Improving the Efficacy and Safety of G-M Virus-Specific T cells for Solid Tumors

Malcolm Brenner
T lymphocytes for cancer

Specific – and (maybe) better than MAb

- Recognize internal antigens (if processed)
- Good bio-distribution - Traffic through multiple tissue planes
- Multiple effector mechanisms
- Self amplifying
Chimeric Antigen Receptor (CAR) Expression in T cells

Tumor Ag

Monoclonal Antibody

HRS3-scFv

Linker

Spacer

TcR-complex

αβ

γεδζζ
Chimeric Antigen Receptor T cells (CAR-T)

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

- Incorporate more co-stimulatory domains
  - CD28
  - CD28 and OX40
  - CD28 and 4-1BB
Chimeric receptor-mediated interaction between T cell and tumor cell
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 EBV+ HD/NHL, PTLD
EBV CTL to treat and prevent PTLD after Transplant

- Extensive (>3 logs) in vivo expansion
- Long term (>10 years) persistence
- No disease in >120 high risk patients receiving CTL prophylaxis versus 12% of controls
- Complete and sustained resolution of tumor in 11/13 patients with resistant lymphoma
- US Orphan drug designation granted 2007. Approval under discussion
Clinical Responses After LMP-CTL Therapy

Patients with resistant disease

- CR = 9
- PR = 1
- SD = 1
- NR = 2

n = 11
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

Chimeric TCR

Tumor Ag specific

Native TCR

EBV-specific

Cognate help

EBV-specific

CD28

CD28

B7

B7

EBV-specific
EBV-specific CD4+ T cell

EBV-Specific CD8 T cell

TA-specific Chimeric TCR

Native TCR EBV-specific

Cognate help
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
Are CAR-cytotoxic T lymphocytes (CTLs) better than CAR-activated T cells (ATC)?

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

Patient One

Activated T cell

Patient Two

EBV specific CTL
Phenotype of cell product

CAR-CTLs

CD4
CD56

CD8

CAR-ATC

CD4
CD56

CD8
What should CAR-CTL do?

• Persist longer at higher levels than CAR-activated T cells (ATC)
Percent gene modified EBV CTL or ATC in PBMNC

![Graph showing the mean % infused cells detected over time with a peak at Day 1 and a decline to near zero by 6 weeks. The graph includes a legend indicating CAR-ATC.]
What do we want for CAR T cells?
Persistence of ATC versus CTL

9 year old with relapsed neuroblastoma
Remains in CR 18 months post T cell infusions
Clinical Responses

• 5/10 patients with active relapsed/resistant disease had tumor response/regression
• 3 Complete remissions (2 sustained >4yrs, >12 Months)
Increasing Value of CAR-CTLs

• Increase Range of Solid Tumors Treated
  – Her2Neu+
    Medulloblastoma; Glioma; Non-Small Cell Lung Cancer
Case Report of a Serious Adverse Event Following the Administration of T Cells Transduced With a Chimeric Antigen Receptor Recognizing ERBB2

Richard A Morgan, James C Yang, Mio Kitano, Mark E Dudley, Carolyn M Laurencot and Steven A Rosenberg

Molecular Therapy 18, 843-851 (April 2010)
Small molecule/MAb toxicities generally improve with time.

Toxicities from cells persist and worsen.
Acute GVHD skin
CD34 Selected Haploidentical Transplants (BCM 1999-2004)

Survival

Years post transplant

46%
Causes of Failure

- Regimen related mortality  5%
- Relapse                  21%
- Infection               21%

Slow immune recovery due to T cell depletion
Selective Depletion Of Alloreactive Cells

Donor PBMC + Recipient EBV-lymphoblastoid cell line (Irradiated) → Alloreactive cells upregulate CD25 → Murine IgG1 anti-CD25 (RFT5) conjugated to deglycosylated ricin A chain → Allodepleted T-cells
Suicide Gene Therapy to Control Toxicities from Cell Therapy

- Herpes Simplex Thymidine Kinase most tested suicide gene
- Phosphorylates pro-drug (e.g. Ganciclovir) to triphosphonucleoside
- Inhibits DNA polymerase/Host cell DNA synthesis
Herpes Simplex Virus Thymidine Kinase (HSVtk) works, but has disadvantages:

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HSVtk</th>
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<tbody>
<tr>
<td>Source</td>
<td>Foreign (\rightarrow) Immunogenic</td>
</tr>
<tr>
<td>Activating drug</td>
<td>Ganciclovir. Widely used to treat CMV.</td>
</tr>
<tr>
<td>Mechanism</td>
<td>Inhibits DNA synthesis – slow killing even of dividing cells</td>
</tr>
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</table>
Inducible caspase 9

FKBP domain

iCaspase 9

CID (AP1903)

Caspase 3

Activated caspase 3
## Suicide gene

<table>
<thead>
<tr>
<th></th>
<th>HSVtk</th>
<th>iCasp9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>Foreign → Immunogenic</td>
<td>Human derived → less immunogenic</td>
</tr>
<tr>
<td>Activating drug</td>
<td>Ganciclovir. Widely used to treat CMV.</td>
<td>Non therapeutic small molecule</td>
</tr>
<tr>
<td>Mechanism</td>
<td>Dividing Cells (DNA synthesis)</td>
<td>All cells by apoptotic pathway. Rapid killing.</td>
</tr>
</tbody>
</table>
Retroviral vector
SFG.iCasp9.2A.ΔCD19
CASPALLO: Eligibility criteria

- Haploidentical stem cell transplantation
- Hematologic malignancy
- Lymphoproliferative disorders
  - HLH, FLH, VAHS, SCAEBV, XLP
CASPALLO: Study objectives

