Extending the range and applicability of antigen-specific T cells for viral infections
Viral infections post-transplant

• 40% deaths after alternative donor transplant due to viral infections

• Antiviral drugs
  – Costly
  – Significant side effects
  – Often ineffective

• Alternative - Adoptive T cell transfer
Immunotherapy for viral infections

1. Blood draw
2. Antigen Specificity
3. T-cell product generation
4. Infusion
Immunotherapy for viral infections

- Virus-specific T cells as prophylaxis and treatment
  - EBV
  - Adv/EBV (bivirus)
  - Adv/EBV/CMV (trivirus)
Generation of trivirus-specific T cell lines using Ad5f35 vectors

PBMC

B95-8 EBV virus

EBV LCL

Ad5f35pp65 vector

Ad5f35pp65 transduced EBV LCL

+IL2

Restimulation

PBMC

Trivirus CTL

6 wk
Clinical Outcome Summary – Donor-specific setting

- *In vitro* expanded donor-derived virus-specific T cells targeting Adv, EBV, CMV
  - Safe
  - Reconstituted antiviral immunity for EBV, CMV and Adv
  - Effective in clearing disease
  - Considerable expansion *in vivo*

*Leen et al, Nat Med. 2006
Leen et al, Blood. 2009*
The problem

- Individualized products
  - impractical for widespread/urgent use

- Manufacture is complicated
The problem

- Individualized products
  - impractical for widespread/urgent use

- Manufacture is complicated
Assess whether banked virus-specific T cells (VSTs) produced clinical benefit in partially HLA-matched 3rd party recipients
3rd party VST therapy

Blood donor

Trivirus VST

EBV activity – B8, DR1
CMV activity – A24
Adv activity – A1, A24, DR15

MDACC
EBV – A1, 11; B8, 35; DR8
3rd party VST therapy

Blood donor

Trivirus VST

EBV activity – B8, DR1
CMV activity – A24
Adv activity – A1, A24, DR15

MDACC
EBV – A1, 11; 
B8, 35; DR8
3rd party VST therapy

Blood donor

EBV activity – B8, DR1
CMV activity – A24
Adv activity – A1, A24, DR15

Trivirus VST

MDACC
EBV – A1, 11; B8, 35; DR8

Boston
CMV – A2, 24; B7, 27; DR1, 15

CHLA
Adv – A1, 11; B7, 8; DR3, 11
VST Product

• Most closely HLA-matched trivirus-specific VSTs
  – Some VST lines already made for previous studies
  – New VST lines made from donors with common alleles (PACT)

• 32 lines

• Multicenter study (TCH, TMH, MDACC, Duke, Dana Farber, CHLA, Hackensack, Mass General, Uni. Of Miami, Boston Children’s Hospital)
Phenotype of VSTs

% Positive cells

CD3  CD4  CD8  Effector memory  Central memory
Trivirus specificity of VSTs
Clinical protocol

- Treatment of refractory EBV, CMV, or Adv
- Patients receive $2 \times 10^7$ VSTs/m$^2$
- If partial response may receive additional doses at 2+ weekly intervals
Screening

• 82 patients screened
  – 23 - Bone marrow
  – 33 - Peripheral blood stem cells
  – 12 - Single cord
  – 13 - Double cord

• Line identified for 74/82
  – Suitable line if matched at least one antigen with activity against infecting virus
Screening

- 24 patients with line not on study
  - subsequently not eligible due to other infections
  - improved
  - progressed and died prior to infusion
  - declined
Patients Treated on Study

- 50 patients infused – 45 evaluable
  - 19 received VSTs for CMV
  - 17 received VSTs for Adv
  - 9 received VSTs for EBV
Are the VSTs safe?

- Acute GVHD within 45 days first infusion
  - 8 patients developed GVHD (6 prior history)
    - 6 Grade I; 1 Grade II; 1 Grade III
  - 1 chronic GVHD flare (discontinued immunosuppression)

- 2 developed transplant-associated microangiopathy (both on sirolimus)
Do the VST produce clinical benefit?

