CAR T cells: Therapy of B cell malignancies and future development

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Conflict of Interest Disclosure
Renier Brentjens MD PhD

• **Stockholder:** Juno Therapeutics (scientific co-founder)
• **Royalties:** Juno Therapeutics
• **Honoraria:** none
• **Research Funding:** Juno Therapeutics
• **Consultant fees:** Juno Therapeutics
Generation of a tumor targeted chimeric antigen receptor (CAR)

**Diagram:**

- **α-TAA mAb**
- **TCR complex**
- **α-TAA scFv—CD8-ζ**
- **CAR retroviral vector**

**Diagram Details:**

- **VH** and **VL** domains
- **α** and **ζ** chains
- **VH** and **VL** scFv
- **CD8** chain
- **5’ LTR** and **3’ LTR**
- **SD** and **SA**
- **ψ** symbol
Generation of TAA-targeted T cells for treatment of Cancer

1. Construct a chimeric antigen receptor (CAR)
2. Subclone CAR gene into a retroviral vector (SFG)
3. Transduce and expand patient T cells \textit{ex vivo}
4. Infuse transduced T cells to eradicate TAA+ tumor cells

\(\alpha\)TAA scFv

\(\text{CD8}\)

\(\text{CD3} \, \zeta\)

TAA CAR

Native TCR

TAA
Evolution in CAR design

First-Generation CAR
scFv-CD3ζ

Second-Generation CAR
scFv-CD28-CD3ζ

Third-Generation CAR
scFv-CD28-4-1BB-CD3ζ
scFv-CD28-OX40-CD3ζ
2nd generation CARs: \textit{in vivo}

![Survival curve](image)

- 19z1 (n=10)
- 19-28z (n=16)
- Pz1 (n=8)

P<0.003

Brentjens et al Clin Cancer Res. 2007 Sep 15;13(18 Pt 1):5426-35
Clinical trials using CD19 targeted T cells in relapsed B cell ALL
A Phase I trial of precursor B cell Acute Lymphoblastic Leukemia (B-ALL) treated with autologous T cells genetically targeted to the B cell specific antigen CD19

• **Inclusion Criteria:**
  – Adult patients, age ≥18
  – Relapsed or refractory CD19+ B-ALL
  – Relapsed after allogeneic HSCT allowed

• **Exclusion Criteria:**
  – Active CNS disease
  – Active GvHD requiring immunosuppressants
  – Significant heart disease (MI ≤ 6 months or NYHA III/IV CHF or EF <40%)
Study Design

Leukapheresis

T Cell Production

Salvage Chemo

BMB

LP + IT Chemo

CTX Conditioning

19-28z CAR T Cell Infusion (1-3x10^6 CAR T cells/kg)

Day -2

Day 1

Post-Treatment Follow UP

Disease Assessment
## Patient characteristics and treatment outcomes

<table>
<thead>
<tr>
<th>Patient ID*</th>
<th>Age (years)</th>
<th>FISH/cytogenetics</th>
<th>Initial therapy</th>
<th>Duration of CR1</th>
<th>Salvage therapy</th>
<th>Disease response to salvage therapy †</th>
<th>Disease response to cell therapy</th>
<th>Steroids</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSK-ALL01</td>
<td>66</td>
<td>Normal karyotype</td>
<td>Mito/Cy → Vinc/Pred → Cy → Etop/Cy</td>
<td>27 weeks</td>
<td>Vinc/Pred/Peg</td>
<td>MRD⁺</td>
<td>MRD⁻</td>
<td>N</td>
<td>Allo-SCT</td>
</tr>
<tr>
<td>MSK-ALL03</td>
<td>56</td>
<td>Normal karyotype</td>
<td>Hyper-CVAD</td>
<td>45 weeks</td>
<td>Inotuzumab oxogamicin → Vinc/Pred/Peg</td>
<td>MRD⁻</td>
<td>MRD⁻</td>
<td>N</td>
<td>Allo-SCT</td>
</tr>
<tr>
<td>MSK-ALL04</td>
<td>59</td>
<td>t(9;11), 9p21 deletion</td>
<td>ECOG2993 (24)</td>
<td>5 weeks</td>
<td>Vinc/Pred</td>
<td>Refractory disease, 63% blasts in BM</td>
<td>MRD⁻</td>
<td>Y</td>
<td>Ineligible for Allo-SCT, relapse 90 days</td>
</tr>
<tr>
<td>MSK-ALL05†</td>
<td>58</td>
<td>9p21 deletion</td>
<td>ECOG2993</td>
<td>28 weeks</td>
<td>HIDAC/Mito</td>
<td>Refractory disease, 70% blasts in BM</td>
<td>MRD⁻</td>
<td>Y</td>
<td>Allo-SCT</td>
</tr>
<tr>
<td>MSK-ALL06</td>
<td>23</td>
<td>Normal karyotype</td>
<td>NYII (25)</td>
<td>34 months</td>
<td>Modified NYII consolidation I (25)</td>
<td>MRD⁺</td>
<td>MRD⁻</td>
<td>N</td>
<td>Allo-SCT</td>
</tr>
</tbody>
</table>

