Driving CD19 CAR T Cells Forward: Nanomanagement Strategies

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http://laplacian.files.wordpress.com/2009/01/t-cell2.jpg
Objectives

- CD19 CAR T cells
  - Promising clinical data – ALL, lymphoma
  - Basic process for manufacturing, infusing
- Areas for optimization
- Nanotechnology-based approaches
  - In vivo and in vitro labeling/imaging
  - Nanoparticle-mediated ablation for enhancing antigen presentation
  - Efficient transfection for manufacturing
Basic Process

A. Collection

B. Transduction with CAR

C. Expansion ex vivo

D. Lymphodepletion followed by administration

CAR T Cell Construct

**Immunosuppressive Players:**

1. Regulatory T Cells (FoxP3+)
2. Myeloid-Derived Suppressor Cells (MDSCs)
The Promise of CD19 CAR T Cells

<table>
<thead>
<tr>
<th>Center</th>
<th>Disease</th>
<th># of pts</th>
<th>% NED/CR at restaging</th>
<th>PFS for pts with CR at restaging</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSKCC</td>
<td>B-ALL</td>
<td>44</td>
<td>36/43 (84%)</td>
<td></td>
</tr>
<tr>
<td>NCI</td>
<td>B-ALL</td>
<td>39</td>
<td>20/39 (51%)</td>
<td>45.5% @ 18 mo</td>
</tr>
<tr>
<td>CHOP/UPenn</td>
<td>B-ALL</td>
<td>53</td>
<td>45/53 (85%)</td>
<td>44% @ 12 mo</td>
</tr>
<tr>
<td>FHCRC</td>
<td>B-ALL</td>
<td>29</td>
<td>24/26 (93%)</td>
<td></td>
</tr>
<tr>
<td>NCI</td>
<td>DLBCL</td>
<td>9</td>
<td>4/7 (57%)</td>
<td>2/7 (29%) = PR</td>
</tr>
<tr>
<td>UPenn</td>
<td>NHL</td>
<td>24</td>
<td></td>
<td>ORR = 68%</td>
</tr>
<tr>
<td></td>
<td>• DLBCL</td>
<td>15</td>
<td></td>
<td>7/15 (47%)</td>
</tr>
<tr>
<td></td>
<td>• MCL</td>
<td>2</td>
<td></td>
<td>1/2 (50%)</td>
</tr>
<tr>
<td></td>
<td>• Follicular</td>
<td>11</td>
<td></td>
<td>8/11 (73%)</td>
</tr>
</tbody>
</table>

Toxicities of Therapy

- **Cytokine release syndrome**
  - Intensity corresponds with expansion kinetics of CAR T cells
  - IL-6 mediated → tx’ed with dexamethasone +/- tocilizumab

- **Neurotoxicity**

- **B cell lymphodepletion**
CAR T Cell Limitations

- Phenotype/Quality of collected T cells

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CAR T Cell Limitations

- Phenotype/Quality of collected T cells

CAR T Cell Limitations

- Dosing – tumor burden vs toxicity
  - Lower doses for higher tumor burden
  - Higher doses for lower tumor burden
- CAR receptor design – spacer length
- Manufacturing – time consuming, expensive
CAR T Cell Limitations

- Immune rejection
  - Rejection of the CAR T cells via CD8+ mediated responses against the murine ScFv → lack of CAR T cell persistence
  - Addition of fludarabine to cyclophosphamide in lymphdepletion regimen (Turtle et al Blood 2015 126:3773)
  - Fully human scFV (Sommermeyer et al Leukemia 2017 e-pub ahead of print)

- Tumor microenvironment
  - Modifying CAR T cells to produce pro-inflammatory cytokines such as IL-12
  - Addition of checkpoint inhibitors

- Relapses
  - CD19- escape
Nanotechnology

http://nano.prochimia.com/
Targeted Ablations to Enhance Antigen Presentation

- Directly inject PEG-coated HAuNS into the primary B16F10 tumors
- Ablate the primary tumor with the NIR laser
- Inject pmel T cells that target gp100 in the B16F10 tumor 24 hours after ablation

Bear et al. (2011) PlosOne
Improved melanoma lung metastasis control

Bear et al. (2011) PlosOne
Imaging Applications

Carpin et al. Breast Cancer Research and Treatment (2011)
Basic Process

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T-cell targeting ligands

DNA encoding leukemia-specific CAR

Nanoparticle binds to T cell

Endocytosis

Endosomal escape

mRNA

Leukemia-specific CARs

T cells capable of recognizing and destroying leukemia cells

Smith et al. (2017) Nature Nanotech
Design and manufacture of lymphocyte-programming nanoparticles

International Patent WO 2014153114 A1

Smith et al. (2017) Nature Nanotech
DNA nanocarriers selectively bind lymphocytes \textit{in vitro}

Smith et al. (2017) Nature Nanotech
Mouse splenocytes were incubated with the nanoparticle assemblies \textit{in vitro}.

Smith et al. (2017) Nature Nanotech
Bioluminescence imaging of in situ-programmed CAR-T cells

Untreated control group

Control nanoparticles (encoding PSMA-specific P4-1BBz CARs)

Nanoparticles carrying leukemia-specific 194-1BBz CARs)

Day 0

Day 3

Day 6

Day 9

Day 12

Day 30

Smith et al. (2017) Nature Nanotech
Mice injected with 1941BBz programming nanoparticles show tumor regression

DNA particles can program T cells in quantities that are sufficient to bring about tumor regression with efficacies that are similar to conventional infusions of T cells transduced ex vivo with CAR-encoding viral vectors.

Smith et al. (2017) Nature Nanotech
Thank you!

- Matthias Stephan
- Cameron Turtle