PRIMARY

• Highest dose of allogeneically depleted donor T cells with grade III-IV acute GvHD rate ≤ 25%
  (Range 10^6 to 5 x 10^7/kg)

• Biological and clinical effects of administration of AP1903
CASPALLO: Protocol overview

- **Allodepletion**
- **ΔCD19 Selection**
- **Haplo SCT**

- **Day 3** iCasp9.ΔCD19 Transduction
- **Day 6**
- **Day 10**
- **30-90** Infusion
Use icasp9 for Additional Cell Types

• Post mitotic
e.g. progeny of mesenchymal stromal cells
Extend Dimerizer to Mesenchymal Stromal Cells

• Site-directed delivery
  – Injury repair
    • Cartilage? (Black et al., Vet Ther 2007)
    • Myocardium? (Chen et al., Chin Med J 2004)
    • Spinal cord? (Moviglia et al., Cytotherapy 2006)

• Systemic delivery
  – Congenital deficiencies
    • Osteogenesis imperfecta (Horwitz et al., Nat Med 1999)
  – Injury repair
    • Stroke? (Bang et al., Ann Neurol 2005)
    • GvHD
MSC differentiation

Expansion medium (alk phos/methylene blue)

Expansion medium (oil red/methylene blue)

Osteodiff medium (alk phos/methylene blue)

Adipodiff medium (oil red/methylene blue)
iCasp9-MSC are multipotent and killed by exposure to CID.
In vivo delivery

• Left flank: MSC only
• Right: MSC/iCasp9
50 µg CID q24h × 2 on day 0/+1
Use icasp9 for Additional Cell Types

• Post mitotic
e.g. progeny of mesenchymal stromal cells
• Prevent neoplasia
  hESC
  iPS
Summary

• EBV-specific cytotoxic T lymphocytes (CTLs) can be modified to express CAR against solid tumors
• CAR-CTLs can survive long term and produce CR in neuroblastoma even in absence of lymphoablation - ?added benefit
• Extending approach beyond neuroblastoma
• Safety may be enhanced by fast acting suicide gene icasp9
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CASPALLO: Fate of residual iCasp9 T cells

QUESTION #4:
• Do residual iCasp9 T cells re-expand without causing GvHD?
Residual iCasp9 T cells re-expand after AP1903 without GvHD (pt 1)
Naïve, CM, EM reconstitution after infusion (pt 1)

Days after T cell infusion

AP1903

cells/µl

EM
EMRA
Naive
CM

0 6 14 17 21 28 61 89 147 209
QUESTION #5:

• Do residual surviving iCasp9 T cells retain response to AP1903 long-term after infusion?
iCasp9 T cells remain sensitive to dimerizer >6 months after infusion

No Drug AP1903

CD3 FITC

CD19 PE

iCas9 copies/10e2 ng DNA

No Drug

AP1903

4.35%

0.15%
QUESTION #6:

• Do iCasp9 T cells contribute to reconstitution of antiviral immunity?
CMV specific response from patient #1
PBMC 6 days pre AP1903 administration

Survivin
0.1%

CMV (pp65/IE1)
1.06 %
CMV specific CD3+CD19+T cells 7 days after AP1903

Survivin

0.67%

CMV (pp65/IE1)

1.98 %
Phenotype of Transduced ATC and CTL

A

B

% of positive cells

0 20 40 60 80 100

CD62L CD27 CD28 CCR7 CD45RA CD45RO

CD62L CD27 CD28 CCR7 CD45RA CD45RO

% of positive cells

0 20 40 60 80 100

CCR4 CCR5 CXCR4 CXCR3 CCR2 CD162 CD54 CD38 CD106 CD11a CD18 CD11c

CCR4 CCR5 CXCR4 CXCR3 CCR2 CD162 CD54 CD38 CD106 CD11a CD18 CD11c

*
<table>
<thead>
<tr>
<th>Pt (dose level)</th>
<th>SCT -last f/u (days)</th>
<th>Disease status at last f/u</th>
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<tbody>
<tr>
<td>1 (1)</td>
<td>219</td>
<td>CR</td>
</tr>
<tr>
<td>2 (1)</td>
<td>167</td>
<td>CR</td>
</tr>
<tr>
<td>3 (2)</td>
<td>170</td>
<td>CR</td>
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### CASPALLO: patients on study

<table>
<thead>
<tr>
<th>Pt (dose level)</th>
<th>Sex/ (Y)</th>
<th>Age (Y)</th>
<th>Dx</th>
<th>Status at SCT</th>
<th>SCT-infusion (days)</th>
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<tbody>
<tr>
<td>1 (1)</td>
<td>M (3)</td>
<td>MDS/ AML</td>
<td>CR2</td>
<td></td>
<td>63</td>
</tr>
<tr>
<td>2 (1)</td>
<td>F (17)</td>
<td>B-ALL</td>
<td>CR2</td>
<td></td>
<td>80/111</td>
</tr>
<tr>
<td>3 (2)</td>
<td>M (8)</td>
<td>T-ALL</td>
<td>CR1 (PIF)</td>
<td></td>
<td>109</td>
</tr>
</tbody>
</table>
Naïve, CM, EM reconstitution after infusion (pt 2)

Days after T cell infusion

cells/µl

API 903
Naïve, CM, EM reconstitution after infusion (pt 3)
CASPALLO: Immune-reconstitution (pt 1)
CASPALLO: Immune-reconstitution (Pt 2)

Days after T cell infusion

Cells/µl

- CD3+CD19 +
- CD4+CD19 +
- CD8+CD19 +
- CD3+CD19 neg

AP1903