*MSK-ALL02 patient was removed from the study before the planned T cell infusion because he deferred T cell infusion for an allo-SCT. †Disease status within 1 week of infusion with CD19-targeted T cells.  ‡This patient’s T cells were harvested while in remission. All other patients listed had their T cells harvested while they had relapsed disease.
Rapid tumor elimination and recovery of normal bone marrow after 19-28z CAR T cell therapy
Study Progress

• As of 30 March 2015, 39 adult patients with relapsed/refractory ALL treated with 19-28z CAR T cells at MSKCC
  – 39 patients evaluable for toxicity assessment
  – 38 patients evaluable for response assessment with ≥1 month follow up
## Summary of Clinical Outcomes

<table>
<thead>
<tr>
<th></th>
<th>Number of Patients, N=38</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overall CR Rate, n (%) [95% CI]</strong></td>
<td>33/38 (87%) [72, 96]</td>
</tr>
<tr>
<td><strong>MRD Negative CR Rate, n (%) [95% CI]</strong></td>
<td>26/32 (81%) [64, 93]</td>
</tr>
<tr>
<td><strong>Median Time to CR (Range)</strong></td>
<td>23.0 days (8 – 46)</td>
</tr>
</tbody>
</table>
UPenn studies of relapsed B-ALL

- 25 pediatric and 5 adult relapsed or refractory B-ALL patients treated
- 19-4-1BBz CAR design
- 90% CR
- 6 month EFS 67%
- 6 month OSR 78%
NCI studies of relapsed B-ALL

- 20 pediatric and young adult relapsed or refractory B-ALL patients treated.
- 19-28z CAR design
- 70% CR (14/20)
- 60% MRD- CR
- 5 month EFS 78% in MRD- patients
## UPenn clinical trial results

<table>
<thead>
<tr>
<th>Patient</th>
<th>Prior Chemotherapy</th>
<th>Conditioning Chemotherapy</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fludarabine, Rituximab, Alemtuzumab, R-CVP, Lenolidomide, PCR</td>
<td>Bendamustine</td>
<td>CR (3+ years)</td>
</tr>
<tr>
<td>2</td>
<td>Alemtuzumab</td>
<td>Bendamustine/Rituximab</td>
<td>PR (7 months)</td>
</tr>
<tr>
<td>3</td>
<td>Rituximab/Fludarabine, Rituximab/Bendamustine, Alemtuzumab</td>
<td>Pentostatin/Cytoxan</td>
<td>CR (3+ years)</td>
</tr>
</tbody>
</table>
Updated UPenn Trials in CLL (ASH 2013)

• Abstract 4162
  – CD19 CAR T cells treating relapsed/refractory CLL
    • Utilizing a 4-1BBz CAR construct, 14 CLL patients treated
    • 3/14 patients obtained CR (21%), 5/14 patients obtained PR (36%), 6/14 patients with no response (43%)
    • 6/14 patients with persistent detectable CAR T cells (5-35 months)
    • No CR patients with reported relapsed disease
    • No dose response reported

• Abstract 873
  – Dose randomized dose optimization trial of CLL patients with either high or low dose CAR T cell infusions
    • Utilizing a 4-1BBz CAR construct, 27 CLL patients treated
    • Patients randomized to either low dose ($5 \times 10^7$ CAR T cells) or high dose ($5 \times 10^8$ CAR T cells)
    • No dose response benefit seen in these treated patients
    • Overall response rate (CR + PR) was 40%
    • No correlation with CRS and RR was observed
MSKCC clinical trial results: CLL

