different conditions.

From B. Levine

rHIV7-shI-TAR-CCR5rz

Day of Culture
Screening & Enrollment

Leukapheresis

CD4 Selection

T Cell Infusions

Days 0 1 2 3 7 14 28
Months 1

#1 #2 #3

Days 0 1 2 3 7 14 28
Months 2

Days 0 1 2 3 7 14 28
Months 3

Days 0 1 2 3 7 14 28
Months 6 12 18 24 36 48 60…

rHIV7-shI-TAR-CCR5RZ Transduction

T Cell Expansion

Cryopreservation Release Testing
Stem Cell Gene Therapy for HIV/AIDS: Ethical considerations

- Partial or complete myeloablation of bone marrow is considered essential for successful engraftment.
- Toxicity of stem cell transplantation must be justifiable.
- Autologous setting is safer than allogeneic setting.
- Use of AIDS patients undergoing autologous HCT is an justifiable population in which to evaluate a new vector system.
Autologous HCT for High Risk AIDS Lymphoma: COH Experience

Surv Time (Months) Post-ASCT

% Overall Survival

Overall Survival

95% Conf. Interval

Outcome

With HCT

Expected Outcome

Without HCT

Treatment of High Risk AIDS Lymphoma

Dose-intense chemotherapy followed by autologous stem cell “rescue” is the treatment of choice for non-HIV positive patients with relapsed lymphoma.

This approach has been extended to high-risk ARL, as follows:

- Standard therapy cycles are completed (e.g. CHOP)
- After the last cycle, the patient’s peripheral blood stem cells are collected using G-CSF
- After carmustine (BCNU), etoposide (VP16), and cyclophosphamide dose-intense chemotherapy, the cells are infused as part of autologous HCT
Treatment of AIDS Lymphoma with Autologous HCT

- 35 patients with high-risk ARL
- Patients had a median of 2 chemo regimens prior to SCT
- Median CD4 = 174/uL; median HIV load = 26,120 gc/ml at Dx
  median HIV load at HCT = <500 gc/ml
- Most were on PI-based regimens and some on NRTI/NNRTI-based regimens
- \(10.6 \times 10^6\) CD34+ cells/kg collected by apheresis
- Median time to engraftment = 11 days [day 23 in one pt on AZT]
Treatment of AIDS Lymphoma with Autologous HCT

- 2 died of early relapse disease; 1 died of cardiomyopathy and renal failure at day +22 post-HCT (age 68)
- HAART continued thru the 3-week HCT hospitalization but <50% patients maintained compliance; HAART resumed in all patients on day 21
- CD4 counts recovered to pre-transplant levels at 1 year and median = 450/uL at 2 years
- HIV load increased during first 2 months in association with non-compliance but was undetectable in 76% at 1 year
Specific Aims:

The primary objective is to determine **safety and feasibility** of lentivirus-transduced hematopoietic stem cells in the setting of autologous HCT for the treatment of AIDS lymphoma.

The secondary objective is to determine the **quantity and duration of vector-marked** peripheral blood cells after marrow engraftment.
Study #1: AIDS Lymphoma

Lymphoma Rx

G-CSF (10 ug/kg)

1 2 3 4 5 6 7 8......
HPC-A Mobilization (days)

Aphereses

#1 #2 #3 #4

CD34+ Selection

Fraction A

Cryopreservation Untransduced

Fraction B

Cryopreservation

Transduction with HIV-shI-TAR-CCR5RZ

Conditioning Regimen:

BCNU  BCNU  BCNU  VP16

Days Pre-and Post-transplant

0 +1
Eligibility Criteria

- Age 18-60 years

- AIDS related lymphoma:
  - Intermediate grade or high grade non-Hodgkin’s lymphoma (working formulations D-H and J), and > partial response or first relapse after remission with standard chemotherapy
  - Hodgkin's lymphoma any subtype except nodular L&H lymphocyte predominant, and partial response or less, or relapse after standard chemotherapy

- HIV load < 50,000 gc/ml on anti-HIV chemotherapy, off AZT

- On appropriate prophylactic antibiotics, if CD4 < 200/uL

- Organ functions consistent with routine transplantation screens
Exclusion Criteria

