Evaluation of T Cell Products – LMP-specific T cell therapies for Lymphoma

CM Bollard

The Long Winding Road to Successful T cell Therapy

- Tumor specific T cells in the clinic (CAR modified)
- Priming naïve T cells in vitro
- Low volume GMP grade culture systems
- Improved APC generation
- Incorporating CD4 and CD8 responder
- Proof of principle Virus specific T cells
- Leukemia-specific T cells
- Multi-virus specific T cells in the clinic
- Improved growth conditions – rapid manufacture
- Target antigen identification

2000

1995
Bench to Bedside

Can CTLs be used to treat "real" malignancies that occur in normal individuals?

Rationale of Immunotherapy for EBV-positive Lymphoma

- Significant failure rate of therapy for advanced stage or recurrent disease
- Long-term side effects of chemotherapy and radiation
- EBV antigens expressed by up to 40% of lymphomas are potential targets for T cell immunotherapy
Types of EBV Latency

Type 3
EBV lymphoma post transplant
Lymphoblastoid cell lines (LCL)

Type 2
Hodgkin’s disease
Nasopharyngeal carcinoma

Type 1
Burkitt’s lymphoma

EBV Specific Cytotoxic T Lymphocytes (CTL)
Control EBV Infection in vivo

EBV Infected B cells

PBMC

EBV +ve Lymphoma Cell

Inhibitory factors
EBV specific T cell Generation

**Step 1: LCL generation**
- EBV-infected B cells (LCL)
- 4-6 weeks

**Step 2: CTL expansion**
- EBV-infected B cells (LCL)
- IL-2
- PBMC
- 4-7 weeks

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

EBV Specific CTL in EBV+ve Hodgkin Lymphoma

- Gene Marked T-cells persisted for 12 months max
- EBV-CTL lines 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 CTL by *in situ* PCR at tumor site

Bollard et al, J Exp Med 2004
Straathof et al, J Immunol 2005
**Bench to Bedside**

How can we improve and expand tumor specific T cells?

- EBV+ Lymphomas post BMT
- EBV+ Hodgkin’s disease

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**LMP1 and LMP2A-specific CTL For Hodgkin and non-Hodgkin Lymphoma**

- LMP1 and LMP2A are potential CTL targets

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

Adherent PBMC
GM-CSF
IL-4
IL-1b
IL-6
TNF-a
PGE-2

PBMC

More recently Substituting pepmixes for ad vectors

LMP-specific Cytotoxic T Lymphocytes (CTL)

Bollard et al, JIT 2004, Straathof et al, JI 2005

Phenotype of Autologous LMP-CTL Lines Expanded from Lymphoma Patients

- 45%+/−15% CD45RA- 62L-
- 34%+/− 5% CD45RA- 62L+

% 0 20 40 60 80 100 120

CD19 CD3 TCRαβ TCRγδ CD4 CD8 CD3+ CD56+ CD56+CD3neg CD16+ CD3neg
LMP1 & LMP2–Specific Activity in LMP-CTL from a Patient with Hodgkin Lymphoma

![Graph showing specific activity and lysis percentages for different LMP targets and LMP-specific tetramers.]

LMP2-specific Cytotoxic T Lymphocytes (CTL) are CD4+ and CD8+

![Bar graph showing specific cell lysis and stimulation with different targets and LMP-specific tetramers.]

CD8+ T cells
CD4+ T cells
Broad LMP2-specific Activity Present in CTL line

Overlapping peptide pool number

HLA type: A2;29/B7;15(62)
A2 = FLYALALLL (4+5+20)
A2 CLG
A29 = ILLARLFY (4+20)

Broad LMP1-specific Activity Present in CTL line

Overlapping peptide pool number

HLA type: A2;29/B7;15(62)
Autologous LMP2-CTL product (n=17)
OR LMP1 and LMP2 CTL product (n=33)

- Dose escalation
  - Level 1: 4x10^7/m^2,
  - Level 2: 1.2x10^8/m^2
  - Level 3: 3x10^8/m^2

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>LMP2 CTLs</th>
<th>LMP1/2 CTLs</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hodgkin’s Lymphoma</td>
<td>9</td>
<td>14</td>
<td>22</td>
</tr>
<tr>
<td>NK/T lymphoma</td>
<td>3</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Diffuse large B cell lymphoma</td>
<td>3</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>PTLD (lung, kidney, liver)</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>32</td>
<td>50</td>
</tr>
</tbody>
</table>

**Age range 8-79 years**
Immune Reconstitution of LMP1 and LMP2-specific T cells in Patients Treated with LMP1/2-CTL

No difference between DL1 and DL3 patients

LMP2-tetramer Positive CTL Detected in LN

Supraclavicular Lymph Node

Peripheral Blood
Rise in LMP-specific T cells Post Infusion and Accumulation in Lymph Node

Clinical Responses post LMP-CTL
**Clinical Responses post LMP-CTL**

**Relapsed Disease Arm (n=21)**

- **No toxicity**
- **11 CR (1 also given Rituximab)** (includes 1PR→CR)
- **2 very good partial responses** (up to 36 mths)
- **8 progressive disease (2-8 wks)**

Median clinical response: 1.5y
(range: >6 to >40 mths)

**Patients with disease at CTL infusion**

- **n = 21**

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**Clinical Responses post LMP-CTL in Patients with Active Disease**

**60% Disease Free Survival at 2 years**

- **n = 21**

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**Graphical representations showing clinical responses and survival rates**
Clinical Responses post LMP-CTL- As Adjuvant Therapy

Adjuvant Therapy
n=29
- No toxicity
- 14 patients post BMT
- 15 post chemo alone
- 1 died of cardiac disease (at <8wks)
- 27 remain in remission
  - 1 relapsed 8 wks post CT
  - CR median of 2.5 years

Patients high risk for relapse at CTL infusion

Relapse

Remain in remission

Progression Free Survival Probability in LMP2-CTL vs LMP1/2-CTL groups

Patients who received CTL as Adjuvant Therapy

P=0.741
Patients who received T cells as Treatment

Patients who received T cells as Adjuvant Therapy

Cause of death specific probability: All subjects

Deaths from Other Causes

In adjuvant Group 8/26 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 – especially when used as adjuvant therapy
- Accumulation of LMP-CTL at disease sites
- Anti-tumor effects seen (13/21 patients PR/CR)
- And what about the patients who relapse??

