Rapid reconstitution of immunity to infectious pathogens post stem cell transplant

David Gottlieb

University of Sydney
Blood and Marrow Transplant Unit Westmead Hospital
Recipient

Stem cell donor

CD34+ cells

Haemopoietic progenitor cells

T cells
NK cells
NKT cells
\(\gamma\delta\) T cells
DC

- anti-host
  - anti-major/minor MHC
  - anti-tissue specific antigens
- anti-viral/bacterial/fungal
- anti-tumour specific antigens
## COMMENTS
This product meets the requirements for product distribution.
Transplant date: 20/04/11.
Neg bag cryopreserved into 4x 50ml bags.
T-Cell Depletion of HLA-Identical Transplants in Leukemia

T cell depletion

T cells
- cures leukaemia

Removing T cells
- cures GvHD
- GvHD/GVT
- Rejection, relapse, viral, fungal infection
Adoptive T cell therapy for the prevention of viral reactivation and disease

CMV specific T cells infused into HSCT transplant recipient to rapidly restore immunity

Blood taken from donor prior to transplant

Donor

STEM CELL TRANSPLANT

CMV specific T cells selectively activated and expanded \textit{ex vivo}

Immunity transplant

CMV specific T cells infused into HSCT transplant recipient to rapidly restore immunity

HSCT recipient
Restoration of Viral Immunity in Immunodeficient Humans by the Adoptive Transfer of T Cell Clones

Stanley R. Riddell,* Kathe S. Watanabe, James M. Goodrich, Cheng R. Li, Mounzer E. Agha, Philip D. Greenberg
INFUSIONS OF DONOR LEUKOCYTES TO TREAT EPSTEIN–BARR VIRUS–ASSOCIATED LYMPHOPROLIFERATIVE DISORDERS AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION

Esperanza B. Papadopoulos, M.D., Marc Ladanyi, M.D., David Emanuel, M.D.,
Stephen Mackinnon, M.D., Farid Boulad, M.D., Matthew H. Carabasi, M.D.,
Hugo Castro-Malaspina, M.D., Barrett H. Childs, M.D., Alfred P. Gillio, M.D.,
Trudy N. Small, M.D., James W. Young, M.D., Nancy A. Kernan, M.D.,
and Richard J. O’Reilly, M.D.
Memorial Sloan-Kettering Cancer Center, New York.


DONOR T CELLS TO TREAT EBV-ASSOCIATED LYMPHOMA

Helen E. Heslop, M.D.
Malcolm K. Brenner, M.B.
Cliona M. Rooney, Ph.D.
St. Jude Children’s Research Hospital

Effect of EBV specific CTL on EBV Reactivation

EBV DNA versus neo DNA

Neo DNA

Rooney et al, Lancet 1995
Einsele et al Blood 2002

Peggs et al Lancet 2003
Is it possible to reconstitute immunity to multiple opportunistic pathogens (and tumours) after stem cell transplant to prevent rather than to treat disease?
The T cell response to CMV is strongly directed towards the pp65 protein

T cell therapy with CD8 cytotoxic T cells recognising HLA A2 restricted CMV epitope
Generation of CMV specific CTL

Peripheral Blood

IL-4 & GM-CSF

Monocyte Derived DCs
Transfected with Ad5F35pp65

Foster et al. Biol Blood Marrow Transplant 2004; 10; 761-771
Generation of CMV specific CTL

Peripheral Blood T-cells

Day 0  Day 7  Day 14

IL-2

Enriched CMV-specific T-cells

Foster et al. Biol Blood Marrow Transplant 2004; 10; 761-771
Time post transplant

BMT

control

CTL

CMV specific T cell infusion

0 28 56 84 110

Time post transplant
Stem cell transplant CMV seropositive donors 2003-2011
n=237

Transplant according to standard institutional protocols

Donor derived CTL lines available for infusion from Day 28 under phase II study
n=60

- CTL infusion n=50
- Not infused n=10

No CTL line available (controls)
n=177

- CTL line available n=128

Landmark analysis time point day 28 post transplant
Patients not fulfilling criteria for landmark analysis excluded**

- CTL infusion n=45
- Not infused n=9

- No CTL line available n=128

CTL cohort
n=54

Control cohort
n=128

**Exclusion criteria for the landmark analysis were: death, relapse, severe acute GVHD and CMV reactivation prior to day 28
Cohorts used for comparative analysis

