Shortening the time to manufacture CAR+ T cells with Sleeping Beauty system supports T-cell engraftment and anti-tumor effects in patients with refractory CD19+ tumors

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1st generation: First-in-human Sleeping Beauty-modified T cells

Electroporation of DNA plasmids coding for Sleeping Beauty (SB) transposon (CD19-specific CAR) and transposase (SB11) results in:

- Published data
  - Stable integration of CAR;
  - In vivo persistence of genetically modified T cells;
  - Anti-tumor effects after hematopoietic stem-cell transplantation.

- New data (2017 ASH #2059)
  - Long-term persistence of infused T cells (currently, detected for up to 4 years in some recipients)
  - Long-term multi-year survival of patients with NHL (OS 100%) and ALL (OS 49%)

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2\textsuperscript{nd} generation: Improvements to the SB system

- CAR design
- Shorten T-cell manufacture on feeder cells
- Shorten release testing

Irradiated feeder cells derived from K-562 genetically modified to co-express CD19 and co-stimulatory molecules
Study Design: Clinical Trial # NCT02529813

- Adult and pediatric patients, 1-80 years
- Active CD19\(^+\) lymphoid malignancies
- Fludarabine (FLU) & Cytoxan (CTX) lymphodepletion
- Standard 3+3 design with Dose Levels from \(>10^5\) to \(\leq 10^9\) CD3\(^+\)CAR\(^+\)/kg

**T-cell and Adult Patient Summary**

Characterization of the infusion product: CAR⁺ T cells were generated by co-culture with feeder cells and cytokines. Total cell manufactured along with percent expression (by flow cytometry) of CD3 and CAR is shown for each patient.

<table>
<thead>
<tr>
<th>Pt #</th>
<th>Dose Level</th>
<th>Dx</th>
<th>Age</th>
<th>Lymphodepletion*</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>-1</td>
<td>DLBCL</td>
<td>36</td>
<td>Regimen A</td>
</tr>
<tr>
<td>2</td>
<td>+1</td>
<td>CLL</td>
<td>68</td>
<td>Regimen B</td>
</tr>
<tr>
<td>4</td>
<td>+1</td>
<td>ALL</td>
<td>40</td>
<td>Regimen A</td>
</tr>
<tr>
<td>8</td>
<td>+1</td>
<td>ALL</td>
<td>41</td>
<td>Regimen A</td>
</tr>
<tr>
<td>16</td>
<td>+1</td>
<td>ALL</td>
<td>16</td>
<td>Regimen B</td>
</tr>
<tr>
<td>9</td>
<td>+2</td>
<td>ALL</td>
<td>29</td>
<td>Regimen A</td>
</tr>
<tr>
<td>13</td>
<td>+2</td>
<td>ALL</td>
<td>47</td>
<td>Regimen A</td>
</tr>
<tr>
<td>14</td>
<td>+2</td>
<td>DLBCL</td>
<td>72</td>
<td>Regimen A</td>
</tr>
</tbody>
</table>

*Regimen A: FLU 30 mg/m², CTX 500 mg/m² x 3 days
Regimen B: FLU 25 mg/m², CTX 250 mg/m² x 3 days
# 2013-1018 Interim Adult Patient Summary

<table>
<thead>
<tr>
<th>Pt #</th>
<th>Dose (CD3^+CAR^+/kg)</th>
<th>Dose Level</th>
<th>Dx</th>
<th>STIMs*</th>
<th>Best Response**</th>
<th>Survival Status</th>
</tr>
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<tbody>
<tr>
<td>6</td>
<td>7.7x10^4</td>
<td>-1</td>
<td>DLBCL</td>
<td>2</td>
<td>NR</td>
<td>Deceased</td>
</tr>
<tr>
<td>2</td>
<td>1x10^6</td>
<td>+1</td>
<td>CLL</td>
<td>4</td>
<td>NR</td>
<td>Alive, 1 yr</td>
</tr>
<tr>
<td>4</td>
<td>1x10^6</td>
<td>+1</td>
<td>ALL</td>
<td>3</td>
<td>CR, 3m</td>
<td>Alive, 1 yr</td>
</tr>
<tr>
<td>8</td>
<td>1x10^6</td>
<td>+1</td>
<td>ALL</td>
<td>3</td>
<td>CR, 1m</td>
<td>Alive, 3m</td>
</tr>
<tr>
<td>16</td>
<td>1x10^6</td>
<td>+1</td>
<td>ALL</td>
<td>2</td>
<td>TBD</td>
<td>Alive, 1m</td>
</tr>
<tr>
<td>9</td>
<td>1x10^7</td>
<td>+2</td>
<td>ALL</td>
<td>2</td>
<td>NR</td>
<td>Alive, 1m</td>
</tr>
<tr>
<td>13</td>
<td>9x10^6</td>
<td>+2</td>
<td>ALL</td>
<td>2</td>
<td>CR, 3m***</td>
<td>Alive, 3m</td>
</tr>
<tr>
<td>14</td>
<td>1x10^7</td>
<td>+2</td>
<td>DLBCL</td>
<td>3</td>
<td>CR, 1m</td>
<td>Alive, 1m</td>
</tr>
</tbody>
</table>

