Using Gene Transfer to Retarget Cytotoxic T lymphocytes

Malcolm Brenner
Epstein Barr Virus

• Infects >90% population
• Acute infection is followed by life-long latency
• Expression of limited array of viral latency proteins
• Usually benign
Epstein Barr Virus

- Infects >90% population
- Acute infection is followed by life-long latency
- Expression of limited array of viral latency proteins
- Usually benign
- Latent virus can produce malignant transformation in B/T lymphocytes and epithelial cells
EBV-associated Malignancies

**Latency/Malignancy**

**Type 3**
- Post transplant lymphoma
- HIV-associated lymphoma

**Type 2**
- Hodgkin’s lymphoma
- NHL
- Nasopharyngeal carcinoma

**Type 1**
- Burkitt’s lymphoma
- Gastric adenocarcinoma

**Gene Expression**

- EBNA1
- EBNA1LP
- LMP2
- LMP1
- EBNAs 2, 3a, 3b, 3c
- LP

**Immunogenicity**
Types of EBV Latency

Type 3 Latency
Post Transplant Lymphoma
5-25% of T cell depleted SCT recipients
Generation of EBV Specific Cytotoxic T lymphocytes (CTLs)

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

Step 2: CTL expansion
- 4-7 weeks
- IL-2
- PBMC

Step 3: QA/QC
- Sterility
- HLA type
- Phenotype
- Cytotoxicity
Successful T Cell Therapy of Cancer

*Minimal Requirements*

Effector Cells need to be

- Plentiful (Proliferate)
- Persistent
- Present in tumor
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Gene Mark neo-MVV
PCR for Neo shows CTL become plentiful

<table>
<thead>
<tr>
<th>UPN</th>
<th>Pre</th>
<th>Post</th>
<th>Control</th>
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<tr>
<td>293</td>
<td></td>
<td></td>
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<tr>
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<td>239</td>
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<tr>
<td>230</td>
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<td>10%</td>
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</table>
Marking Detection – CTLs Persist

Marking detection for each patient over time
Donor-derived CTLs *Present at tumor site*

Marked CTL by in situ PCR at tumor site
CTLs for EBV PTLD

Improving CTL Therapy – Attack Targets that are Present

Type 2
Hodgkin's disease
Nasopharyngeal carcinoma

EBNA-1
LMP 1
LMP 2
Increasing LMP2 tetramer-positive cells using Ad-LMP2 vector

LMP2 tetramers
FLY
LLW
EBNA3C tetramer
RRI

LCL
CTL
Recombinant Ad5f35 with LMP2
Chimeric Ad5F35 LMP2
Over-expression and innate immune response make a weak antigen strong

Bollard et al, J Immunother 2004
Increasing LMP2 tetramer-positive cells using Ad-LMP2 vector

### LMP2 tetramers
- **FLY**
  - LMP2 CTL: 5.93%
  - LCL CTL: 0.01%

- **LLW**
  - LMP2 CTL: 2.38%
  - LCL CTL: 0.02%

### EBNA3C tetramer
- **RRI**
  - LMP2 CTL: 0.11%
  - LCL CTL: 12%
Resolution of Bony Lesions In HD

Pre CTL

3mth Post CTL
Complete Radiological Response
EBV+ve NK-T NHL

Pre CTL

Post CTL

EBV DNA

EBV T cells

Graph showing SFC per 10^5 cells and EBV copies/ug DNA over time (pre, 1wk, 2wks, wk6).
Immunohistochemistry
Left Carytenoid

Pre CTL

Post CTL

EBER 10x

CD4 40x
CTL Studies targeting EBV antigens in EBV+ve lymphoma

42 Patients with Active Rel. Disease

- CR 17 (41%)
- PR 6 (15%)
- SD 7 (17%)
- NR 12 (28%)
NPC Clinical Response post EBV-CTL: Reduction of FDG uptake in metastases
Complete Remission of Refractory NPC

Pre-CTL

Post-CTL

Absent uptake of F-18 fluorodeoxyglucose (FDG) 8 weeks post CD45 MAbs and EBV-CTL infusion
Complete Remission of Refractory NPC

Pre CTL: EBV pos  Post CTL: EBV neg
Conclusions

- Anti-tumor activity seen in 12/24 patients with active NPC treated with EBV-CTL

No Response

Complete Response (6)

Partial Response (2)

Stable Disease (4)
Broadening the Applicability of EBV-CTLs

• Manufacturing is robust (98% success rate in >200 clinical lines)

• “Exportable” concept

  O’Reilly; MSKK         Khanna; QIMR, Brisbane
  Lucas; UAB            Volk; Charite, Berlin
  Wang; HMS             Amrolia: ICH/GOS, London
  Commoli; Pavia        Crawford; Univ. Edinburgh
Broadening the Applicability of EBV-CTLs

• Manufacturing is robust (98% success rate in >200 clinical lines)
• “Exportable Concept”
• Accelerate and simplify production –
  \( \text{Was } >10\text{wks: Now } <10 \text{ days} \)
• Increase range of diseases to be treated
Chimeric Antigen Receptor (CAR) Expression in T cells

Monoclonal Antibody

HRS3-scFv

Linker

Spacer

Tumor Ag

Tumor

T Cell

TcR-complex

αβ

γεε δζζ
Chimeric Primary T cells (CAR-PTC)

- Recognize unmodified tumor antigens in MHC unrestricted manner- bypass many tumor immune evasion strategies
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- Tumor cells have other problems in presenting antigen (e.g. lack co-stimulator molecules, inhibit induction of effector phenotype)
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- Consequence – poor in vivo persistence, expansion and function
Overcoming poor costimulation to CAR-PTC

- Incorporate more co-stimulatory domains

CD28 and OX40 (Pule et al. Mol Therapy 2005)
Chimeric receptor-mediated interaction between T cell and tumor cell

Tumor cell

Complete signal?