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Diagnosis-Patient</th>
<th>Age at Diagnosis (years)</th>
<th>Age at Treatment (years)</th>
<th>Sex</th>
<th>Indication for Treatment</th>
<th>Prior Therapies</th>
<th>Genetic Abnormalities/ IgV H Mutation Status</th>
<th>WBC (x10^3/ul)</th>
<th>ALC (x10^3/ul)</th>
<th>Hgb (g/dL)</th>
<th>PLT (x10^5/ul)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLL-1</td>
<td>44</td>
<td>51</td>
<td>M</td>
<td>Bulky LAD</td>
<td>PCR, PCRM</td>
<td>del11q</td>
<td>200.6</td>
<td>196.6</td>
<td>7.1</td>
<td>26</td>
</tr>
<tr>
<td>CLL-2</td>
<td>66</td>
<td>72</td>
<td>M</td>
<td>Bulky LAD</td>
<td>FR, RCVP, PCRM</td>
<td>Unmutated IgV H</td>
<td>4.2</td>
<td>3.4</td>
<td>9.9</td>
<td>60</td>
</tr>
<tr>
<td>CLL-3</td>
<td>62</td>
<td>73</td>
<td>F</td>
<td>Bulky LAD</td>
<td>Chlorambucil, PCR, PCRM</td>
<td>Normal karyotype</td>
<td>136.4</td>
<td>132.3</td>
<td>8.9</td>
<td>100</td>
</tr>
<tr>
<td>CLL-4</td>
<td>63</td>
<td>69</td>
<td>M</td>
<td>Bulky LAD</td>
<td>R, PCRM</td>
<td>del11q</td>
<td>187.1</td>
<td>174</td>
<td>9.9</td>
<td>189</td>
</tr>
<tr>
<td>CLL-5</td>
<td>65</td>
<td>68</td>
<td>M</td>
<td>Bulky LAD</td>
<td>PCRM</td>
<td>del11q, trisomy 12</td>
<td>76.3</td>
<td>66.4</td>
<td>10</td>
<td>162</td>
</tr>
<tr>
<td>CLL-6</td>
<td>56</td>
<td>68</td>
<td>M</td>
<td>Bulky LAD</td>
<td>RCVP, PCR, Bendamustine</td>
<td>del11q, inv1, unmutated IgV H</td>
<td>97.1</td>
<td>92.2</td>
<td>8.9</td>
<td>174</td>
</tr>
<tr>
<td>CLL-7</td>
<td>52</td>
<td>62</td>
<td>M</td>
<td>Bulky LAD</td>
<td>CVP, RC, PCR, PCRM</td>
<td>del17p, unmutated IgV H</td>
<td>1.9</td>
<td>1</td>
<td>10</td>
<td>61</td>
</tr>
<tr>
<td>CLL-8</td>
<td>58</td>
<td>61</td>
<td>M</td>
<td>Bulky LAD</td>
<td>RCVP, Alemtuzumab</td>
<td>del17p, monosomy 14, monosomy 15</td>
<td>5.4</td>
<td>3.3</td>
<td>11.6</td>
<td>41</td>
</tr>
</tbody>
</table>

Table 5. Summary of patient responses

<table>
<thead>
<tr>
<th>Diagnosis-Patient</th>
<th>Response to T-cell infusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLL-1</td>
<td>No objective response</td>
</tr>
<tr>
<td>CLL-2</td>
<td>No objective response</td>
</tr>
<tr>
<td>CLL-3</td>
<td>No objective response</td>
</tr>
<tr>
<td>CLL-4</td>
<td>Not evaluable</td>
</tr>
<tr>
<td>CLL-5</td>
<td>Marked reduction in lymphadenopathy at 3 months subsequently stable for 6 months</td>
</tr>
<tr>
<td>CLL-6</td>
<td>Progressive disease</td>
</tr>
<tr>
<td>CLL-7</td>
<td>Stable disease, lasting 4 months</td>
</tr>
<tr>
<td>CLL-8</td>
<td>Stable disease, lasting &gt;8 weeks</td>
</tr>
</tbody>
</table>

Brentjens et al Blood. 2011 Nov 3;118(18):4817-28
CAR T cells: New Targets
## Challenges targeting MM

<table>
<thead>
<tr>
<th>Target</th>
<th>Questionable efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD19</strong> NCT02135406</td>
<td>~2% MM patients express CD19 Even when expressed, not on most MM cells</td>
</tr>
<tr>
<td><strong>Kappa</strong> NCT00881920</td>
<td>Light chains are secreted, but not retained on the surface of most MM cells</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Target</th>
<th>Potential “on target, off tumor” toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD138</strong> (Syndecan 1) NCT01886976</td>
<td>Bronchial epithelia</td>
</tr>
<tr>
<td><strong>CD38</strong> (ADP-Ribosyl Cyclase)</td>
<td>Hematopoietic stem cells, NK cells, dendritic cells, prostate, pancreas islet cells, others</td>
</tr>
<tr>
<td><strong>CD56</strong> (Neural Cell Adhesion Molecule 1)</td>
<td>CNS Neurons, NK cells</td>
</tr>
<tr>
<td><strong>SLAMF7</strong> (CS1)</td>
<td>monocytes, macrophages, dendritic cells, NK cells, smooth muscle cells</td>
</tr>
<tr>
<td><strong>BCMA</strong> (B cell maturation antigen) NCT02215967; NCT02546167</td>
<td>Differentiated B/plasma cells (likely well tolerated)</td>
</tr>
</tbody>
</table>

G-protein coupled receptor C5D (GPRC5D)

- Orphan receptor
- Survival inversely correlated with MM BM mRNA expression
- Nl expression likely limited to PCs & seminiferous tubules of testis
- First GPR targeted by a CAR

Venkateshaiah S. ASH. 2013
B cell maturation antigen (BCMA)

- Plasma cell differentiation & long term survival
- BAFF/APRIL -> NFkB/MAPK signaling
- Secreted
- Nl expression likely limited to differentiated B cells & PCs
- mAb therapy in clinical trials