*The every 7- to 10-day addition of feeder cells is a "stim". Feeder cells derived from K-562 genetically modified to co-express CD19 and co-stimulatory molecules.

**Best Response: CR= Complete Response, NR= No Response, PD=Progressive Disease

*** MRD+
Summary of Related Adverse Events

No dose limiting toxicities

<table>
<thead>
<tr>
<th>Dose Level</th>
<th>Patient</th>
<th>Event</th>
<th>Grade</th>
<th>Serious</th>
<th>Attribution</th>
<th>Onset (Days after Infusion)</th>
<th>Resolved</th>
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</thead>
<tbody>
<tr>
<td>-1</td>
<td>6</td>
<td>CRS: Fever</td>
<td>1</td>
<td>-</td>
<td>PO</td>
<td>5</td>
<td>Y</td>
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<tr>
<td>1</td>
<td>2</td>
<td>Hypotension</td>
<td>2</td>
<td>-</td>
<td>PR</td>
<td>0</td>
<td>Y</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>Sinus Tachycardia</td>
<td>1</td>
<td>-</td>
<td>Def</td>
<td>0</td>
<td>Intermittent</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>Fever</td>
<td>1</td>
<td>-</td>
<td>Def</td>
<td>0</td>
<td>Y</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>Chills</td>
<td>1</td>
<td>-</td>
<td>Def</td>
<td>0</td>
<td>Y</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>Sinus Tachycardia</td>
<td>1</td>
<td>-</td>
<td>Def</td>
<td>1</td>
<td>Y</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>CRS: Fever</td>
<td>1</td>
<td>-</td>
<td>Def</td>
<td>9</td>
<td>Y</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>CRS: Fever, cardiac arrhythmia</td>
<td>2</td>
<td>Yes</td>
<td>Def</td>
<td>10</td>
<td>Y</td>
</tr>
</tbody>
</table>

Attribution: PO=Possible, PR=Probable, Def=Definitely
**In vivo** persistence of SB-modified T cells

Digital droplet polymerase chain reaction (ddPCR)

Flow cytometry

Each symbol represents an individual patient and horizontal bar the ‘mean’.

*Two outliers omitted*
Detection of T cells and loss of B cells

Patient 8 - ALL

Patient 14 - DLBCL

Percent CAR was calculated from CD3+ gated T cells and CD19 was calculated from viable (CD45+) lymphocyte gate.
Summary and Conclusions

- Shortened T-cell manufacturing on feeder cells to approximately 2 weeks
- Modifying testing to rapidly release products
- Persistence of SB-modified CAR+ T cells by ddPCR and flow cytometry
- Encouraging safety profile and anti-tumor effects
- Study is ongoing
- 2nd-generation SB-modified CD19-specific CAR+ T-cell trial serves as platform, providing data supporting 3rd-generation trial for very-rapid (<2 days) T-cell manufacture under point-of-care (P-O-C)
**Goal:** Implement in 2018 a new approach to very rapidly manufacture genetically modified CAR⁺ T cells in under 2 days (termed “point-of-care”)

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**1st generation SB complete: CAR⁺ T cells**
- Manufacture ~4 weeks
- Cryopreserve
- Release testing 14 days
- Safety, feasibility & efficacy of SB system
- Publication #: 2059

**2nd generation SB ongoing: CAR⁺ T cells**
- Manufacture →~2 weeks
- Cryopreserve
- Release testing 14 → 7 days
- Revise CAR design, shorten manufacture and release testing

**3rd generation planned 2018: CAR⁺mbIL15*Switch⁺ T cells**
- Manufacture ≤ 2 days
- Release testing Same day
- Very rapid (< 2 days) manufacture without need for feeder cells, administer fresh T cells
- Publication #: 1324
It takes a village…

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