Targeting tumors with genetically enhanced T lymphocytes

- Redirect T cell specificity towards selected antigens
  – to overcome self-tolerance and antigen escape

- Enhance antigen receptor-mediated T cell activation
  – to increase T cell expansion and survival

- Increase intra-tumoral T cell activation
  – to augment tumor rejection
Physiological and chimeric antigen receptors

- Extra and intra-cellular antigens
- Adapted to CD3 complex
- Restricted by patient’s HLA spec
- No ligand on HLA-negative tumor
- Strength of signal often weak?
- Heterologous pairing

- Extra-cellular ag only; incl. glycolipids
- No peptide processing required
- Not restricted by patient HLA
- Not limited by HLA down-regulation
- Designer signaling domain
- Immunogenicity?
19z1⁺ and Pz1⁺ T cells specifically lyse CD19⁺ and PSMA⁺ targets, respectively.
QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.
Intravenous administration of Pz1 T cells can eradicate pulmonary RM1.PGLS

Gade et al., Cancer Res, 2005
Monitoring tumor burden by positron emission tomography (PET): eradication of established systemic Raji by i.v. 19z1+ T cells
T cell expansion conditions determine whether 19z1 T cells eradicate established systemic Raji in SCID/bg mice

All survive to day 300

RAJI

NALM/6

Days since tumor injection

NALM/6-B7

Survival

Days since tumor injection

What costimulation?
How to safely and effectively provide it?

- Classic costimulation
- Integrated costimulation
- On-demand costimulation

Figure from Kim et al, *Nat Biotechnol*, 2004
Pz1+ CD8+ PBL proliferation on AAPCs expressing CD80 and/or 4-1BBL
Combined CD80 and 4-1BB signals enhance effector functions

INF-γ

GM-CSF

TNF-α

Granzyme B

Combined CD80 and 4-1BB signals enhance effector functions
What costimulation and how to safely and effectively provide it

- Classic costimulation
- Built-in costimulation
- On-demand costimulation
Construction of CD28/CD3ζ receptor

Four weekly T cell infusions greatly enhance survival (NALM6)
Retroviral vector production

Plasmid DNA

Patient cell expansion - Wave bio

Patient cell transduction

Patient cell selection
A Phase I Trial for the Treatment of Purine Analog-Refractory Chronic Lymphocytic Leukemia using Autologous T cells Genetically Targeted to the B cell Specific Antigen CD19 (IRB 06-138)

- **Enrollment Criteria:**
  Patients with purine analog-refractory CLL disease

- **Protocol Design:**
  - A 2 step design:
    - Step I: Standard dose escalation of modified T cells
    - Step II: MTD from phase I in combination with dose escalating lympho-depleting cyclophosphamide chemotherapy

- **Treatment Protocol Phase 1:**
  - Dose escalation of 19-28z T cells
  - 3 dose levels will be studied (3 x 10⁷ T cells/kg, 1 x 10⁸ T cells/kg, and 3 x 10⁸ T cells/kg)

- **Treatment Protocol Phase 2:**
  - Dose escalation of cyclophosphamide prior to MTD dose of T cells established in phase I.
  - 3 dose levels of cyclophosphamide will be tested (1.5g/m², 2.2g/m², and 3.0g/m²)

- **Anticipated patient enrollment**
  - 24-36 patients
“Third generation” chimeric antigen receptors

Proliferation of PSMA_redirected CD8+ T cells
(3T3-PSMA AAPC with 20 U/ml IL-2)
What costimulation and how to safely and effectively provide it

- Classic costimulation
- Integrated costimulation
- On-demand costimulation
Tumor antigen-specific T lymphocytes may serve themselves as providers of costimulatory ligands if they constitutively express these ligands and thus offset costimulatory deficiency within the tumor microenvironment.

Constitutively expressed costimulatory ligands could in principle directly engage their respective receptors eventually following their induced upregulation (Auto-costimulation).

… as well as deliver bystander costimulation to other tumor infiltrating lymphocytes. (Trans-costimulation)
QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.
Costimulatory ligands on the Tumor cell

Costimulatory ligands on the T cell

Costimulatory receptor(s)
Costimulatory ligand(s)

Pz1

PSMA
Days after initial tumor antigen contact

ON TUMOR

ON T CELL

TNFL

CD8\(^+\) PZ1\(^+\) T cell number

CD8\(^+\) PZ1\(^+\) T-cell number

Days after initial tumor antigen contact

4-1BBL  OX40L  CD70  CD30L  LNCaP only
Supplementary Figure 4
DAY 2

19ζ-CONTROL

PZ1

PZ1-CD80

PZ1-4-1BBL

PZ1-4-1BBL CD80
DAY 10

19ζ-CONTROL

PZ1

PZ1-CD80

PZ1-4-1BBL

PZ1-4-1BBL

CD80
Days after tumor injection

Survival (%)