CMV Colitis Responds to VSTs – pt69

Pre VSTs

Post-VSTs

Ulcers on endoscopy

Viral Inclusion

Immunostain for CMV

Normal endoscopy

No Viral Inclusions
Clinical response correlates with increase in VSTs.
Overall CMV responses

- 19 patients treated for CMV
  - 17/19 responded to VSTs
    - 9 CR
    - 8 PR
Clinical benefit - Adv

**Stool**

Copies/ml

- Pre
- wk1

**Blood**

- Pre
- wk2
- wk4
- wk6
Clinical benefit - Adv

**Stool**

- **Copies/ml**
  - Pre: 2.0 x 10^6
  - wk1: 1.0 x 10^6

**Blood**

- **Copies/ml**
  - Pre: 1.5 x 10^6
  - wk2: 1.0 x 10^6
  - wk4: 0
  - wk6: 0

**Adv T cells**

- **SFC/2x10^5**
  - Pre: 5.0 x 10^5
  - wk2: 1.0 x 10^6
  - wk4: 1.0 x 10^6
  - wk6: 0
Overall Adv responses

• 17 patients treated for Adv
• 14/17 responded to VSTs
  • 7 CR
  • 7 PR
Clinical Responses – EBV (pt37)

Pre VSTs

1 month post VSTs
Immune Responses - EBV (pt37)

- EBV T cells

SFC/2x10^5

Pre  wk2  wk4

0  20  40  60  80
Overall EBV responses

• 9 patients treated for EBV
  • 6/9 responded to VSTs
    • 2 CR
    • 4 PR
Do VSTs Persist?

- Deep sequencing TCRs
- Detect specificities in both donor and recipient

PT37

Week 2

Week 4
Overall response rate

- Overall 74%
  - 74% - CMV
  - 67% - EBV
  - 79% - Adv

- Durable
  4 subsequent progression/recurrence
What happened to patients without a line?

- Line identified for 74/82
- 8 patients without a suitable line
  - 6 died of progressive infection
  - 1 failed multiple antivirals but eventually cleared post-DLI
  - 1 PR by day 42
What happened to patients without a line?

- Line identified for 74/82
- 8 patients without a suitable line
  - 6 died of progressive infection
  - 1 failed multiple antivirals but eventually cleared post-DLI
  - 1 PR by day 42
- Response rate 13% vs 74% VST group

Leen et al, Blood, in press
Summary of 3rd Party VST Trial

- Low attributable toxicity
- VSTs effective in clearing EBV/Adv/CMV disease
- T cell expansion seen in approx. 50% of responders
- May require several infusions to sustain benefit
- Persistence for 12 weeks in a recipient of a 4/6 matched line
Solutions

- Individualized products
  Administration of “off the shelf” T cells

- Manufacture is complicated
The problem

- Individualized products
  Administration of “off the shelf” T cells

- Manufacture is complicated
The problem

- Individualized products
  Administration of “off the shelf” T cells

- Manufacture is complicated
  - Cost
  - Complexity
  - Antigenic competition
Cost

PBMC → B95-8 EBV virus

EBV LCL → Ad5f35pp65 vector

Ad5f35pp65 transduced EBV LCL → +IL2

PBMC → Ad5f35pp65 vector

Restimulation → Trivirus CTL

6 wk
**Complexity**

- **Day 0** – CTL initiation: $2.4 \times 10^7$
- **Day 9** – restim: $2 \times 10^7$
- **Day 12** – IL2 feed
- **Day 16** – harvest and reseed: $6 \times 10^7$
- **Day 20** – split + IL2 feed
- **Day 23** – harvest and reseed
- **Day 27** – split + IL2 feed
- **Day 30** – harvest/freeze: $3.6 \times 10^8$

The diagram illustrates the progression and cell counts at each stage.
Antigenic competition

*Precludes extension of this approach to additional clinically relevant viruses*
Solutions

(i) Cost

(ii) Complexity

(iii) Antigenic competition
Replacing virus/vector with Overlapping peptide libraries