Shapiro-Shelef M. Nat Rev Immunology 2005
Gardam S. Front Immunology 2014
Cell surface staining of GPRC5D & BCMA on MM patient BMA

Gated on viable cells
### scFv Identification

#### Human Antibody Phage Library
- Fully-human phage library containing >6x10^{10} unique scFv’s

#### Validation of Positive Phage clones
- Specificity: Protein ELISA or FACS
- Diversity: DNA sequencing

#### Cell Surface Binding of Phage Clones by FACS

#### scFv Cloning into CAR vector
- scFv-Fc production and characterization

#### Panning
- BCMA ECD-IgG1 Fc Panning
- GPRC5D overexpressing 3T3 Cell Panning

#### FACS Sort of GPRC5D+ 3T3 Cells

### Diagram Notes
- 25
- 33
CAR surface expression on T cells
αBCMA & αGPRC5D CAR T cells specifically lyse HMCL (Time 0)

CAR:
- 19-28z
- GPRC5D(8)-28z
- BCMA(24)-28z

SET2 (AML)
BCWM1 (LPL)
L363 (MM)
αBCMA & αGPRC5D CAR T cells specifically lyse HMCL (36h)

CAR:
- 19-28z
- GPRC5D(8)-28z
- BCMA(24)-28z

**SET2** (AML)
- Data Set 6: SET 1928z 099
  - M: 69.02%

**BCWM1** (LPL)
- Data Set 3: WM 1928z 102
  - S: 19.27%
- Data Set 2: WM 8 103
  - T: 72.16%

**L363** (MM)
- Data Set 9: L363 1928z 096
  - G: 56.75%
- Data Set 8: L363 8 097
  - H: 2.06%
αBCMA & αGPRC5D CAR T cells specifically lyse all human MM cell lines tested
U266 murine xenotransplant model (day 7 post CAR T cell injection)

Untreated:

19-28z:

BCMA-28z:
Expansion and validation of this approach: A solid tumor model

Adoptive immunotherapy of ovarian carcinoma utilizing MUC-16 targeted autologous T cells
Rationale

• Ovarian Carcinomas are immunogenic tumors
  – Enhanced T cell infiltration of tumor islets is associated with improved prognosis (Zhang et al NEJM 2003, 348:203-13)
  – Increased levels of the immunosuppressive cytokine TGFβ secretion by tumor increases T cell conversion to Tregs (Li et al Can Letters 2007, 253: 144-53)
The CA125 (MUC16) structure

The CA125 (also known as MUC16) ovarian cancer antigen, is a high-molecular-weight mucin overexpressed on 80% of ovarian cancers, which has three major domains:

1. Extracellular amino terminal domain
2. Large multiple repeat domain
3. Carboxyl terminal domain with transmembrane and short cytoplasmic domain

- **MUC-CD**: retained extracellular and cytoplasmic domains of the MUC16 protein with Ab binding epitopes
- **4H11, 4A5.3, 9B11, VK8**: Ab’s purified to bind various epitopes on MUC16
Generation and *in vitro* analyses of MUC-CD targeted CARs

**A**

![Diagram of CAR constructs](image)

**B**

![Graph showing T cell count](image)

**C**

![Graphs showing IL-2 and IFN-γ production](image)

4H11-28z modified T cells exhibit enhanced *in vitro* anti-tumor efficacy, proliferation, and eradicate autologous tumor cells

**A**

![Graph showing T cell counts over time for different cell lines](image)

**B**

![Graph showing lysis percentage for different cell lines](image)

**C**

![Bar graph showing IL-2 and IFN-g levels](image)

**D**

Healthy donor  Patient #1  Patient #2  Patient #3

MUC-CD targeted T cells eradicate i.p. OVCAR3(MUC-CD) tumor cells

Syngeneic mouse tumor model

A

ID8

ID8(MUC-CD)

C

% IPEM

days since tumor injection

B

ID8(MUC-CD) (n=10)

Syngeneic mouse tumor model
4H11mz versus 4H11m28mz

A

B

C

19m28mz 1d (n=10)
4H11mz 1d (n=8)
4H11m28mz 1d (n=10)
control (no T cells) (n=10)
Armored CAR T cells: New Approaches
The hostile tumor microenvironment

The tumor microenvironment contains multiple inhibitory factors designed to potentially suppress effector T cells.