ICOS
CD28
CD4, CD8
OX-40R
4-1BB
LFA-1
LFA-2

B7-H3?
B7-2?
B7-1?
LICOS?
OX-40?
4-1BBL?
ICAM-1?
CD58?
CD59?
Using EBV Infected Target Cells as source of co-stimulation

• EBV targets express all relevant co-stimulator molecules and are present lifelong

• EBV-CTL
  – Expand in vivo
  – Have effector phenotype
  – Persist long term
  – Eradicate bulky tumors
EBV-infected B cell

EBV-specific CD4+ T cell

EBV peptides on MHC class II

EBV peptides on MHC class I

EBV-specific CD8 T cell

Tumor Ag specific

Chimeric TCR

Native TCR

EBV-specific
Neuroblastoma

- Commonest extracranial solid tumor of childhood
- May respond to intensive therapies
- High relapse risk in advanced disease
- Neural crest tumor and expresses many developmental antigens
- Lack MHC molecules – problem for CTL
Neuroblastoma Target antigen: GD2

- Disialoganglioside expressed in tumors of neuroectodermal origin
- Expressed at high density on almost all neuroblastoma cells
- Poorly expressed or absent from most normal tissue
- MAb has been used with clinical responses
Killing of Neuroblastoma and Autologous LCL by PTC/CTL

% transduction

GD2 CTL  GD2 PTC

Neuroblasts  Auto EBV-LCL
Are CAR-CTL better than CAR-PTC in neuroblastoma patients?

Transduce patient PTC and CTL with a vector encoding identical receptor but distinct oligonucleotide for each population.
Vectors in Clinical Study

Patient One

LTR  GD2S  GD2  LTR

Primary T cell

Patient Two

LTR  GD2L  GD2  LTR

EBV specific CTL

LTR  GD2S  GD2  LTR
Phase I Dose Escalation Study

- Relapsed/Refractory or incompletely treated NB patients
- Evaluate safety of GD2 redirected T-cells (T-GD2)/EBV CTL (CTL-GD2)
- Compare persistence of CTL-GD2 and T-GD2
- Evaluate clinical outcome
Patient Details

- 11 Patients with relapsed disease
- Age 3yrs - 15 yrs (Median 10yrs)
- 3 Received dose level 1 \((10^7)\)
- 6 received dose level 2 \((5 \times 10^7)\)
- 2 received dose level 3 \((10^8)\)
Clinical Product Transduction Efficiency

EBV-CTLs  PBTs
Phenotype of cell product

EBV-CTLs

CD4

CD8

CD56

PBTs

CD4

CD8

CD56
T-GD2 Cells Kill Neuroblastoma In-Vitro; No Killing Of Autologous LCL

% Cytotoxicity

T-GD2

NB Cell Line

Auto LCL
CTL-GD2 Kill Both Neuroblastoma And Autologous LCL

![Graph showing cytotoxicity of CTL-GD2 and T-GD2 against NB Cell Line and Auto LCL. The graph indicates higher cytotoxicity for CTL-GD2 compared to T-GD2.]
Safety of Infusions

No severe adverse effects attributable to study agent
What should CAR-EBV CTL do?

• Persist longer at higher levels than CAR-Primary T cells (PTC)
Percent Gene Modified EBV CTL or Primary T cells in PBMNC

Mean % infused cells detected

T-GD2

1 Day 1 week 2 weeks 4 weeks 6 weeks
Percent gene modified EBV CTL or Primary T Cells in MNC

Mean % infused cells detected

- CTL-GD2
- T-GD2

Day, 1 week, 2 weeks, 4 weeks, 6 weeks
Successful T Cell Therapy of Cancer

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Increase persistence

- Depletion of lymphocytes enhances homeostatic proliferation of transferred cells
- Autografting is standard of care for high risk Neuroblastoma
- Give modified CTL after autograft
Successful T Cell Therapy of Cancer

Minimal Requirements

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- Plentiful (Proliferate)
- Persistent
- Present in tumor
Increase Persistence

• TGFβ secreted by many tumors including HD and neuroblastoma
• Transduction of Dominant Negative receptor blocks TGFβR trimer formation and downregulation of CTL in vitro/vivo
• Clinical trial of DNR approved and awaiting final vector release
Successful T Cell Therapy of Cancer

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Expression of Chemokine Receptors on EBV-Specific CTL

Chemokine Receptor Expression

Chemokine
- CCL2
- CCL4
- CCL5
- CCL21
- CXCL12

Chemokine Receptor
- CCR2
- CCR4
- CCR5
- CCR7
- CXCR4

CD4
CD8
CCR2b-T Cells Homing

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Day 3</th>
</tr>
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<tbody>
<tr>
<td>NT  CCR2b</td>
<td>NT  CCR2b</td>
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ROI 1=16335
ROI 2=19167
ROI 1=5907.3
ROI 2=20348
Summary
Gene Transfer to retarget CTLs

- Retroviral gene marking confirms EBV-CTL’s effective against post-transplant lymphoma.
- Adviral vectors enhance specificity of CTL for weak tumor antigens – HD and NPC
- CAR gene transfer allows CTL to effectively bear alternative anti-tumor specificities- Solid tumors
- Further engineering should enhance clinical efficacy
Immunotherapy

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