EBV T cells for EBV-associated Lymphoma

Patrick J. Hanley, PhD
Director, GMP for Immunotherapy
Children’s National Health System
The George Washington University
School of Medicine and Health Sciences
DISCLOSURE

Nothing to disclose
Types of EBV Latency

**Type 3**
- EBV lymphoma post transplant
- Lymphoblastoid cell lines

**Type 2**
- Hodgkin’s Lymphoma
- Nasopharyngeal carcinoma

**Type 1**
- Burkitt’s lymphoma
EBV Lymphoma After BMT

- Incidence 1-25% following mismatched or unrelated donor BMT
- Predisposing factors:

[Diagram showing EBV proteins and their interactions]
**EBV-specific T cell Generation**

**Step 1: LCL generation**
4-6 weeks

**Step 2: T-cell expansion**
4-7 weeks

**Step 3: QA/QC**
- Sterility
- HLA type
- Phenotype
- Cytotoxicity

EBV-infected B cells (LCL)

EBV-specific T cells

Retroviral vector-neo gene
Donor-derived EBV-specific T cells for Stem Cell Transplant Recipients

- **Prophylaxis:**
  - 110 high risk patients
  - None developed EBV lymphoma

- **Therapy**
  - 14 patients treated with active disease
  - 12 attained CR
  - No recurrences

*Leen et al, Nat Med 2006 and Heslop et al, Blood 2010*
Hematopoietic Stem Cell Transplantation and Virus Infection

- High incidence of viral infections (not just EBV) post-transplant

- Cytomegalovirus (CMV), Epstein-Barr virus (EBV), adenovirus (Ad)

- Highest incidence when donor seronegative (i.e. cord blood)
Expanding T cells targeting CMV, EBV and adenovirus (Multivirus-specific CTL)

Ad5f35-pp65

EBV-Lymphoblastoid cell lines (LCL)

EBNA-1
LMP2
LMP1
EBNA-2
EBNA
3a,3b,3c
LP
Generating Multi-Virus-Specific T cells (VSTs) From Peripheral Blood (PB) of Seropositive Donors

Ad5f35CMVpp65 vector

EBV LCL

Antigen Stimulation

PBMC

IL-2

Multivirus-specific T cells (VSTs)

Leen et al, Nat Med 2006
The New: Generating Multi-Virus-Specific T cells (VSTs) From Seropositive Donors

Peptide mixtures
CMV IE1
CMVpp65
EBV EBNA1
EBV LMP2
Ad hexon
Ad Penton

Dr. Mike Keller, CNMC
EXTENDING TO HIV
(Lam et al, Mol Ther 2014)

Vera, JIT 2009
Papadopoulou, STM 2014
Multivirus specificity of VSTs after 10 days

+Actin

+SEB

+pp65/IE1

+EBNA1/LMP2

+Hexon/Penton
Generating Multivirus-Specific T cells (VSTs) From Cord Blood (CB) Using Same Methodology

- Ad5f35CMVpp65 vector
- EBV LCL
- Antigen Stimulation
- IL-2
- Multivirus-specific T cells (VSTs)
Successfully Generating Multivirus-Specific T cells (VSTs) From Cord Blood

Eligibility Criteria

- Prophylaxis and Treatment
- Day +30 post HSCT or CBT
- GvHD <grade III at enrollment
- In addition for Cord Blood:
  - $2.5 \times 10^7$ TNC/kg
  - Fractionated cord blood unit
Multivirus specific T-cells (VSTs) from Peripheral and Cord Blood

38 generated from donor peripheral blood (8 with rapid manufacture)
10 generated from cord blood

- Peripheral Blood VST Study:
  - $1 \times 10^7/m^2$
  - $5 \times 10^7/m^2$
  - $1 \times 10^8/m^2$

- Cord Blood VST Study:
  - $5 \times 10^6/m^2$
  - $1 \times 10^7/m^2$
  - $1.5 \times 10^7/m^2$
  - $2.5 \times 10^7/m^2$
VSTs Derived from PB and CB Express Effector and Central Memory Markers After Expansion