- CD4$^+$ CD25$^\text{hi}$ FoxP3$^+$ regulatory T cells (Tregs)
- MDSCs
- TAMs
- Expression of inhibitory ligands by tumor (PD-L1)
- Tumor secretion of T cell suppressive cytokines (TGF-β and IL-10)
19z1+ Tregs abrogate anti-tumor efficacy of 1928z+ effector T cells

Lee et al Can Res 2011
Armored CAR T cells: A new approach
Armored CAR T cells version 1.0: IL-12 secreting CAR T cells
IL-12

- A heterodimeric cytokine secreted by activated APCs, neutrophils and macrophages.
- Induces Th1 CD4\(^+\) T cell response enhancing IL-2 and IFN-\(\gamma\) secretion
- Enhances T cell clonal expansion and effector function in concert with TCR signaling (signal 1) and CD28 co-stimulation (signal 2), serving as a signal 3.
- Avoids/reverses T cell anergy
- May overcome Treg mediated effector T cell inhibition
- Recruits and activates NK cells
- Clinical trials in cancer using systemic IL-12 therapy has been limited by severe inflammatory side effects
IL-12 inhibits Treg mediated suppression of effector T cell proliferation and cytotoxicity in vitro
IL-12 secreting CAR T cells
IL-12 secreting CAR T cells

A

B

C

D

% GzmB+ CD8 T cells

% Prs+ CD8 T cells

Specific Lysis (%)

NALM-6 cell number (x10^6)

19-28z / 19-28z/miL-12

E:T ratio

1:1 2.5:1 5:1 10:1 20:1 40:1

*
IL-12 secreting CAR T cells *in vivo* efficacy
IL-12 secreting CAR T cells are resistant to Treg mediated inhibition *in vitro*
IL-12 secreting CAR T cells are resistant to Treg mediated inhibition \textit{in vivo}
Syngeneic EL4(hCD19) tumor model

- Assess T cell eradication of tumor
- Assess T cell homing to tumor
- Assess long-term survival of T cells
- Assess memory T cell response to rechallenge with tumor
- Assess T cell proliferation in vivo
- Assess the efficacy of suicide vectors
- Determine the side effects of therapy
- Retroviral transduction with chimeric receptor
- Harvest splenocytes

```
EL4(hCD19)
```

IV injection

```
mCD19−/− hCD19+/−
```

```
mCD19+/− hCD19+/−
```

```
53%
```

Counts

```
0 10 10^2 10^3 10^4
FL1-H
```

Determine the side effects of therapy
Lymphodepletion enhances anti-tumor efficacy of 19z1⁺ T cells.
Cyclophosphamide lymphodepletion reduces Tregs and induces IL-12 and IFNγ secretion

A

B

Pegram et al Blood 2012
19z1IRESIL-12 modified T cells secrete biologically active IL-12 and exhibit enhanced targeted cytotoxic function and resistance to Tregs.

Pegram et al Blood 2012
Syngeneic IL-12 secreting CD19 targeted T cells induce B cell aplasias and tumor eradication.
Complete eradication of ID8(MUC-CD) ovarian tumors in mice with MUC16 targeted T cells expressing IL-12

A. ID8(MUC-CD) lysis by CAR+ T cells

B. IFN-γ (pg/ml)

C. Survival (7 days after tumor injection)

D. Survival (14 days after tumor injection)
IL-12 genetically modified T cells: Armored CAR T cells

Enhanced CM phenotype, enhanced cytotoxicity, enhanced persistence. Resistance to Treg and TGFβ inhibition.

IL-12 secretion, Targeted tumor cytotoxicity. CAR-RES IL-12, IL-12 secretion, Enhanced CM phenotype, enhanced cytotoxicity, enhanced persistence. Resistance to Treg and TGFβ inhibition.
Armored CAR T cells version 2.0: Constitutively expressing CD40L CAR T cells
Armored CAR T cells: A new approach
Armored CARs v2.0: CD40L (CD154)

- Type II transmembrane protein (TNF gene superfamily)
  - Trimer (most active form)
- Expression on activated T cells
  - Inflammation/Infection/Injury
- Rapid upregulation (peak 6 hours)
- Cleavage (sCD40L)
  - Biologically active
CD40L genetically modified T cells: Armored CAR T cells v2.0

1. CD40L$^+$ CAR$^+$ T cell
2. Tumor
3. DC
4. NK cell
5. T$_{reg}$
CD40L+ Armored CAR T cells v2.0: Enhanced proliferation and cytokine secretion

![Graphs and diagrams showing enhanced proliferation and cytokine secretion over time.]
CD40L+ Armored CAR T cells v2.0: Enhanced immunogenicity of tumor cells

CD40+ DoHH2

CD40- NALM6
CD40L+ Armored CAR T cells v2.0: CD40L+ T cells modify autologous CLL tumor cells
CD40L+ Armored CAR T cells v2.0: CD40L+ T cells mature autologous DCs
CD40L+ Armored CD19 targeted CAR T cells v2.0: Enhanced anti-tumor efficacy
CD40L+ Armored CD19 targeted CAR T cells v2.0: Enhanced *in vivo* anti-tumor efficacy

![Graph showing survival rates over time with P<0.05 significance](image-url)
Armored CAR T cells version 3.0: scFv secreting CAR T cells
Armored CAR T cells: A new approach

