Next-generation non-viral gene transfer to redirect T-cell specificity

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Overview

To improve therapeutic potential and shorten the time for *ex vivo* manufacture of T cells genetically modified using the *Sleeping Beauty* system to stably express CD19-specific CARs
**Sleeping Beauty (SB) system transposon/transposase**

- **Transposon DNA plasmid**
  - IR/DR
  - hEF1α
  - CAR

- **Transposase DNA plasmid**
  - CMVIE
  - SB11 Transposase

Co-delivery into cells by nucleofection (Lonza)

**Diagram:**
- **Nucleus**
- **Cytoplasm**
- **Transposase**
- **CAR**
- **Transposon**
- **Co-delivery**

Transposon DNA plasmid (or in vitro transcribed mRNA)
Non-viral gene transfer in compliance with current good manufacturing practice (cGMP)

Blood draw → Sleeping Beauty → AaPC & IL-2/21 → Products

- Electroporation of DNA plasmids
- 28 days
- 4 (stim) additions of γ-irradiated AaPC
Non-viral delivery: *Sleeping Beauty* CAR$^+$ T-cell platform (first-in-human studies)

Long term follow-up data from 1st generation *Sleeping Beauty* platform in two trials infusing CAR$^+$ T cells after hematopoietic stem-cell transplantation (HSCT)

- Showed approximate doubling PFS or OS in both autologous and allogeneic cohorts
- Non-viral *Sleeping Beauty* T-cell survival compared favorably versus viral approaches

*CAR* = CD19RCD28  

<table>
<thead>
<tr>
<th>CAR</th>
<th>VL</th>
<th>VH</th>
<th>IgG4-Fc</th>
<th>CD28</th>
<th>CD3z</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD19RCD28</td>
<td></td>
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Designs of CARs

- CARs signal through chimeric CD28 and CD3-ζ
- CARs differ in the type and length of extracellular spacer

<table>
<thead>
<tr>
<th>Design</th>
<th>Structure</th>
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<tbody>
<tr>
<td>CD19RCD28</td>
<td>VL</td>
</tr>
<tr>
<td>CD19R*CD28</td>
<td>VL</td>
</tr>
<tr>
<td>CD19RCD8CD28</td>
<td>VL</td>
</tr>
<tr>
<td>CD19R12aaCD28</td>
<td>VL</td>
</tr>
</tbody>
</table>

- EQ mutant CARs: contain L235E and N297Q mutations in the CH2 region of the IgG4 Fc spacer.

Wild type IgG4 Fc spacer

EQ mutant CARs

CD8 alpha

IgG4 Hinge

12aa Spacer
Phenotype of 28-day AaPC (4-stim) propagated CAR⁺ T cells
Phenotype of 28-day AaPC (4-stim) propagated CAR+ T cells
Specificity for CD19 by CAR+ T cells after 28 days (4-stim) of co-culture on AaPC
CD8 stalk improves *in vivo* anti-tumor effect of CAR⁺ T cells propagated for 28 days (4 stim) on AaPC
Shorten *ex vivo* time in culture from 28 to 14 days

Blood draw → *Sleeping Beauty* → AaPC & IL-2/21 → Products

- 28 days
- 4 (stim) additions of $\gamma$-irradiated AaPC

Blood draw → *Sleeping Beauty* → AaPC & IL-2/21 → Products

- 14 days
- 2 (stim) additions of $\gamma$-irradiated AaPC
Phenotype of CAR⁺ T cells propagated for 28- (4 stim) versus 14- (2 stim) days on AaPC

CD19RCD8CD28

Inferred CAR⁺ Cell Counts

Days of culture

Day 28
4x AaPC
28 days

Day 28
2X AaPC
14 days

Day 1
Propagation of CAR⁺ T cells after 14 days (2-stim) on AaPC leads to improved outgrowth of naïve/memory populations

Cells gated on CAR⁺ cells

Propagation of CAR$^+$ T cells after 14 days (2-stim) on AaPC maintains a naïve-memory and less exhausted transcriptional profiles

Exhaustion/Inhibition

Naïve/Memory

Effector Molecules
Propagation of CAR$^+$ T cells after 14 days (2-stim) on AaPC improves anti-tumor effect

CD19$^+$ NALM-6 tumor model in NSG mice

**p<0.01
Propagation of CAR+ T cells after 14 days (2-stim) on AaPC improves persistence

CD19RCD8CD28-4x
C2-0; Day 43

Blood

CD3-APC

GFP

100 101 102 103 104

GFP

98.14% 0.00%
1.44% 0.43%

Spleen

CD3-APC

GFP

100 101 102 103 104

GFP

99.60% 0.01%
0.39% 0.00%

BM

CD3-APC

GFP

100 101 102 103 104

GFP

98.81% 0.05%
0.80% 0.35%
Propagation of CAR⁺ T cells after 14 days (2-stim) on AaPC improves persistence

CD19RCD8CD28-2x
D1-2; Day 50

Blood

Spleen

BM

CD3-PE

anti-CD19scfv(136.20.1)-AF647

GFP

GFP (Tumor)

CD3

CD3

0.00% 0.00%
2.25% 97.75%
97.97%
20.70%
79.30%
18.29%
2.48%
1.99%
0.01%
0.00% 0.00%
0.00% 0.00%
0.00% 0.00%
SB11 was not detected in genetically modified T cells recovered from mice

Tissues from mice infused with CAR\(^+\) T cells after 14 days (2-stim) on AaPC

**Diagram:**
- M
- SB
- G
- SB
- G
- SB
- G
- SB
- G
- SB
- G
- SB

**Legend:**
- G = GAPDH Primers
- SB = SB11 Primers
Summary

• CD8 stalk improves anti-tumor effect of CD19-specific CAR⁺ T cells

• Propagating with 2-stim (14-day) on γ-irradiated AaPC results in improved:
  • Outgrowth of T cells with naïve/memory phenotype and genotype
  • Persistence of administered T cells
  • Anti-tumor effect in vivo
Acknowledgments