GM-CSF Blockade during Chimeric Antigen Receptor T Cell Therapy Reduces Cytokine Release Syndrome and Neurotoxicity and May Enhance Their Effector Functions

Hefazi M¹, Sterner RM¹,², Sakemura R¹, Cox MJ¹, Yang N¹, Khadka RH¹, Forsman CL¹, Hansen MJ³, Jin F³, Ayasoufi K³, Schick KJ⁴, Walters DK³, Ahmed O⁵, Chappell D⁵, Sahmoud T⁵, Durrant C⁵, Nevala WK³, Patnaik MM¹, Pease LR³, Hedin KE³, Kay NE¹, Johnson AJ³, Kenderian SS¹,³.

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CRS and neurotoxicity post CAR-T cell therapy

- CAR-T cell therapy is limited by the development of CRS and NT
- CRS is related to elevation of cytokines and T cell expansion
- Exact NT mechanism unknown

**Modified from Locke et al, ASH 2017**

**Neelapu et al, NEJM 2017**

**Gust et al, Can Discov 2017**

**Santomasso et al, Can Discov 2018**

**Cell Population**

<table>
<thead>
<tr>
<th></th>
<th>NE Grade 0-2 (N=15)</th>
<th>NE Grade ≥3 (N=10)</th>
<th>NE Grade ≥3 / Grade 0-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD14+ (myeloid cells)</td>
<td>18 (11, 436)</td>
<td>306 (125, 1063)</td>
<td>17</td>
</tr>
</tbody>
</table>

*Modified from Locke et al, ASH 2017*
GM-CSF depletion during CAR-T cell therapy

Does GM-CSF blockade impair CAR-T cell function?

Does GM-CSF blockade ameliorate CAR-T cell associated toxicities?
GM-CSF neutralization does not inhibit CAR-T cell effector functions

- Anti-human GM-CSF ab 10mg/kg daily x 10 days
- Control antibody 10mg/kg daily x 10 days
- 1-1.5x10^6 CART19 or UTD
- Anti tumor effect
- Survival
GM-CSF neutralization does not inhibit CAR-T cell effector functions

- Anti-human GM-CSF ab 10mg/kg daily x 10 days
- Control antibody 10mg/kg daily x 10 days
- UTD + control ab
- UTD + GM-CSF ab
- CART19 + control ab
- CART19 + GM-CSF ab

Graph showing survival rates and anti-tumor effect over time.
GM-CSF neutralization enhances CAR-T cell effector function in a patient-derived xenograft model

- **Patient ALL 2x10^6**
- **NSG mice 10-13 weeks**
- **Bleeding**
- **Anti-human + anti-murine GM-CSF ab 10mg/kg daily x 10 days**
- **PBMCs 1x10^6**
- **2.5x10^6 CART19**
- **Control antibodies 10mg/kg daily x 10 days**
- **2.5x10^6 CART19**

**Graph:**
- **Before Treatment**
- **Day 10 Post CART**
- **Day 35 Post CART**
- **Before Treatment**
- **Day 10 Post CART**
- **Day 35 Post CART**

- **CD19+ cells/ul**
- **CART19 + control ab**
- **CART19 + GM-CSF ab**
- **Significance:**
  - **ns**
  - ****
  - ****
  - ****
GM-CSF CRISPR knockout CAR-T cells exhibit reduced production of GM-CSF and enhanced anti-tumor activity
GM-CSF depletion after CAR-T cell therapy

Does GM-CSF blockade impair CAR-T cell function?

Does GM-CSF blockade ameliorate CAR-T cell associated toxicities?
GM-CSF neutralization in a patient-derived xenograft model for CRS and NT

Busulfan
NSG mice
Patient B-ALL Blasts 1-3x10^6

CART19
High Dose

anti-human + anti-murine GM-CSF ab or control antibodies

Bleeding Engraftment

MRI Brain Tissue
Weight Cytokines

~10–13 weeks

High Burden
GM-CSF neutralization *in vivo* prevents CRS after CART19 therapy in a xenograft model.

### Human Cytokines and Chemokines

<table>
<thead>
<tr>
<th>Untreated</th>
<th>IgG</th>
<th>GM-CSF Ab</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF</td>
<td>0.8</td>
<td>0.6</td>
</tr>
<tr>
<td>FGF-2</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>IL-12</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>IFN-α</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>IL-13</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>IL-18</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>IL-17α</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>IL-5</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>MIP-1b</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>MCP-1</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>IP-10</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>KC</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>MIP-1α</td>
<td>0.4</td>
<td>0.4</td>
</tr>
</tbody>
</table>

### Mouse Cytokines and Chemokines

<table>
<thead>
<tr>
<th>Untreated</th>
<th>IgG</th>
<th>GM-CSF Ab</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM-CSF</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>IL-1α</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>IL-1β</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>IL-2</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>IL-4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>IL-9</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>IP-10</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>KC</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>MCP-1</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>MIG</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>
GM-CSF neutralization *in vivo* ameliorates NT after CART19 therapy in a xenograft model

**Graph:**

- Untreated
- CART19 + Isotype Control
- CART19 + GM-CSF Ab

**Day 5**

**Legend:**

- **Gadolinium enhanced T1-hyperintensity** (cubic mm)
- **Bar chart** showing statistical significance:
  - Untreated
  - CART19 + Isotype Control
  - CART19 + GM-CSF Ab

**Significance:**

- **** indicates significant difference (2-tailed t-test, **p** < 0.01)
- * indicates significant difference (2-tailed t-test, *p* < 0.05)
GM-CSF neutralization *in vivo* ameliorates NT after CART19 therapy in a xenograft model

- Untreated xenografts
- CART19 + control ab
- CART19 + GM-CSF ab

hCD3+ cells/hemisphere

- Untreated xenografts
- CART19 + control ab
- CART19 + GM-CSF ab

Murine CD11b bright%

- Untreated xenografts
- CART19 + control ab
- CART19 + GM-CSF ab

CD19+ cells/hemisphere
Conclusions

- GM-CSF blockade does not impair CAR-T cell functions
- GM-CSF blockade enhances CAR-T cell activity in certain models
- GM-CSF blockade ameliorates CAR-T cell associated toxicities

Clinical trials of CART19 combination with lenzilumab are planned
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Questions and Discussion
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