- History of grade III cystitis due to cyclophosphamide
- CNS lymphoma
- Prior other malignancy except treated basal cell ca, cervical ca, or squamous cell ca
- HIV-associated encephalopathy; dementia; seizures in past year
- No active bacterial, fungal, CMV infection; no OI in past year except treatment-responsive MAI, candida, HSV, VZV, CMV
- Other AIDS-related syndromes, infectious or otherwise, perceived to cause excessive risk for morbidity post-HCT
- Inability to undergo blood stem cell mobilization or any contraindication for undergoing HCT
Transduction Method

- CD34+ G-CSF mobilized peripheral blood progenitor collected off ART and cells selected on a CliniMax® column (Miltenyi)
- Cells thawed, centrifuged thru HSA to remove DMSO, and cultured overnight in X-Vivo 15 media supplemented with-- SCF 100 ng/ml
  TPO 10 ng/ml
  Flt-3L 100 ng/ml
- Cells are transduced at MOI = 5 in a Retronectin® (Takara)-coated T-75 flask for 16 hr
- Final suspension in PBS with Ca/Mg, 0.5% EDTA, 0.5% HSA
Cell Product Release Tests

- Endotoxin assay
- Gram stain
- Viability >70%
  >5 x 10e6/kg viable CD34+ cells
## AIDS Lymphoma Study Recruitment

<table>
<thead>
<tr>
<th>UPN #</th>
<th>Diagnosis</th>
<th>Status</th>
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<tbody>
<tr>
<td>0301</td>
<td>Diffuse Large B cell</td>
<td>Cell Product failed release</td>
</tr>
<tr>
<td>0302</td>
<td>Burkitt</td>
<td>Failed eligibility due to infection</td>
</tr>
<tr>
<td>0303</td>
<td>Burkitt</td>
<td>Failed eligibility due to low mobilization of PBPC</td>
</tr>
<tr>
<td>0304</td>
<td>Diffuse Large B cell</td>
<td>Transplanted Feb 19, 2008</td>
</tr>
<tr>
<td>0305</td>
<td>Diffuse Large B cell</td>
<td>Transplanted Mar 13, 2008</td>
</tr>
</tbody>
</table>
CD34 Frequency

Starting CD34: 4%
Pre-Column CD34: 4%
CD34: 34%
Fold Enrichment: 110

From D. DiGiusto and L. Li
Summary CD34 Recovery

From D. DiGiusto and L. Li
## Cell Yield

### Total Cell Number and Viability of CD34 Transduction Cultures Over Time

<table>
<thead>
<tr>
<th>Date Processed</th>
<th>Date Processed</th>
<th>Date Processed</th>
<th>Date Processed</th>
<th>Date Processed</th>
<th>Date Processed</th>
<th>Date Processed</th>
<th>Date Processed</th>
</tr>
</thead>
<tbody>
<tr>
<td>9/2/2007</td>
<td>UPN0301</td>
<td>9.84E+07</td>
<td>1.23E+08</td>
<td>6.23E+07</td>
<td>76.6</td>
<td>33.3</td>
<td>62</td>
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<tr>
<td>2/2/2008</td>
<td>UPN0304</td>
<td>1.16E+08</td>
<td>1.83E+08</td>
<td>1.36E+08</td>
<td>95.9</td>
<td>72.4</td>
<td>62.8</td>
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<tr>
<td>2/21/2008</td>
<td>UPN0305</td>
<td>1.52E+08</td>
<td>1.42E+08</td>
<td>1.31E+08</td>
<td>95</td>
<td>57.9</td>
<td>52.4</td>
</tr>
</tbody>
</table>

### Viable Cell Number

<table>
<thead>
<tr>
<th>Date Processed</th>
<th>Date Processed</th>
<th>Date Processed</th>
<th>Date Processed</th>
<th>Date Processed</th>
<th>Date Processed</th>
<th>Date Processed</th>
<th>Date Processed</th>
</tr>
</thead>
<tbody>
<tr>
<td>9/2/2007</td>
<td>UPN0301</td>
<td>7.54E+07</td>
<td>4.08E+07</td>
<td>3.86E+07</td>
<td>51%</td>
<td>77%</td>
<td>48% yield</td>
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<tr>
<td>2/2/2008</td>
<td>UPN0304</td>
<td>1.11E+08</td>
<td>1.32E+08</td>
<td>8.54E+07</td>
<td>71%</td>
<td>76%</td>
<td>48% yield</td>
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<tr>
<td>2/21/2008</td>
<td>UPN0305</td>
<td>1.44E+08</td>
<td>8.20E+07</td>
<td>6.90E+07</td>
<td>72%</td>
<td>79%</td>
<td>52% yield</td>
</tr>
</tbody>
</table>

Table 1. Total Cell Number and Viability of CD34 transduction cultures over time.