Percentage of Live Cells

Mean CB VSTs
Mean PB VSTs
CB VSTs
PB VSTs

CD4
CD8
CD45RA-CD62L-
CD45RA-CD62L+
CD3-CD56+
CD3-CD19+
**VSTs Recognize Multiple Viruses**

**IFN-γ ELISPOT: Spots per 1x10^5 cells**

<table>
<thead>
<tr>
<th>T-cell Line</th>
<th>Adeno</th>
<th>EBV</th>
<th>CMV</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral Blood VST</td>
<td>86</td>
<td>183</td>
<td>648</td>
<td>14.5</td>
</tr>
<tr>
<td></td>
<td>(9-542)</td>
<td>(0-351)</td>
<td>(28-1278)</td>
<td>(3-65)</td>
</tr>
<tr>
<td>Cord Blood VST</td>
<td>83</td>
<td>117</td>
<td>36</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>(1-504)</td>
<td>(4-339)</td>
<td>(1-110)</td>
<td>(1-28)</td>
</tr>
</tbody>
</table>

- 33/40 CTL were Ad-specific
- 38/40 CTL Lines were EBV-specific
- 35/40 CTL Lines were CMV-specific
- 8/8 rapid manufacture VSTs – all tri-virus specific
### Patient Characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>Median Age (range)</th>
<th>Alternative Donors</th>
<th>Campath or ATG in vivo</th>
<th>Median day VST infused</th>
<th>Off Immune Suppression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral blood VST</td>
<td>10 years (1-62)</td>
<td>84%</td>
<td>90%</td>
<td>+84 (35-164)</td>
<td>63%</td>
</tr>
<tr>
<td>Cord Blood VST</td>
<td>1.5 years (0.6-5)</td>
<td>100%</td>
<td>0</td>
<td>92 (84-146)</td>
<td>0</td>
</tr>
</tbody>
</table>

- Most patients received transplant for malignant disease
- Haploidentical donors included (n =14)
- Cord Blood donors (n= 10)
- 10 patients had Adv infection
- 19 patients had CMV infection/reactivation
- 11 patients had EBV reactivation
Multivirus T cells Protect Against EBV

- 11/58 patients had EBV reactivation
- 11/11 patients had decrease in EBV viral load with coinciding elevation in EBV-specific T cells in PB
- No additional antiviral therapy required
Rapidly Manufactured Multi-Virus T cell Protect and Treat EBV Lymphoma

Multivirus T cells (Targeting LMP2 and EBNA1)

Rituximab

EBV T cells

EBV Viral Load

Spots per 200,000 cells

Day -16  Day -13  day -10  Day -3  Day 0  Day 4  Day 7  Day 14  Day 21

DNA Copies/mL Blood

0  50,000  100,000  150,000  200,000  250,000

0  50,000  100,000  150,000  200,000  250,000

Courtesy of Dr. Mike Keller
Duration of T cell Persistence Depends on Viral Reactivation

No Reactivation (P3275)

CMV, adeno Reactivation (P2891)

% PBMC TCRs

Clones in the product

Patient P3275 – No Viral Reactivation

Patient 2891 – CMV and Adenovirus Reactivation

Pre Infusion | Month 1 | Month 6

Clones in the patient
Infused VST Clones Persist and Expand \textit{in vivo}

![Graph showing amino acid clone tracker sequences for samples. Possible sequences include CASSIKGNNNSP.](image)
# Minimal Related Toxicity – No GVHD

<table>
<thead>
<tr>
<th>Study</th>
<th>Donor/Recipient Matching</th>
<th># of Patients</th>
<th>Acute GvHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral Blood VST</td>
<td>Haplo</td>
<td>14</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>MUD</td>
<td>6</td>
<td>1 Grade I</td>
</tr>
<tr>
<td></td>
<td>MRD</td>
<td>10</td>
<td>1 Grade I</td>
</tr>
<tr>
<td>Cord Blood VST</td>
<td>CBT: 5/6</td>
<td>6</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>CBT: 6/6</td>
<td>4</td>
<td>None</td>
</tr>
</tbody>
</table>