<table>
<thead>
<tr>
<th></th>
<th>All CTL infused patients n=50</th>
<th>CTL cohort n=54</th>
<th>Control n=128</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median length of follow-up Months (range)</td>
<td>26 (2-80)</td>
<td>26 (2-80)</td>
<td>25 (1-98)</td>
<td>0.79</td>
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<tr>
<td>Number of patient treated with CTL</td>
<td>50</td>
<td>45</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Median day of infusion Days post-transplant (range)</td>
<td>45 (29-115)</td>
<td>45 (29-115)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CMV reactivation n (%)</td>
<td><strong>Total</strong> 26 (52)</td>
<td>25 (46)</td>
<td>77 (60)</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>Pre-infusion 14 (28)</td>
<td>9 (16)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Day of infusion 7 (14)</td>
<td>4 (8)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Post-infusion 5 (10)</td>
<td>6 (11)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>CMV in uninfused patients -</td>
<td>6 (11)</td>
<td>77 (60)</td>
<td>-</td>
</tr>
<tr>
<td>Peak titre (median)</td>
<td>Positive below linear limit of assay 0</td>
<td>600</td>
<td>-</td>
<td>0.04</td>
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[1] p-values refer to comparisons between columns 3 and 4, all are 2 sided, and values <0.05 are considered significant.
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<tr>
<td><strong>IV treatment</strong> n (%)</td>
<td>9 (18)</td>
<td>9 (17)</td>
<td>46 (36)</td>
<td>0.01</td>
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<tr>
<td>Days of IV treatment (days per patient in cohort)</td>
<td>4.08</td>
<td>3.4</td>
<td>8.9</td>
<td>0.03</td>
</tr>
<tr>
<td>CMV disease n (%)</td>
<td>1 (2)</td>
<td>3 (6)</td>
<td>11 (9)</td>
<td>0.76</td>
</tr>
<tr>
<td>Death from CMV disease n (%)</td>
<td>1 (2)</td>
<td>1 (2)</td>
<td>1 (1)</td>
<td>0.51</td>
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<tr>
<td>aGVHD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II-IV</td>
<td>12 (24)</td>
<td>13 (24)</td>
<td>24 (18)</td>
<td>0.42</td>
</tr>
<tr>
<td>III-IV</td>
<td>4 (8)</td>
<td>4 (7)</td>
<td>9 (7)</td>
<td>1.0</td>
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Peak CMV titre, number of days of therapy

**Graph 1:**
- **Y-axis:** Peak CMV titre (copies/ml plasma)
- **X-axis:** Number of treatment days
- **Legend:**
  - CTL
  - Control
- **Statistical Significance:**
  - p = 0.04

**Graph 2:**
- **Y-axis:** Number of treatment days
- **X-axis:** Peak CMV titre (copies/ml plasma)
- **Legend:**
  - CTL
  - Control
- **Statistical Significance:**
  - p = 0.03
CMV reactivation

Days post-transplant

Percent CMV reactivation

CTL infusion (median)

Landmark day 28

p=0.17
Influenza in HSCT recipients

- Immunity relies on CD4+ and CD8+ T cell responses and on serological responses to haemagglutinin and neuraminidase.
- For proven A/H1N1 infection in SCT patients, lower RTI in 32.5%, mechanical ventilation in 11.5% and mortality in 6.3% (Ljungman 2011).
- Results of serological responses to influenza vaccination (including A/H1N1) using inactivated influenza vaccine typically poor in HSCT patients.
**Fluvax- CSL**

- Fluvax- inactivated viral fragments of flu virus strains
  - H1N1
  - H3N2
  - Brisbane
- Vaccine updated annually
  - 2013 vaccine contains
    i. A (H1N1)
    ii. A (H3N2)
    iii. B Wisconsin /1/2010 like virus
- Approved for human use

**Ingredients**

*Active ingredients:*
- Purified, inactivated virus fragments from influenza types:
  - H1N1 strain - 15 micrograms
  - H3N2 strain - 15 micrograms
  - B strain - 15 micrograms

*for the Southern Hemisphere winter season 2011.*

*Other ingredients:
- Sodium chloride
- Sodium phosphate - monobasic
- Sodium phosphate - dibasic anhydrous
- Potassium chloride
- Potassium phosphate - monobasic
- Calcium chloride*

*Fluvax® vaccine may also contain trace amounts of egg proteins, neomycin, polymyxin, sucrose and detergent (sodium taurodeoxycholate).*
Generation of influenza specific T cells

**B**

Fold Expansion vs. Day of Culture

**C**

% of Cells vs. CD3, CD4, CD8, CD19, CD56, Tcm, Tn, Treg

**A**

Flow cytometry analysis of IFNγ, TNFα, IL-2 levels in Control and Fluvax groups.

- IFNγ: 0.787, 0.792, 0.739
- TNFα: 42.8, 39, 32.2
- IL-2: 0.739, 0.792, 0.787
Antigen Specificity

B

CD3

% CD3 Cells

Control

Fluvex

C

CD4

% CD4 Cells

IFNγ

TNFα

IL-2

D

CD8

% CD8 Cells

IFNγ

TNFα

IL-2

A

IFNγ

0.476

0.185

0.461

Control

TNFα

4.27

1.46

2.86

H1N1

IL-2

5.9

1.99

3.62

H3N2

IFNγ

7.69

2.22

4.92

Brisbane
BK virus (polyoma hominis 1)

- Non enveloped, encapsulated Papova DNA virus
- Other family members JC virus and SV40 (70% homology)
- Around 90% of normal adults are seropositive for BKV
- After primary infection, enters a latent state in the urothelium
- Post BMT syndromes include
  - Haematuria
  - Haemorrhagic cystitis
  - Interstitial nephritis
  - Ureteral stenosis
  - Renal failure
BKV Structure
Targets of BKV specific CTL activity