<table>
<thead>
<tr>
<th>First generation CAR</th>
<th>Second generation CAR</th>
<th>Third generation CAR</th>
<th>“Armored” Fourth generation CAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>scFv (Vα + Vβ)</td>
<td>Transmembrane domain</td>
<td>ζ signaling domain</td>
<td>Costimulatory ligand</td>
</tr>
<tr>
<td>CD28 costimulatory domain</td>
<td>Flexi-cytokine</td>
<td>41BB costimulatory domain</td>
<td>Costimulatory ligand</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Secretable scFv</td>
</tr>
</tbody>
</table>

[Diagram of CAR T cells evolution from first to fourth generation, showing the addition of scFv, costimulatory ligand transgene, and secretable scFv.]
PD-1 antagonistic scFv secreting CAR T cells

<table>
<thead>
<tr>
<th>SP</th>
<th>CD19 or Muc-CD specific scFv</th>
<th>hCD28 TM and signaling</th>
<th>hCD3zeta signaling</th>
<th>P2A</th>
<th>SP</th>
<th>V_H domain</th>
<th>G_S</th>
<th>V_L domain</th>
<th>tag</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PD-1 blocking scFv</td>
<td></td>
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</table>

![Graphs showing 1928z and 1928z-E27]
Retained CAR T cell function
PD-1 antagonistic scFv secreting CAR T cells retain CAR T cell function in the context of PD-L1
PD-1 antagonistic scFv secreting CAR T cells retain CAR T cell function in the context of PD-L1
PD-1 antagonistic scFv secreting CAR T cells retain CAR T cell function in the context of PD-L1
PD-1 antagonistic scFv secreting CAR T cells retain CAR T cell function in the context of PD-L1
Conclusions

• Autologous CD19 targeted CAR modified T cells have demonstrated very promising anti-tumor efficacy in B cell ALL with more modest responses in patients with low grade B cell malignancies.

• Etiologies of CAR T cell resistance may be related to the hostile tumor microenvironment.

• Application of CAR T cell therapy for low grade B cell malignancies as well as moving forward towards application to solid tumor malignancies requires “armored” CAR T cells designed to both overcome the hostile tumor microenvironment and exhibit enhanced anti-tumor efficacy and long term persistence.

• Variations of “armored CAR” T cells appear to have enhanced anti-tumor efficacy based on pre-clinical tumor models.

• Future studies using “armored” CAR T cell technology will focus on translation of these armored CAR T cells to the clinical setting both in the context hematological as well as solid tumor malignancies.
The MSKCC Cellular Therapeutics Center: Current and Planned Clinical Application
Cellular Therapeutics Center (CTC): Clinical Faculty
CTCEF Production Capacity: New Facility

- Current GMP Facility: 6 cleanrooms
- New GMP facility opening 2015: 13 cleanrooms
- Cellular products: T cells, Dendritic cells, NK cells, HSCs, Cord Blood, ES cells
- Other products: RNA vectors, plasmid DNA
- Phase I/II clinical trials
- Multicenter trials with other academic centers
CTCEF Production Capacity: Moving Forward into the New Facility

- >100 subjects infused with CAR T cells
- 2016 Projection: 75 CAR T cell products manufactured

Brentjens et al. *Blood*, 2011. 8 CLL/1 ALL patients
Brentjens et al. *STM*, 2013. 5 ALL patients
Davila et al. *STM*. 2014. 16 ALL patients
Currently Enrolling CTC Clinical Trials (2015)

**PARK 06-138**: A Phase I/IIa Trial For The Treatment of Relapsed or Chemotherapy Refractory Chronic Lymphocytic Leukemia or Indolent B Cell Lymphoma Using Autologous T Cells Genetically Targeted to the B Cell Specific Antigen CD19

**PARK 09-114**: A Phase I Trial of Precursor B Cell Acute Lymphoblastic Leukemia (B-ALL) Treated with Autologous T Cells Genetically Targeted to the B Cell Specific Antigen CD19(CAR)

**PARK 11-048**: A Phase I Trial of Consolidation Therapy with Autologous T Cells Genetically Targeted to the B Cell Specific Antigen CD19 in Patients with Chronic Lymphocytic Leukemia Following Upfront Chemotherapy with Pentostatin, Cyclophosphamide and Rituximab

**PARK 15-099**: Phase II Study – Single-arm, Multicenter Trial to Determine the Efficacy and Safety of JCAR2015 in Adult Subjects with Relapsed or Refractory B-Cell Acute Lymphoblastic Leukemia

**SAUTER 12-117**: A Phase I Trial of High Dose Therapy and Autologous Stem Cell Transplantation Followed by Infusion of Chimeric Antigen Receptor (CAR) Modified T-Cells Directed Against CD19+ B-Cells for Relapsed and Refractory Aggressive B Cell Non-Hodgkin Lymphoma

**CURRAN 13-052**: A Phase I Trial of Autologous T-Lymphocytes Genetically Targeted to the B-Cell Specific Antigen CD19 in Pediatric and Young Adult Patients with relapsed B-Cell Acute Lymphoblastic Leukemia

