Cord Blood CAR NK Cell Therapy

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Disclosures

• License agreement and research agreement with Takeda to develop CB-CAR NK cells for the treatment of B-cell malignancies and other cancers.

• Educational grant:
  – Affimed; Pharmacyclics

• SAB:
  – Virogen; Adicet Bio
**NK Cells**
- Innate immune system
- CD56+CD3-
- Differentiate in the BM
- No antigen priming
- Primarily in blood
- No/low risk of GVHD
- Recognition takes place through complex array of receptors

**T Cells**
- Adaptive immune system
- CD3+CD4+ or CD3+CD8+
- Differentiate in the thymus
- Antigen priming required
- Antigen specific
- Allogeneic T cells induce GVHD
- Recognize targets through TCR rearrangement
# Advantages of NK cells over T cells for CAR therapy

## CAR-T

- **Autologous Product**
  - Production time
  - Cost
  - 1 patient, 1 product

- If allogeneic: GVHD Risk

- Toxicity: cytokine release syndrome; neurotoxicity (50% need ICU care)

- CAR-mediated killing

## CD19 CAR-NK

- **Allogeneic Product**
  - “Off the shelf”
  - Potential low cost
  - 1 cord, > 100 doses

- Low/absent GVHD

- CAR + NK Receptor mediated
NK cell immunotherapy for the treatment of cancer

• **Improve persistence**

• **Antigen-specificity**

• **Logistics:** NK cells need to be collected on an individual case basis:
  • From a healthy donor (allogeneic source) – haploidentical donor or **cord blood** (MDACC CB Bank) – **we have treated >50 patients with doses of >10e8/kg CB-NK with no toxicity**
  • Others use NK92 cell line, HSC or iPSCs
  • From the patient (autologous- **less effective**
Higher expression of genes involved in cell cycle, cell division and DNA replication in cord blood (CB) versus peripheral blood (PB) NK cells

Li et al. Blood Advances 2019; 3 (23)
CAR NK cells persist & control Raji tumor in NSG mouse model

Armored CAR

LTR → iCasp9 → 2A → scFv → CD28 → CD3ζ → 2A → OhIL-15 → LTR

- Suicide switch
- CAR.19
- Signaling cytokine

Day 70 Post Infusion

Blood

- hCD45: 24%
- hCD19: 0.01%
- hCD56: 87.3%
- CAR: 92.3%

Liu et al. Leukemia 2017
**Clinical CAR-NK Transduction & Expansion**

**Day 0**
- Frozen cord Blood unit
- Thaw
- Ficoll
- CD3/14/19 depletion (CliniMACS)
- Negatively-selected NK cells

**Day 9**
- Culture condition: γ-irradiated K562-based feeder (mbIL21, 41BBL, CD48) + IL-2 200u/ml

**Day 15**
- Flow cytometry CAR
  - CD56
  - CD16
  - CD3
  - CD19
  - CD14
  - CD45

**Day 6**
- Transduce with retroviral supernatant
Characteristics of iC9/CAR.19/IL15-transduced CB-NK cells generated from 5 different CB units after 14 days of culture

<table>
<thead>
<tr>
<th></th>
<th>Starting Cell Number ($\times 10^6$)</th>
<th>NK Fold Expansion</th>
<th>NK Absolute Count at Day14 ($\times 10^8$)</th>
<th>CAR Transduction Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAR-CBNK#1</td>
<td>20</td>
<td>564.3</td>
<td>113</td>
<td>66.6</td>
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<tr>
<td>CAR-CBNK#2</td>
<td>20</td>
<td>843.7</td>
<td>170</td>
<td>87.4</td>
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<tr>
<td>CAR-CBNK#3</td>
<td>20</td>
<td>7369.6</td>
<td>1530</td>
<td>64.4</td>
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<tr>
<td>CAR-CBNK#4</td>
<td>20</td>
<td>2514.3</td>
<td>500</td>
<td>47.8</td>
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<tr>
<td>CAR-CBNK#5</td>
<td>20</td>
<td>2221.8</td>
<td>440</td>
<td>67.5</td>
</tr>
<tr>
<td>Median</td>
<td>20</td>
<td>2221.8</td>
<td>440</td>
<td>66.6</td>
</tr>
</tbody>
</table>

>100 doses of CAR NK cells can be generated from one cord blood unit
CAR NK Cells in Patients With Relapsed/Refractory B-lymphoid Malignancies (CLL, NHL, ALL)  
PI: Katy Rezvani MD, PhD; MD Anderson Cancer Center