Table 2. Viable cell number in CD34 transduction cultures over time.
Competition for Engraftment: transduced: untransduced cells

- >2.5 x 10^6 CD34/kg frozen unmanipulated
- ~2.5 x 10^6 CD34/kg frozen & thawed for transduction
- Post-Miltenyi column, freeze thaw, transduction process yield = ~50-60%
- Final CD34 infusion =
  - 1 x 10^6/kg transduced cells
  - 2.5 x 10^6/kg unmanipulated cells
  - Ratio-- 1:2.5 = 0.4
## Total Cell Infusion

<table>
<thead>
<tr>
<th>UPN</th>
<th>Cell Product</th>
<th>% Viable Cells in Cell Product</th>
<th>Viable cells/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Non-Transduced</td>
<td>Transduced</td>
</tr>
<tr>
<td>0301</td>
<td>$3.86 \times 10^7$</td>
<td>62</td>
<td>62</td>
</tr>
<tr>
<td>0304</td>
<td>$8.54 \times 10^7$</td>
<td>67</td>
<td>64.5</td>
</tr>
<tr>
<td>0305</td>
<td>$6.9 \times 10^7$</td>
<td>53.7</td>
<td>52.4</td>
</tr>
</tbody>
</table>
Phenotype Kinetics from 28D Culture of CD34+ Cells

Liquid Culture

Stromal Culture

From D. DiGiusto and L. Li
Phenotype of Liquid Culture CD34+ Cells

Day 7

Day 14

From D. DiGiusto and L. Li
Marking of Bulk Cultures

\[
\text{WPRE/ApoB}
\]

Condition

Untd S D7  TD S D7  UTD L D7  TD L D7  UTD L D14  TD L D14  UTD L D21  TD L D21

From D. DiGiusto and L. Li
Marking of Lineage Specific Cells

From D. DiGiusto and L. Li
## Transduction Analysis of Cultured CD34+ Cells

<table>
<thead>
<tr>
<th></th>
<th>D8</th>
<th>D13</th>
<th>D22</th>
<th>D29</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liquid Untd</strong></td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td><strong>Liquid TD</strong></td>
<td>23%</td>
<td>3%</td>
<td>1%</td>
<td>1%</td>
</tr>
<tr>
<td><strong>Stroma Untd</strong></td>
<td>ND</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td><strong>Stroma TD</strong></td>
<td>ND</td>
<td>2%</td>
<td>3%</td>
<td>1%</td>
</tr>
</tbody>
</table>

**Bulk culture**

<table>
<thead>
<tr>
<th></th>
<th>CD15</th>
<th>CD14</th>
<th>GlyA</th>
<th>Lin-</th>
<th>CD10+</th>
<th>CD10-</th>
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<tbody>
<tr>
<td><strong>Liquid Untd D13</strong></td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td><strong>Liquid TD D13</strong></td>
<td>1%</td>
<td>1%</td>
<td>8%</td>
<td>11%</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Stroma Untd D29</strong></td>
<td>0%</td>
<td>0%</td>
<td>ND</td>
<td>0%</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Stroma TD D29</strong></td>
<td>1%</td>
<td>1%</td>
<td>ND</td>
<td>1%</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

From D. DiGiusto and L. Li
qPCR assay for anti-tat/rev siRNA expression in patient cells

patient 304 1M

From J. Rossi
Gene Marking in PBMC: Q-PCR primary data
Primer: WPRE, Standard Curve: H9c1/PBMC (100%-0.002% H9c1-positive)

A. Standard Curve

B. Ct curves

C. Melting Curve analysis
Outcome Pending

- Safe engraftment in 10 days in two patients
- Gene marking in blood:

  Months:  1  2  3  6  12  18  24
  Patient #1  +  +  ......................
  Patient #2  +  +  ......................

- Patients continue to be enrolled and followed
Conclusions

- Strategic gene therapy design would treat early after HIV infection
- Multiplex lentivirus vector can be used in HIV-related projects
- shRNA-containing vector can be used in clinical studies
- Further follow-up continues for ongoing studies
- Development of T-cell based and stem cell based methods deserve further evaluation
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Zaia, John A.

UPENN:
C. June
B. Levine

NIAID; NHLBI;
NCI; NCRR

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