**ADUSUMILLI 15-007**: A Phase I Clinical Trial of Malignant Pleural Disease Treated with Autologous T Cells Genetically Engineered to Target the Cancer-Cell Surface Antigen Mesothelin

**O’CEARBHAILL 15-014**: A Phase I Clinical Trial of Cyclophosphamide Followed by Intravenous and Intraperitoneal Infusion of Autologous T Cells Genetically Engineered to Secrete IL-12 and to Target the MUC16ecto Antigen in Patients with Recurrent MUC16ecto+ Epithelial Ovarian, Fallopian Tube or Primary Peritoneal Cancer
<table>
<thead>
<tr>
<th>Open Protocols (n=8)</th>
<th>Total Subjects Planned for Treatment</th>
<th>Total Accrual</th>
<th>Total Treated</th>
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<tbody>
<tr>
<td>06-138 (Phase I/II R/R CLL) Jae Park</td>
<td>56</td>
<td>40</td>
<td>22</td>
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<tr>
<td>09-114 (Phase I ALL) Jae Park</td>
<td>120</td>
<td>82</td>
<td>47</td>
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<tr>
<td>11-048 (Phase I CLL) Jae Park</td>
<td>18</td>
<td>13</td>
<td>8</td>
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<tr>
<td>12-117 (Phase I NHL) Craig Sauter</td>
<td>24</td>
<td>17</td>
<td>15</td>
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<tr>
<td>13-052 (Phase I ALL Pediatrics) Kevin Curran</td>
<td>24</td>
<td>33</td>
<td>11</td>
</tr>
<tr>
<td>15-007 (Phase I Meso) Prasad Adusumilli</td>
<td>24</td>
<td>2</td>
<td>1</td>
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<tr>
<td>15-014 (Phase I Ovarian) Roisin O’Cearbhaill</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15-099 (Phase II ALL) Jae Park</td>
<td>10</td>
<td>5</td>
<td>2</td>
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<tr>
<td><strong>Total as of Q4 2015</strong></td>
<td><strong>306</strong></td>
<td><strong>189</strong></td>
<td><strong>106</strong></td>
</tr>
</tbody>
</table>
15-099: The ROCKET Study: A Phase 2, Single-arm, Multicenter Trial to Determine the Efficacy and Safety of JCAR015 in Adult Subjects with Relapsed or Refractory B-Cell Acute Lymphoblastic Leukemia

**Important Inclusion/Exclusion Criteria:**

- Relapsed or refractory CD19+ B-ALL
  - Prior allo HSCT allowed if no active GvHD
  - ≥ 5% blasts in bone marrow at time of consent
- Excludes patients with:
  - Isolated extramedullary disease, CML in blast crisis
  - Active CNS disease
  - Prior treatment with any gene therapy product

**Treatment Scheme:**

**Part A: Apheresis/Cell Production**

- Leukapheresis
- JCAR015 Product Generation
- Cytoreductive chemotherapy
- Bone marrow biopsy and group assignment
- Part B screen failure, or unsuccessful product generation

**Part B Screening**

- Day -2
- Day 1
- Day 14-21
- Day 42-55
- Month 3 – Month 12

**Part B: JCAR015 Treatment and Follow-up**

- Group 1: morphologic disease (ITT population)
  - CPM 1-3 g/m²
  - JCAR015
  - Dose K1 (1.0 x 10⁸ CAR+ cells/kg)
- Group 2: MRD-only
  - CPM 1-3 g/m²
  - JCAR015
  - Dose K2 (3.0 x 10⁸ CAR+ cells/kg)

**Accruals:**

- Patients Enrolled: 4
- Patients In Screening: 2
- Patients Treated: 2

**Participating Institutions:**

- Dana Farber Cancer Institute
- Mass General Hospital
- Roswell Park
- Northwestern
- U. Alabama
- U. Miami
- City of Hope
- MD Anderson
- U. Nebraska Medical Center
- Johns Hopkins
- Washington University
- Cleveland Clinic
- UCSF

**PI:** Jae Park  
**Co-PIs:** Craig Sauter  
**Research NP:** Elizabeth Halton  
**Research RN:** Claudia Diamonte
15-007: A Phase I Clinical Trial of Malignant Pleural Disease Treated with Autologous T Cells Genetically Engineered to Target the Cancer-Cell Surface Antigen Mesothelin (2015)

**Important Inclusion/Exclusion Criteria:**
- Malignant pleural disease (MPD)
  - Mesothelioma
  - Lung Cancer
  - Breast Cancer
- Previously treated with at least 1 prior treatment regimen
- Mesothelin expression in the tumor determined either by tumor IHC or serum measurement
- Free flowing pleural effusion requiring management by placement of pleural catheter
- Excludes patients with any prior history of brain metastases

**Accruals:**
- Total # patients screened: 40
- Screening Consent Accrual: 10
- Treatment Consent Accrual: 2
- Patients Treated: 1