CB-derived NK cells

Transduction of NK Cells with retroviral vectors

Antigen Specific CAR+ NK cells

Expansion of CAR NK cells

Cyclophosphamide, 300 mg/m²

Fludarabine 30 mg/m²

Day -15

Day -9

Day -4

Day -3

Day -2

Day 0

3 dose levels. 1x10⁵/kg, 1x10⁶/kg, 1x10⁷/kg

Cell Infusion

Banked umbilical cord blood

no HLA match

KIR-ligand mismatch

Use of CAR-Transduced Natural Killer Cells in CD19-Positive Lymphoid Tumors

Enli Liu, M.D., David Marin, M.D., Pinaki Banerjee, Ph.D., Homer A. Macapinlac, M.D., Philip Thompson, M.B., B.S., Rafet Basar, M.D., Lucila Nassif Kerbauy, M.D., Bethany Overman, B.S.N., Peter Thall, Ph.D., Mecit Kaplan, M.S., Vandana Nandivada, M.S., Indresh Kaur, Ph.D., Ana Nunez Cortes, M.D., Kai Cao, M.D., May Daher, M.D., Chitra Hosing, M.D., Evan N. Cohen, Ph.D., Partow Kebriaei, M.D., Rohtesh Mehta, M.D., Sattva Neelapu, M.D., Yago Nieto, M.D., Ph.D., Michael Wang, M.D., William Wierda, M.D., Ph.D., Michael Keating, M.D., Richard Champlin, M.D., Elizabeth J. Shpall, M.D., and Katayoun Rezvani, M.D., Ph.D.
<table>
<thead>
<tr>
<th>Dose level</th>
<th>Diagnosis</th>
<th>Age/Sex</th>
<th>Cytogenetics</th>
<th>Lines of treatment</th>
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</thead>
<tbody>
<tr>
<td>Dose level 1</td>
<td>Relapsed transformed double-hit DLBCL</td>
<td>47/M</td>
<td>Double hit (C-MYC and BCL-2)</td>
<td>3 (including ASCT)</td>
</tr>
<tr>
<td>1 x 10e5 CAR</td>
<td>Refractory DLBCL</td>
<td>59/M</td>
<td>complex cytogenetics</td>
<td>7</td>
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<tr>
<td>NK/kg</td>
<td>CLL</td>
<td>59/F</td>
<td>17p del</td>
<td>4 (including ibrutinib and venetoclax)</td>
</tr>
<tr>
<td>Dose level 2</td>
<td>CLL</td>
<td>56/M</td>
<td>17p del</td>
<td>4 (including ibrutinib)</td>
</tr>
<tr>
<td>1 x 10e6 CAR</td>
<td>CLL/Richter’s transformation</td>
<td>61/M</td>
<td>Trisomy 21, unmuted</td>
<td>5 (including ibrutinib) Progressive disease on HyperCVAD prior to admission</td>
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<tr>
<td>NK/kg</td>
<td>CLL/Richter’s transformation</td>
<td>60/F</td>
<td>17p del</td>
<td>5 (including ibrutinib and venetoclax)</td>
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<tr>
<td>Dose level 3</td>
<td>CLL</td>
<td>66/F</td>
<td>del ATM +SPEN, +SF3B1,</td>
<td>4 (including ibrutinib)</td>
</tr>
<tr>
<td>1 x 10e7 CAR</td>
<td>Refractory DLBCL</td>
<td>64/M</td>
<td>complex cytogenetics</td>
<td>11 (including ASCT)</td>
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<tr>
<td>NK/kg</td>
<td>Relapsed transformed double-hit DLBCL</td>
<td>70/M</td>
<td>complex cytogenetics</td>
<td>4 (including ASCT)</td>
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<tr>
<td></td>
<td>Relapsed Follicular lymphoma</td>
<td>61/F</td>
<td>t14;18</td>
<td>4 (including ASCT)</td>
</tr>
<tr>
<td></td>
<td>Relapsed Follicular lymphoma</td>
<td>60/M</td>
<td>14;18</td>
<td>4 (Progressed before ASCT).</td>
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</tbody>
</table>
Clinical Response to NK-CAR therapy

<table>
<thead>
<tr>
<th>Time from infusion (months)</th>
<th>OS Probability</th>
<th>PFS Probability</th>
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<tbody>
<tr>
<td>0</td>
<td>1.0</td>
<td>0.8</td>
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<tr>
<td>3</td>
<td>0.8</td>
<td>0.6</td>
</tr>
<tr>
<td>6</td>
<td>0.6</td>
<td>0.4</td>
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<tr>
<td>9</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>12</td>
<td>0.2</td>
<td>0.0</td>
</tr>
<tr>
<td>15</td>
<td></td>
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</tr>
<tr>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Patient 5 Achieved Complete Response in Richter’s
(1 x 10e6/kg)

Selective depletion of B cells after CAR NK infusion
Patient #6- achieved CR. CAR NK cell traffic to sites of disease

Pre-admission

Day 30 post CAR NK
CAR NK cells are detectable up to 12 months post infusion.
The cytokine profile after CAR NK cell therapy does not support CRS

• CB-NK cells can be engineered to express a CAR to redirect their specificity and a cytokine to enhance their *in vivo* proliferation and persistence

• A first-in-human clinical trial of CAR19/IL15 transduced cord blood NK cells resulted in responses in 8/11 patients with no CRS or neurotoxicity

• CB CAR NK against other targets under development