Immune Evasion Strategies in Hodgkin’s Lymphoma - Do CTL have a chance?
Bench to Bedside

Can Tumor-specific CTL Be Genetically Modified to Become Resistant to Inhibitory Effects of Lymphoma Cells?

TGFβ Effects on CTL

- Inhibits CTL proliferation
- Inhibits cytotoxicity
  - ↓ perforin
- Inhibits cytokine production
  - ↓ IFN γ
Creating a Mutant TGFβ Receptor II

Wild type Receptor → Truncated TGFβ Receptor II (DNR) → Dominant Negative Receptor

Transmembrane domain

Stop codon 597

Retroviral vector SFG

SFG:DNR

MoMLV

U3 R U5

NcoI/BamHI

SD PBSQ SA

DNR

Bollard et al, Blood 2002

Rendering LMP-specific T cells Resistant to TGFβ

Ad5f35 LMP1-I-LMP2

EBV-LCL

DC

PBMC

IL-2

DNR-transduced LMP CTLs

CD4 and CD8 T cells are DNR-transduced

DNR-transduced CD4 and CD8 T cells are Predominantly Effector Memory

n=6 CTL lines
DNR-Transduced CTL are LMP-specific

![Graph showing IFNγ release SFC per 1x10^6 cells](chart1.png)

DNR-Transduced LMP-CTL are Functional *in vitro*

![Graph showing CTL proliferation](chart2.png)
Patients Studied

- 5 females and 1 male
- EBV+ HL
  - 5 – relapsed post autologous SCT
  - 1 – relapsed post allogeneic SCT
- Two previously treated with LMP-CTL alone
- All refused additional chemotherapy

DNR-transduced T-cells Persist in vivo for 5-23 months
DNR-transduced T-cells Persist \textit{in vivo} for 5-23 months

Patient 4

Pre-infusion

Patient 5 – Partial Response

Pre

Post
**Patient 2 – Complete Response**

*PRE CR 8 wk*

Response durable for over 34mths

![Graph showing copy numbers in 10^3 ng DNA](image)

**Patient 1 – Mixed Response to CR**

*Patient 1 – Mixed Response to CR*

![Graph showing copy numbers in 10^3 ng DNA](image)

**Residual Tumor Cells**

LMP2neg Post CTL

**Tumor Cells**

LMP2pos PreCTL

EBV T-cells

DNR-trans T-cells

Anti-CD25 RIT

Mixed Response

CR
Patient 3 – Partial Response

LMP-specific CTL Accumulate in Tumor Sites

CD3 T-cells infiltrating tumor sites post CTL

Residual LMP2+ Tumor Cells Present post CTL

Tumor infiltrating T-cells are LMP-specific

SFC per 2x10^5 cells

Infused CTL line

Tumor infiltrating CTL

LMP1 peptide pools

Residual LMP2+ Tumor Cells Present post CTL
### Clinical Outcomes after DNR-transduced LMP-CTL

<table>
<thead>
<tr>
<th>Pt ID</th>
<th>Age</th>
<th>Dose</th>
<th># Doses</th>
<th>Response post CTL</th>
<th>Duration of Response</th>
<th>CTL Persistence</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>27</td>
<td>$2 \times 10^7/m^2$</td>
<td>2</td>
<td>Mixed response → CR</td>
<td>14 months</td>
<td>12 months</td>
</tr>
<tr>
<td>2</td>
<td>47</td>
<td>$2 \times 10^7/m^2$</td>
<td>2</td>
<td>Complete response</td>
<td>34 months+</td>
<td>2 months</td>
</tr>
<tr>
<td>3</td>
<td>43</td>
<td>$6 \times 10^7/m^2$</td>
<td>8</td>
<td>Partial response</td>
<td>18 months</td>
<td>23 months</td>
</tr>
<tr>
<td>4</td>
<td>47</td>
<td>$6 \times 10^7/m^2$</td>
<td>6</td>
<td>Stable Disease</td>
<td>12 months</td>
<td>18 months</td>
</tr>
<tr>
<td>5</td>
<td>32</td>
<td>$2 \times 10^7/m^2$</td>
<td>6</td>
<td>Very good PR</td>
<td>10 months +</td>
<td>10 months+</td>
</tr>
<tr>
<td>6</td>
<td>28</td>
<td>$6 \times 10^7/m^2$</td>
<td>4</td>
<td>Stable Disease</td>
<td>7 months</td>
<td>5 months</td>
</tr>
</tbody>
</table>

### Conclusions

- No dose limiting toxicity
- TGFβ-resistant LMP-CTL may beneficial in EBV+ Lymphoma
- DNR-trans LMP-CTL persist up to 12 months
- Enrolment continues...
Conclusions-LMP1/2 data

- Evaluation of T cell products important to perform detailed immune reconstitution analysis and persistence
- Accumulation of LMP-specific T cells at disease sites even when peripheral blood persistence low
- No toxicity – especially when used as adjuvant therapy
- Anti-tumor effects seen (PFS 60% at 2 years)

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