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Dose</th>
<th>Number of doses</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1*</td>
<td>$1 \times 10^5$</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>$3 \times 10^5$</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>$3 \times 10^5$+Cyclophosphamide</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>$1 \times 10^6$+Cyclophosphamide</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>$3 \times 10^6$+Cyclophosphamide</td>
<td>1</td>
</tr>
</tbody>
</table>

**PI:** Prasad S Adusumilli  
**Co-PIs:** Dr. Roisin O'Cearbhaill  
Dr. Charles Rudin  
**Research NP:** Elizabeth Halton  
**Research RN:** Claudia Diamonte  
**Study RSA:** Erin McGee
15-014: A Phase I Clinical Trial of Cyclophosphamide Followed by Intravenous and Intraperitoneal Infusion of Autologous T Cells Genetically Engineered to Secrete IL-12 and to Target the MUC16ecto Antigen in Patients with Recurrent MUC16ecto+ Epithelial Ovarian, Fallopian Tube or Primary Peritoneal Cancer

PI: Dr. Roisin O’Cearbhaill
Co-PIs: Dr. David Spriggs
Research NP: Elizabeth Halton
Research RN: Claudia Diamonte
Study RSA: Christina MacAulay

Important Inclusion/Exclusion Criteria:
• High-grade serous ovarian, primary peritoneal, or fallopian tube carcinoma
• Expression of MUC16^{ecto} antigen
• One prior platinum-based chemotherapeutic regimen for management of ovarian, primary peritoneal, or fallopian tube carcinoma and at least two prior chemotherapy regimens and no more than 5 chemotherapy regimens
• Excludes patients with any prior history of brain metastases

Accruals:
Screening Consent Accrual: 0
Treatment Consent Accrual: 0
Patients Treated: 0

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Dose Level</th>
<th>4H11-28z/fIL-12/EFGRT+ T cell Dose</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>-I</td>
<td>-1</td>
<td>1 x 10^5 cells/kg</td>
<td>3-6 patients</td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>3 x 10^5 cells/kg</td>
<td>3-6 patients</td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>Cyclophosphamide + 3 x 10^5 cells/kg</td>
<td>3-6 patients</td>
</tr>
<tr>
<td>III</td>
<td>2</td>
<td>Cyclophosphamide 1 x 10^6 cells/kg</td>
<td>3-6 patients</td>
</tr>
<tr>
<td>IV</td>
<td>3</td>
<td>Cyclophosphamide + 3 x 10^6 cells/kg</td>
<td>3-6 patients</td>
</tr>
<tr>
<td>V</td>
<td>4</td>
<td>Cyclophosphamide + 1 x 10^7 cells/kg</td>
<td>3-6 patients</td>
</tr>
</tbody>
</table>
The Trident Trial (Juno Therapeutics)

Relapsed refractory CLL patients

- Treat with 19-28z/4-1BBL CAR T cells
- Treat with 19-28z/IL-12 CAR T cells
- Treat with 19-28z/CD40L CAR T cells
Brentjens’ Extended Lab Members
Renier Brentjens  
Hollie Pegram  
Mythili Koneru  
Swarish Rafiq  
Swati Pendeharkar  
James Lee  
Yan Nikhamin  
Jae Park  
Kevin Curran  
Peter Chang

Michel Sadelain  
Marco Davila  
Michael Gong  
Jean Baptiste Latouche

Leukemia Service  
David Scheinberg  
Jae Park  
Mark Frattini  
Peter Maslak  
Mark Heaney  
Joe Jurcic  
Nicole Lamanna  
Marco Davila  
Dan Douer

Cell Therapy and Cell Engineering Facility  
(Isabelle Riviere, Director)

R&D, Manufacturing  
Xiuyan Wang  
(Dan Hollyman)  
Jolanta Stefanski  
Malgorzata Olszewska  
Oriana Borquez-Ojeda  
Clare Taylor  
Teresa Wasielewska  
Jinrong Qu

QA/QC  
Shirley Bartido  
(Mark Przybylowski)  
James Hosey  
Domenick Pirraglia  
Vanessa Capacio

Clinical Research  
Yvette Bernal

Lymphoma Service  
Craig Moskowitz  
Ariela Noy

GYN service  
Samith Sandadi  
Stephen Lee  
Roisin O’ Clearbhail

Adult BMT Service  
Sergio Geralt  
Craig Sauter

Department of Clinical Laboratories  
Lillian Reich  
David Wuest  
Kathy Smith

Biostatistics  
Glenn Heller

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CA59350 (MS); P30 CA-008748 (CT); 3RO1CA138738-02S1(RJB); Alliance for Cancer Gene Therapy; Terry Fox Run for Cancer Research; William H. Goodwin and Alice Goodwin, and the Commonwealth Cancer Foundation for Research and the ETC of MSKCC; Damon Runyon Clinical Investigator Award (RJB); William Lawrence & Blanche Hughes Foundation (RJB); CLL-Global Research Foundation (RJB)