T-cell Therapies for Hematologic Malignancies: Utilizing A Non-Gene Transfer Approach

Catherine Bollard
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 1
Burkitt’s lymphoma
Type 2
Hodgkin’s disease
Nasopharyngeal carcinoma
Type 3
EBV lymphoma post transplant
Lymphoblastoid cell lines (LCL)

EBNA-3a
EBNA-3b
EBNA-3c
EBNA-1
EBNA-2
LMP 1
LMP 2
LP

EBNA 1
LMP 1
LMP 2
EBNA-1

EBNA-3a
EBNA-3b
EBNA-3c
EBNA-1
EBNA-2
LMP 1
LMP 2
LP
EBV Specific Cytotoxic T Lymphocytes (CTL) Control EBV Infection *in vivo*

- **EBV Infected B cells**
- **EBV +ve Lymphoma Cell**
- **Inhibitory factors**
- **CTL**
- **PBMC**
- **LMP1**
- **LMP2A**
- **EBNA 1**
- **EBNA 2**
- **EBNA 3a, b, c**
- **LMP 1**
- **LMP 2**
- **LMP 2**
- **LP**
- **EBNA 3a, b, c**
- **Lytic**
**EBV specific T cell Generation**

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

**Step 2:** CTL expansion
- 4-7 weeks

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

- EBV-infected B cells (LCL)

- LMP2-CTL

- EBV-CTL
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 \textit{in situ} PCR at tumor site

\textit{Bollard et al, J Exp Med 2004}
\textit{Straathof et al, J Immunol 2005}
LMP1 and LMP2A are potential CTL targets 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

rAd5F35

LCL

rAd5F35

LCL

rAd5F35

LCL

IL-15
IL-2
IL-2

PBMC

mDC

More recently Substituting ad vector for pepmixes

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

CD19  CD3  TCRαβ  CD4  CD8  CD56+ CD3+  CD56+ CD3neg  CD4+ RAneg  CD4+ RAneg  CD8+ RAneg  CD8+ RAneg  CD62L+ CD62Lneg  CD62L+
LMP1 & LMP2–Specific Activity in LMP-CTL lines from Patients with EBV+ Lymphoma

- LMP2 CTL line
- LMP1/2 CTL

SFC per 10^5 cells

- LMP1 pepmix
- LMP2 pepmix
LMP1 & LMP2–Specific Activity in LMP-CTL lines from Patients with EBV+ Lymphoma

LMP2-specific T-cell lines

- Mean
- Median

% Cytotoxicity at 20:1 E:T ratio

LMP1/2 specific T-cell lines

- Mean
- Median

% Cytotoxicity at 20:1 E:T ratio

LCLs, PHA blasts +LMP2 pepmix, PHA blasts alone

LCLs, PHA blasts +LMP1 pepmix, PHA blasts +LMP2, PHA blasts alone
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)
Autoolgous 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

Patients received 2 doses (given 2 weeks apart). If stable disease or PR then could receive an additional 6 doses.
## Types of Lymphoma Treated on LMP-CTL Studies

<table>
<thead>
<tr>
<th>Patients Treated as Adjuvant therapy</th>
<th>HL (n)</th>
<th>NHL/Other (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMP2-CTL protocol</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>LMP1/2-CTL protocol</td>
<td>12</td>
<td>8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patients Treated with Active Disease</th>
<th>HL (n)</th>
<th>NHL/Other (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMP2-CTL protocol</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>LMP1/2-CTL protocol</td>
<td>4</td>
<td>9</td>
</tr>
</tbody>
</table>

NHL/Other = NK/Tcell NHL, DLBCL, PTLD, CAEBV, LYG

Age range 8-79 years
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)

Bollard et al, JCO 2013 in press
Clinical Responses post LMP-CTL in Patients with Active Disease

50% Disease Free Survival at 2 years

Proportion disease-free

Year

LMP2-CTL study
LMP1/2-CTL study

P = 0.882

CR
NR
PR

n = 21
Responder vs. Non-responder LMP 2
Responder vs. Non-responder
LMP 1
Immune Reconstitution of LMP1 and LMP2-specific T cells in Patients Treated with Disease

**Responders**

LMP1-T-cells

LMP2-T-cells

**Non-Responders**

LMP1-T-cells

LMP2-T-cells
Evidence of Epitope spreading in Responding Patients Treated with LMP1/2-CTL

**Responde**rs

- **MAGE A4**
- **Survivin**
- **PRAME**

**Non-responders**

- **MAGEA4**
- **Survivin**
- **PRAME**
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 CTL
  - CR median of 2.5 years

Patients high risk for relapse at CTL infusion

Bollard et al, JCO 2013
Progression Free Survival Probability in LMP2-CTL vs LMP1/2-CTL groups

Patients who received CTL as Adjuvant Therapy

LMP2-CTL study

LMP1/2-CTL study

P=0.366
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 – especially when used as adjuvant therapy
- Accumulation of LMP-CTL at disease sites
- Anti-tumor effects seen (13/21 patients PR/CR)
- Now moving to multicenter study with industry support (Cellmedica) for NK/T cell lymphoma
- Developing third party LMP-CTL study for patients with PTLD post SOT (Children’s Oncology Group)
Conclusions-LMP1/2 data

• But....what about the patients who relapse??
Immune Evasion Strategies in Hodgkin’s Lymphoma - Do CTL have a chance?