Melenhorst et al, Blood 2010
Donor-derived Multivirus specific T cells After HSCT
Improve Outcomes without Toxicity

Overall response rate = 93% without pharmacotherapy
(100% for EBV)

Diagnosis
of EBV Lymphoma

2 months after
T-cells – CR

Leen, Myers et al- Nature Medicine. 2006
Hanley et al – Science Translational Medicine. 2015
Summary: CB and PB Multivirus-specific T cells are Protective and Efficacious in vivo

• We can now expand multi virus (including EBV)-specific T cells from TWO donor sources: cord blood and peripheral blood

• Safe to infuse to patients (minimal toxicity)

• Persistence of virus-specific T cells in presence of antigen

• Regardless of source of virus-specific T cells (naïve/memory), both populations appear protective
Targeting EBV – Beyond PTLD
Rationale of Immunotherapy for Lymphoma ....Beyond PTLD


• Significant failure rate of therapy for advanced stage or recurrent disease

• Long-term side effects of chemotherapy and radiation

• EBV antigens expressed by 20-40% of lymphomas are potential targets for T cell immunotherapy
Types of EBV Latency

**Type 3**
- EBV lymphoma post transplant
- Lymphoblastoid cell lines

**Type 2**
- Hodgkin’s Lymphoma
- Nasopharyngeal carcinoma

**Type 1**
- Burkitt’s lymphoma
EBV Specific Cytotoxic T Lymphocytes (CTL) Control EBV Infection *In Vivo*

EBV-infected B cells

PBMC

CTL

Inhibitory Factors

EBV+ Lymphoma Tumor Cell

- **Lytic**
- **EBNA 3a, b, c**
- **LMP2**
- **LP**
- **LMP1**
- **EBNA 2**
- **EBNA 1**
EBV specific T cell Generation

Step 1: LCL generation
4-6 weeks

EBV-infected B cells (LCL)

Step 2: T-cell expansion
4-7 weeks

IL-2

Step 3: QA/QC
Sterility
HLA type
Phenotype
Cytotoxicity

EBV-specific T cells

EBV-infected B cells

PBMC

EBV

PBMC

IL-2

LMP2-CTL
Administration of EBV Specific T-cells to Patients with EBV+ve Hodgkin Lymphoma

- Gene Marked T-cells persisted for 12 months max
- EBV-T cells showed small populations of T cells reactive against LMP2
- Some expansion of LMP2-specific T cells in PB post infusion.
- Anti-tumor effects seen (20% CR/PR)

Marked EBV-T cells by *in situ* PCR at tumor site

*References:

Bollard et al, J Exp Med 2004
Straathof et al, J Immunol 2005*
LMP1 and LMP2A-specific T cells For Hodgkin and non-Hodgkin Lymphoma

- LMP1 and LMP2A are potential T cell targets

Hodgkin R-S Cell/NHL Cell
Making LMP1 and LMP2 Immunodominant Antigens

adherent PBMC

rAd5f35dLMP1-I-LMP2
or Ad5f35LMP2

EBV-infected B cells

GMCSF IL4
IL1b IL6
TNFa PGE2

IL15 IL2 IL2

LMP-specific T cells

Bollard et al, JIT 2004
Straathof et al, J Immunol 2005
LMP1 & LMP2 Specific Activity in LMP-specific T cells from Patients with EBV+ Lymphoma

LMP2-specific T cells

LMP1/2-specific T cells

SFC per 10^5 cells

LMP1 pepmix

LMP2 pepmix

LMP1 pepmix

LMP2 pepmix
Eligibility

• Any age
• EBV+ type III or type II latency lymphoma (EBER and/or LMP1 pos)
• HIV negative
• Either with relapsed disease OR high risk for relapse (e.g. multiply-relapsed patient post chemotherapy or autologous BMT)
• 25 HL and 25 NHL
• Age range 8-79 years
• Autologous LMP-T-cell product
• Dose escalation: $4 \times 10^7$/m$^2$ to $3 \times 10^8$/m$^2$
• Patient received 2 doses (given 2 weeks apart). If stable disease or PR then could receive an additional 6 doses
Clinical Responses Post LMP-specific T cells