<table>
<thead>
<tr>
<th>Virus/Vector</th>
<th>15mer pepmixes</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBV LCL</td>
<td>EBV- EBNA1, LMP2, BZLF1</td>
</tr>
<tr>
<td>Ad5f35pp65</td>
<td>CMV- IE1, pp65</td>
</tr>
<tr>
<td></td>
<td>Adv- Hexon, Penton</td>
</tr>
</tbody>
</table>

Target antigens
Specificity

SFC/2x10^5

Adv  CMV  EBV

HEXON  PENTON  IE1  PP65  EBNA1  LMP2  BZLF1  NO PEP
Solutions

(i) Cost – overlapping peptides

(ii) Complexity

(iii) Antigenic competition
Solutions

(i) Cost – overlapping peptides

(ii) Complexity

(iii) Antigenic competition
• Gas permeable membrane allows CO$_2$/O$_2$ exchange
• Supports cell growth with large volumes of media
• Reduces feeding frequency and manipulation
• No rocking or stirring

Vera et al, JIT, 2010
Superior expansion of CTLs in G-Rex vs 24w plate

Production time halved
(i) Cost – overlapping peptides

(ii) Complexity – G-Rex

(iii) Antigenic competition
Solutions

(i) Cost – overlapping peptides

(ii) Complexity – G-Rex

(iii) Antigenic competition
Overcoming antigenic competition

- Prevent of activation induced cell death
  - Addition of cytokines that reduce AICD w/out inducing non-specific T cell proliferation
  - Corresponding increase in the frequency and repertoire of reactive cells
Superior expansion in cultures supplemented with activating cytokines.
T cell function

No cytokines

CD8+ 5.4%

IL15

17.4%

IL2

20.8%

IL4+7

17.6%

CD4+ 2.7%

0.25%

0.83%

16.31%

Gerdemann et al, Mol. Ther., 2012
(i) Cost – overlapping peptides

(ii) Complexity – G-Rex

(iii) Antigenic competition – IL4/7

Will they work?
ARMS
Administration of Rapidly Generated Multivirus-Specific Cytotoxic T-Lymphocytes for the Prophylaxis and Treatment of EBV, CMV, Adenovirus, HHV6, and BK virus Infections post Allogeneic Stem Cell Transplant

Pepmix
mastermix

EBV – EBNA1, LMP2, BZLF1
CMV – IE1, pp65
Adv – Hexon, Penton
BK – LT and VP1
HHV6 – U11, U14, U90

+IL4/7

T cell stimulation/ expansion
10 days

Overall conclusions

- Generation of broad-spectrum virus-specific T cell lines feasible
- 10 day manufacturing without exposure to biohazards
- Safety being assessed in donor-specific setting
- Extend to 3rd party use
Acknowledgements

TRL Lab PIs
Helen Heslop
Cliona Rooney
Malcolm Brenner
Catherine Bollard

GMP/QC Laboratory
Adrian Gee
Zhuyong Mei
Debbie Lyon
Suzanne Poole

CTL Laboratory
Oumar Diouf
Joyce Ku
Pallavi Mohpatra
Huimin Zhang
Weili Liu

TRL Laboratory
Ulrike Gerdemann
Usha Katari
Anastasia Papadopolou
Jacqueline Kiernan
Joey Tong
Lisa Rollins
Juan Vera

Clinical Research
Bambi Grilley
Bridget Medina
Alician Brown
Yu-Feng Lin

EMMES
Adam Mendizabal
Katherine Christensen

NMDP
Dennis Confer

NHLBI
John Thomas

Transplant Service
Bob Krance
Kathy Leung
Caridad Martinez
George Carrum
Ram Kamble

CHALLAH
J. Antin
B. Dey
D. Avigan
P. Szabolcs
E.J. Shpall
P Kebriaei,
N. Kapoor
S-Y Pai,
S.D. Rowley,
BR Dey,

Specialized Centers for Cell-Based Therapy
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE

SCCT

PACT

Production Assistance for Cellular Therapies

Funding: NCI Program Project Grant, NHLBI Somatic Cell Therapy Center, Lymphoma SPORE, Leukemia and Lymphoma Society Specialized Center of Research, Doris Duke Distinguished Clinical Scientist Award, PACT