Reed-Sternberg cell

- LMP-1
- LMP-2
- EBNA-1
- BARF0

APC

- IL-10

CD8+CTL

- TARC
- TGF-β

CD4+TH2 Cell

- IL-13

CD4+CD25+ Treg Cell

- IL-4
Creating a Mutant TGF\(\beta\) Receptor II

Wild type Receptor

Truncated TGF\(\beta\) Receptor II Dominant Negative Receptor (DNR)

Transmembrane domain

Stop codon 597

Retroviral vector SFG

SFG:DNR

MoMLV

U3 R U5

\(\Psi^+\)

SD PBSQ

NcoI/BamHI

MoMLV

U3 R U5

SA

TM domain

DNR

Bollard et al, Blood 2002
Rendering LMP-specific T cells Resistant to TGFβ

PBMC → DC → EBV-LCL → Ad5f35 LMP1-I-LMP2 → IL-2 → SFG:DNR

DNR-transduced LMP CTLs

DNR-Transduced LMP-CTL are Functional\textit{ in vitro}

The diagram shows a comparison of CTL proliferation in non-transduced (Non trans CTL) and transduced (Transduced CTL) cells, with or without TGF\(\beta\) treatment. The bars indicate higher proliferation in transduced CTL compared to non-transduced CTL, with a further increase when TGF\(\beta\) is added. The y-axis represents CTL proliferation, while the x-axis categorizes the samples as Non trans CTL and Transduced CTL with and without TGF\(\beta\) treatment.
Patients Studied

- 5 females and 3 males
- EBV+ HL
  - 7 – 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 up to 3 years

Patient 1

Patient 3
<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>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27</td>
<td>$2 \times 10^7/m^2$</td>
<td>2</td>
<td>Mixed response $\rightarrow$ 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>36 months+</td>
<td>36 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 $\rightarrow$ CR</td>
<td>24 months +</td>
<td>24 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>
<tr>
<td>7</td>
<td>33</td>
<td>$2 \times 10^7/m^2$</td>
<td>2</td>
<td>Continued CR</td>
<td>6 months+</td>
<td>6 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 3 years+
- Now expanding to Melanoma, lung cancer, Brain tumor, Neuroblastoma setting
Generating T cells for Patients with Lymphoma or Leukemia without Gene Transfer

1st stimulation

PBMC

WT1
PR3
PRAME
MAGE
HNE
NY-ESO
SSX
Survivin

DC

+ IL-7, IL-12, IL-15, IL-6 or IL-27

maintenance and re-stimulation with IL-15 or IL-2

2nd stimulation

DC

+ IL-7

Multi leukemia or lymphoma Antigen-specific CTL

LAAmix *

Weber et al, Leukemia 2013
Weber et al, CCR 2013
Multi TAA-specific T cells block tumor proliferation and survival

Partially HLA matched CTL generated with TAA mix eliminate AML blasts in culture

Weber et al, Leukemia 2013
Gerdemann et al, Mol Ther 2012
Patient TAA-specific T-cells are functional against autologous tumor

Weber et al, Clinical Cancer Research 2013
Demethylation Increases TAA Expression

T-cell Lymphoblastic Lymphoma
Burkitt’s lymphoma
NS Hodgkin’s lymphoma

MAGEA4
NY-ESO
SSX2
Survivin
GAPDH

Shafer et al, Leukemia Lymphoma 2010

- + - + - +
Plus/minus 1uM Decitabine

Demethylating agents increase MAGE expression in primary lymphoma tissue
Can TAA-specific T cells Kill Decitabine Treated HL Targets in vitro?

**A**

- INF-γ ELIspot (SFU)
  - No CTLs
  - MAGEA4
  - Aspf16

**B**

- Viability (%)
  - No CTLs
  - HLA-A2+ CTL (matched)
  - HLA-A2- CTL (mismatched)

**C**

- HDLM2 alone
  - No drug = 25%
  - Decitabine = 28%

- HDLM2 plus MAGEA4-CTL
  - No drug = 25%
  - Decitabine = 74%

**Cruz et al, Clin Cancer Research 2012**
Clinical Response post epigenetic therapy (Vidaza/HDAC)
Tumor was MAGE +

MAGE-specific CTL reactivity

Cruz et al, Clin Cancer Research 2012
Breadth of MAGE-specific T cell response increased after chemo

Cruz et al,
Clin Cancer Research 2012
Clinical Use of MultiTAA-CTL

DC Generation
7 days

Initiation
16 days

Expansion
23 days

Product for Infusion: PRAME/SSX2/MAGEA4/NYESO1/Survivin-specific CTLs

Stimulators
Pepmix-pulsed DCs

Ann Leen
George Carrum
Helen Heslop

PRAME, SSX2, MAGEA4, NY-ESO-1, Survivin

IL7, 12, 15, 6

+IL2/15
+IL7
Can we combine TAA-specific T cell Therapy with Epigenetic Therapy?

- Plan to treat leukemia or lymphoma with epigenetic modifiers→ then give TAA-CTL
Potential for using TAA-specific T cells

Target remission with tumor-specific T cells

Improving outcomes post HSCT/CBT

Opportunity to offer GVL type immunotherapy and avoid transplant or bridge to transplant

A safer alternative for patients under 2 years or over 60 years - avoid TBI with HSCT?
Proof of Principle Studies

- With an optimal approach the goals are:
  - Evaluate safety and feasibility
  - Broaden applicability beyond a few centers
  - **Improve outcomes after BMT**
  - Ultimate goal is to perform studies with COMBINATION therapies… → T cell therapy, vaccines, antibodies, small molecules, chemotherapy, transplant etc….? Avoid RT
Cell therapy beyond HSCT for malignancy – the Vision

Surgery, Chemotherapy
Small molecules
vaccines

DC vaccines
T cells
NK cells
Gene modified T cells
Tumor seeking MSC

Disease burden
Minimal residual disease

cure

Sources: autologous / allogeneic

Lymphoma
Myeloma
Acute leukemia
Chronic leukemia

Melanoma
Breast
Prostate
Colon
Brain tumors
Lung cancer
T-cell Therapies for Heme Malignancies

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