- No toxicity
- 11 CR
- 2 PR
- 8 progressive disease (2-8 weeks)
- Median clinical response: 1.5 years (range: >6 to >40 months)

Bollard et al, JCO 2014
Clinical Responses Post LMP T cells in Patients with Active Disease

50% Disease Free Survival at 2 Years (n=21)

Year
Proportion disease-free
0 1 2 3
0
0.2
0.4
0.6
0.8
1
Alascer
ALCI
P=0.882

LMP2 T cell protocol
LMP1/2 T cell protocol

P=0.882

Bollard et al, JCO 2014
Immune Reconstitution of LMP1 & LMP2-T cells in Patients Treated with Disease

**RESPONDERS**

- **LMP1 T Cells**

**NON RESPONDERS**

- **LMP1 T Cells**

---

- **LMP2 T Cells**

---

- **LMP2 T Cells**
Evidence of Epitope Spreading in Responding Patients Treated with LMP1/2 T cells
Clinical Responses Post LMP-specific T cells as Adjuvant Therapy

- No toxicity
- 14 patients post BMT
- 15 post chemo alone
- 1 died of cardiac disease (at <8 weeks)
- 27 remain in remission
  - 1 relapsed 8 weeks post T cells
  - CR median of 2.5 years

Patients high risk for relapse at infusion (n=29)

Bollard et al, JCO 2014
Progression-Free Survival Probability in LMP2-T cell vs LMP1/2-T cell Groups

Patients who received T cells as adjuvant therapy

Bollard et al, JCO 2014

Proportion disease-free

LMP2-specific T cells

LMP1/2-specific T cells

Year

P=0.366
Cause of Death Specific Probability: All Subjects

Patients who received LMP-T cells as treatment

Patients who received LMP-T cells as adjuvant therapy

Cumulative Incidence Probability

Year

Cumulative Incidence Probability

Year

Lymphoma

Other

0

0.2

0.4

0.6

0.8

1
Deaths from Other Causes

• In adjuvant group, 8/29 patients died
  • 1 relapsed, died in CR after allo SCT
  • 3 second cancers (2 MDS, 1 sarcoma)
  • 3 infection
  • 1 cardiac disease

Confirms need for targeted therapies
Conclusions – LMP1/2 Data

• No toxicity
• Accumulation of LMP-specific T cells at disease sites
• Anti-tumor effects seen (13/21 patients PR/CR)
• Next....how to broaden applicability
How Do We Extend Applicability?

• Use bank of allogeneic partially matched CTLs

• Simplify production patient specific product
LMP-CTL – Moving Beyond Single Center Studies

• Pediatric Lymphoma Cell Therapy Consortium (Funded by St Baldricks Foundation, 7 Centers)
  ➔ Donor-derived LMP-CTL post allo SCT for HL
  ➔ Third party LMP-CTL for EBV+ lymphomas
• Multicenter study through Children’s Oncology Group for PTLD post SOT (ANHL1551)
• Industry support (Cellmedica) NK/T cell NHL
Proof of Principle Studies

• Learn from the bedside back to the bench
• With an optimal approach the goals are:
  - Broaden applicability beyond a few centers
  - Multicenter studies are planned
T-cell Therapies for EBV+ Lymphomas

CAGT Laboratory
A Leen, U Gerdeman
B Savoldo, G Dotti
S Gottschalk, Carlos Ramos
A Gee, B Grilley
Malcolm Brenner
Cliona Rooney
Helen Heslop

MDACC
EJ Shpall
Nina Shah
Katy Rezvani
NIH
John Barrett
Jos Melenhorst
Barts, London
John Gribben

Cath Bollard’s Lab (CAGT-BCM ➔ CETI-CNMC)
Cath Bollard, Stephanie Ku, Russell Cruz, Sharon Lam, Gerrit Weber, Paul CastilloCaro, Yasmin Hazrat, An Lu, JW Blaney, Francesco Saglio, D Jacobsohn, K Williams, A Abraham, Cecilia Baresce, Kaylor Wright, Fahmida Hoq, M Luo, Mike Keller, Renuka Miller, Maria Manso-Martin