19Z-control (ms = 47 days)
PZ1 (ms = 59 days)
PZ1-CD80 (ms = 63 days)
PZ1-4-1BBL (ms = 66 days)
PZ1-CD80-4-1BBL

P = 0.0001*
P = 0.077*
P = 0.52
P < 0.0001*

n=10

P = 0.0001*
P = 0.077*
P = 0.52
P < 0.0001*
12 hrs

19Z-4-1BBL CD80-CONTROL

T-CELL IMAGING

PZ1

PZ1-4-1BBL CD80
DAY 6

19Z-4-1BBL
CD80-CONTROL

PZ1

PZ1-4-1BBL
CD80

T-CELL IMAGING
DAY 18

T-CELL IMAGING

19Z-4-1BBL CD80-CONTROL

PZ1

19Z-4-1BBL CD80-CONTROL

PZ1-4-1BBL CD80
Days after T-cell injection

A = median 85.4-fold ↑ (P = 0.0009)
B = median 84.6-fold ↑ (P = 0.0009)
C = median 31.8-fold ↑ (P = 0.0009)
D = median 1.4-fold ↑ (P = 0.0831)
E = median 40.7-fold ↑ (P = 0.0009)
F = median 14.6-fold ↑ (P = 0.0009)
**Do CD80+4-1BBL+ T cells auto-costimulate?**

<table>
<thead>
<tr>
<th>DIC</th>
<th>FITC-CTB</th>
<th>4-1BBL</th>
<th>4-1BB</th>
<th>Overlay</th>
</tr>
</thead>
</table>

- T cell
- Tumor cell
Figure 4

0 hrs                                   6 hrs                12 hrs                                18 hrs     24 hrs                                30 hrs

PZ1-IRES-4-1BBL, 4-1BB shRNA

PZ1-IRES-4-1BBL, control shRNA (β-globin shRNA)

PZ1-IRES-4-1BBL, 4-1BB shRNA

PZ1+ control shRNA

PZ1+ CD80+ dsRed-4-1BBL+ control shRNA

PZ1+ CD80+ dsRed-4-1BBL+ 4-1BB shRNA

DIC          FITC-CTB      4-1BBL     Granzyme B     Overlay

MFI-PE=230

MFI-PE=39

MFI-PE=243
1) SFG-NIT control vector

2) SFG-4-1BB-T2A-dsRED-4-1BBL-IRES-CD80
3.8-fold increase ($p < 0.0001$)
Can CD80+4-1BBL+ trans-costimulate in the tumor microenvironment?

**Tumor targets**
- RM1.PGLS, Gaussia-luc⁺
- (all mice)
- Raji, Gaussia-luc⁺
- (all mice)

**T cells**
- Pz1⁺Click-luc⁺
- 19z1⁺Click-luc⁺
  - (all mice)
- Pz1⁺ T cells
  - or
- Pz1⁺4-1BBL⁺CD80⁺
  - or
- 19z1⁺4-1BBL⁺CD80⁺
DAY 1

T-CELL IMAGING

PZ1 click
+19Z click
+PZ1

PZ1 click
+19Z click
+PZ1-4-1BBL-CD80

PZ1 click
+19Z click
+19Z-4-1BBL-CD80

PZ1 click
+19Z click
+19Z-4-1BBL-CD80
PZ1 click
+19Z click
+PZ1

PZ1 click
+19Z click
+PZ1-4-1BBL-CD80

PZ1 click
+19Z click
+19Z-4-1BBL-CD80

DAY 3

T-CELL IMAGING
DAY 5 tumor

PZ1 click +19Z click +PZ1

PZ1 click +19Z click +PZ1-4-1BBL-CD80

PZ1 click +19Z click +19Z-4-1BBL-CD80
Conclusions

1. Systemic tumors can be eradicated by CAR-directed human T cells without cytokine support or any other concomitant treatment in SCID mice
2. Xenogeneic tumor models are valuable to assess transduced T cell potency
3. In these models, T cell survival is limiting
4. 4-1BB+CD28 are synergistic in enhancing T cell function and survival
5. T cell potency is proportional to the strength of PI3kinase/Akt activation
   • T cell and tumor localizations can be co-registered using BLI
6. T cells expanded under cGMP (i.v. admin) eradicate systemic tumors in mice
7. Have initiated a first trial targeting CD19 in pts with chemorefractory CLL
8. Constitutive expression of costimulatory ligands in T cells sustains antigen-induced T cell survival and function in vitro
9. Auto- and trans-costimulation are promising approaches to enhance T cell function in vivo
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