Graph showing the percentage of cells producing cytokines (of CD3) for CD8+ and CD4+ cells. The x-axis represents different treatments or stimuli (Neg, All BKV, VP1, VP2, VP3, LTA, sTA), and the y-axis represents the percentage of cells producing cytokines. The graph indicates that BKV-specific CTL activity varies across different stimuli, with some showing a higher percentage of cells producing cytokines.
Multiple cytokines were produced in response to antigen re-challenge

- IFN-γ, TNF, IL-2

Single cell analysis
Expanding specificity of antigen specific cell therapy

<table>
<thead>
<tr>
<th>Pathogen/Antigen</th>
<th>Antigen source/method of cell generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytomegalovirus</td>
<td>Peptides, viral vector</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Adenoviral vector, peptides</td>
</tr>
<tr>
<td>Epstein Barr virus</td>
<td>Adenoviral vector, peptides</td>
</tr>
<tr>
<td>Varicella zoster</td>
<td>Vaccine used in vitro</td>
</tr>
<tr>
<td>Influenza</td>
<td>Vaccine used in vitro, peptides</td>
</tr>
<tr>
<td>BK virus</td>
<td>Peptides</td>
</tr>
<tr>
<td>HHV6</td>
<td>Peptides</td>
</tr>
<tr>
<td>Aspergillus</td>
<td>Lysate, peptides</td>
</tr>
</tbody>
</table>

*Blyth et al Cytotherapy 2012  
**Gaundar et al Cytotherapy 2012  
***Blyth et al Transplantation 2011  
****Gaundar et al Cytotherapy 2012
Next Westmead allogeneic BMT study: donor T cells specific for

- CMV
- Adenovirus
- EBV
- VZV
- Influenza
- BKV
- Aspergillus

AND post infusion patient vaccination with influenza and VZV vaccine
Assessing response to multiple pathogen T cells

• Problems
  – Low incidence clinical syndromes
  – Disease clusters
  – Knock on effects

• Assessing benefits
  – Total antibiotic usage
  – In hospital days
  – Total cost of transplant
  – Quality of life
## Composition of Peripheral blood stem cell products

<table>
<thead>
<tr>
<th>Donor</th>
<th>WCC x10⁹/L</th>
<th>Vol (ml)</th>
<th>CD34 x10⁶/kg</th>
<th>% monocytes</th>
<th>% of product to give 200x10⁶ monocytes</th>
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<tbody>
<tr>
<td>1</td>
<td>344</td>
<td>211</td>
<td>6.7</td>
<td>39.8</td>
<td>0.72</td>
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<tr>
<td>2</td>
<td>185</td>
<td>124</td>
<td>7</td>
<td>25</td>
<td>3.46</td>
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<tr>
<td>3</td>
<td>195</td>
<td>291</td>
<td>12.5</td>
<td>18.5</td>
<td>1.9</td>
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<tr>
<td>4</td>
<td>179</td>
<td>265</td>
<td>7.3</td>
<td>31.5</td>
<td>1.33</td>
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<td>5</td>
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<td>337</td>
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<td>7</td>
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<td>292</td>
<td>3.4</td>
<td>29.2</td>
<td>1.55</td>
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<tr>
<td>12</td>
<td>254</td>
<td>85</td>
<td>33.8</td>
<td></td>
<td>2.74</td>
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<tr>
<td>Mean</td>
<td>214</td>
<td>225</td>
<td>5.9</td>
<td>28.4</td>
<td>1.5</td>
</tr>
</tbody>
</table>
CMV CTL culture from peripheral blood stem cell harvest

21 day culture

Clinical trial CMV CTL culture from same donor – whole blood

21 day culture
Collect 100-500ml of peripheral blood prior to transplant/leukapheresis or 1-2% of G-CSF primed apheresis product

Donor not required to give 100-500ml of additional blood or undergo additional leukapheresis

Collection/transport of cells occurs within the established quality system of the BMT laboratory

Could be applied to transplants with unrelated donors collected at interstate or international collection sites

Reduced processing time (7-8 hours)

Manual buffy isolation

Isolation of PBMC (Ficoll)

CD14 CliniMACS selection

Culture GM-CSF + IL-4 5 days

Mature DC with cytokines

Cryopreserve, phenotype DC, QA

Thaw, peptide pulse and vaccination

Day 1

Day 5

Day 7
Conclusions

• Antigen specific T cells to many clinically relevant opportunistic pathogens can be generated in vitro
• Clinical testing for their efficacy in prophylaxis and treatment will be required
• Designing trials to measure their benefits is likely to require rethinking of endpoints to account for low frequencies of individual pathogen related disease
• Combining effective immune reconstitution with anti-tumour cell therapy may make transplantation redundant
Transplantation and immune suppression after transplant to reduce GVHD risk.Await generation of mature tumor and infectious antigen specific T cells. Opportunistic infection and relapse cause morbidity and mortality.

No immune suppression. Commence infusions of donor derived T cells specific for tumor and infectious pathogens and NK cells, CAR T cells, dendritic cells, specific antibody etc.
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Westmead BMT co-ordinators
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