Eradication of Established CD19+ Leukemia using a Single Injection of Chimeric Immunoreceptor Modified Lentiviral Transduced T Cells in a Xenograft NOG Mouse Model


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Disclosure

- The author has no relevant financial disclosures or conflicts of interest.
- This involves no off label use of any medications.
Overview

• Acute Lymphoblastic Leukemia (ALL) is the most common childhood malignancy, and relapsed ALL is the fifth most common as well as the most common cause of cancer death in children

• Higher doses of chemotherapy for relapsed/refractory disease come with increased toxicity (and not that much more success)

• Donor derived T-cells studied as part of the Graft Versus Leukemia Effect in allogeneic transplant are effective in eradicating hematologic malignancies but at the cost of Graft Versus Host disease

• Chimeric Immunoreceptors are artificial constructs fusing the extracellular antigen-recognition portion of an immunoglobulin with activation and costimulatory domains of T-cell receptors

• Chimeric Immunoreceptors expressed in Autologous T-cells provide the potential for cytotoxic efficacy without Graft Versus Host disease
Chimeric Immunoreceptor

Tumor Ag

MHC
Independent
Antigen
Engagement
and Induction of
Signalling

Proliferation

Cytokine production

APC

CD40L
PD-1L
ICOS-L
CD80/86
MHC
CD137L
CD80/86

T-Cell

CD40
PD-1
ICOS
CTLA-4
TCR-zeta
CD4 or CD8
CD137
CD28

CD19 as a tumor target antigen

- CD19 is expressed by the majority of B-cell derived tumors
- Normal CD19 expression is limited to only B-lineage lymphoid cells
- B-cell deficiency is well tolerated given extensive experience with rituximab (anti CD20 antibody), and is treatable with IV immunoglobulin
Incorporating co-stimulatory signals

- CD28 provides potent activating signals that enhance CD4 and CD8 T cell:
  - Proliferation
  - Survival
  - Cytokine production

- 4-1BB (CD137) provides signals that:
  - Promote long-term proliferation and survival of CD8 T cells
  - Promote cytokine production
  - Enhance CD8+ T cell responses in viral infection and allograft rejection
Pre-clinical Experimental Model

Artificial APC Beads with anti CD3/CD28

CD4+ and CD8+ T-Cells

Leukemia

Self Inactivating Lentiviral vector

promoter
cPPT Long form
partial gag sequence
HIV 5'LTR R/U5
RSV U3
HIV-1 3'LTR w/ deletion
Woodchuck Hep PR E
CAR gene cassette

HIV 5'LTR R/U5 partial gag sequence
Methods

Tail vein injection of $1 \times 10^7$ transduced T-cells (5x$10^6$ CD4 and 5x$10^6$ CD8) of which 70% are CAR +

Tail vein injection of $1 \times 10^6$ Nalm-6

Day 0
Day 7
Day 14
Day 21
Day 28
Day ??

Weight, activity, appearance and absolute counts CD10, CD3, CD4, CD8 and CD19
Chimeric Immunoreceptors

\[ \alpha_{\text{meso-BB-\(\zeta\)}} \]
\[ \alpha_{\text{CD19-\(\zeta\)}} \]
\[ \alpha_{\text{CD19-BB-\(\zeta\)}} \]
\[ \alpha_{\text{CD19-CD28-\(\zeta\)}} \]
\[ \alpha_{\text{CD19-CD28-BB-\(\zeta\)}} \]

- **CD8\(\alpha\) leader**
- **\(V_H\)**
- **GGGSx4 linker**
- **\(V_L\)**
- **CD8\(\alpha\)**
- **CD28**
- **TCR-\(\zeta\)**
- **4-1BB intracellular domain**

TM
ND150 CAR Positive T cells Day of Injection

mock 19-BBz 19-28z

19-Zeta SS1-BBz 19-28BBz

scFv expression
Overall Survival

Percent survival vs Days

- mock
- 19-zeta
- 19-28-zeta
- 19-28-41BB-zeta
- 19-41BB-zeta
- meso-41BB-zeta
- Saline
Overall Survival

![Graph showing overall survival over days with different treatment groups represented by lines and markers.]
Overall Survival

![Overall Survival graph](image)

Legend:
- **mock**
- **19-zeta**
- **19-28-zeta**
- **19-28-41BB-zeta**
- **19-41BB-zeta**
- **meso-41BB-zeta**
- **Saline**
Overall Survival

![Overall Survival Graph]

- **mock**
- **19-zeta**
- **19-28-zeta**
- **19-28-4 BB-zeta**
- **19-4 BB-zeta**
- **meso-4 BB-zeta**
- **Saline**

Days

Percent survival

Overall Survival
Overall Survival

![Graph showing overall survival over time with different treatment groups. The x-axis represents days, ranging from 0 to 200. The y-axis represents percent survival, ranging from 0 to 100. Different lines correspond to different treatment groups, such as mock, 19-zeta, 19-28-zeta, 19-28-41BB-zeta, 19-41BB-zeta, meso-41BB-zeta, and saline.]
Influence of Xenogeneic Graft Versus Host Disease

Overall Survival

Overall Survival – GVHD Excluded

<table>
<thead>
<tr>
<th>Group</th>
<th>Median OS</th>
<th>Median OS (GVHD excluded)</th>
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<tbody>
<tr>
<td>Saline</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Mock</td>
<td>24</td>
<td>24</td>
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<tr>
<td>Meso-41BB-zeta</td>
<td>22</td>
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<tr>
<td>19-zeta</td>
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<td>19-41BB-zeta</td>
<td>41*</td>
<td>57</td>
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<tr>
<td>19-28-41BB-zeta</td>
<td>79*</td>
<td>80*</td>
</tr>
</tbody>
</table>

*p<0.001 (mock)
Total Peripheral Blood T Cells correlate with onset of GVHD
Disease Progression or Relapse

Splenic, Bone Marrow or CNS Relapse

Percent survival

Days

Parent Nalm-6 Relapse

Facial Tumor Spleen

huCD19

huCD3

mock

19-zeta

19-28-zeta

19-28-41BB-zeta

19-41BB-zeta

meso-41BB-zeta

Saline

huCD19

huCD3
Summary

- Immunoreceptor modified T cells generated by lentiviral transfection of \textit{ex vivo} expanded T cells can effectively eradicate human leukemia in a xenograft NOG mouse model with only a single injection of $1 \times 10^7$ T cells.
- Slight survival advantage over other constructs is observed with the 19-28-41BB-$\zeta$ CAR
  - Including apparent eradication of disease (survival $>100$ days post disappearance of circulating T cells, $>60$ days since any relapse in any other group)
- High total circulating T cells correlates with xenogeneic GVHD mortality in the absence of detectable leukemia
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