Supplemental Data

Absolute Lymphocyte Count Kinetics after CAR T Cell Infusion Impacts Response and Relapse

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Short title: ALC and Morphology after CAR infusion

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Supplemental Figure Legends

Supplemental figure 1. Kinetics of lymphocyte activation and WBC recovery after CAR infusion. A. Immunoblasts in day 4 followed by atypical LGL with polarized cytotoxic granules in days 7-17 in representative patient HP-123. B. ALC shows characteristic expansion and contraction in weeks 2 followed by count recovery in weeks 4. C. Representative example of Downey cells and LGLs in acute EBV infection.

Supplemental figure 2. Kinetics and morphology of in-vitro stimulated CAR T cells. A. Kinetics of plate bound anti-CD3/CD28 and CD19-stimulated CD3, CD4 and CD8 CAR T cells and non-transduced controls. Rapid increase in cell volume followed by contraction is noted in plate bound anti-CD3/CD28 and CD19 stimulated CD3 CAR, CD4 CAR and CD8 CAR T cells but not in non-transduced controls (not depicted). B. Concurrent morphological images of plate bound CD19-stimulated CAR CD3, CD4 and CD8 T cells and non-transduced controls showing increase in cell size, basophilic cytoplasm with blebbing at the 72h and 168h time points followed by return to baseline at 216h. Pure red blood cells were added after harvesting of cell cultures to simulate clinical peripheral blood smears.

Supplemental figure 3. ALC kinetics closely track absolute CD3 T cell counts and overall kinetics. A. ALC (red) and absolute CD3 counts (black) in the first month showing close overlap when tested concurrently. Frequent ALC capture variation in kinetics better than infrequent CD3 counts. All patients were initial responders except for HP-132 who was a non-responder and relapsed with CD19-negative disease at 1 month.

Supplemental Figure 4. A. Linear regression analysis of lymphocyte expansion parameters and extent of tumor involvement in whole cohort (left). B. ROC of peak ALC in patients with early
loss versus no loss of B cell aplasia group. C Correlation matrix of ALC parameters and CSF count
Supplemental Figure 1.

A. 
B. 
C.
Supplemental Figure 2.

A. Median cell volume (fl) over time (hours) for CD3, CD4, and CD8 CARs.

B. Images showing cell morphology at 0h, 72h, 168h, and 216h for NTD, CD3, CD4, and CD8 CARs.
Supplemental Figure 3.
Supplemental Figure 4.

A. % Bone marrow involvement pre CAR

B. Sensitivity% vs 100% - Specificity%

C. Heatmap with values:
- Peak ALC
- ALC AUC1mon
- d28 CSF

Values:
- Peak ALC:
  - 1.00
  - 0.78
  - 0.32
- ALC AUC1mon:
  - 0.78
  - 1.00
  - 0.31
- d28 CSF:
  - 0.32
  - 0.31
  - 1.00
<table>
<thead>
<tr>
<th></th>
<th>% Bone marrow disease</th>
<th>Peak ALC/µl</th>
<th>Day of peak</th>
<th>AUC 1mon</th>
<th>Peak ALC expansion</th>
<th>1 mon ALC expansion</th>
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</thead>
<tbody>
<tr>
<td>Non-responder</td>
<td>70 (80)</td>
<td>842.9 (770)</td>
<td>11.7 (11)</td>
<td>8837 (8781)</td>
<td>20.26 (9.6)</td>
<td>8.1 (4.0)</td>
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<td>Sustained responder</td>
<td>13.23 (0)</td>
<td>3034.3 (1428)</td>
<td>11.6 (10)</td>
<td>27607.8 (20355)</td>
<td>47.71 (16.3)</td>
<td>18.4 (8.2)</td>
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<td>CD19-pos MRD/Relapse</td>
<td>25.5 (5)</td>
<td>1242.3 (900)</td>
<td>13.2 (6.3)</td>
<td>16295 (12835)</td>
<td>19.6 (10.4)</td>
<td>13.1 (6.3)</td>
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<tr>
<td>CD19-neg MRD/Relapse</td>
<td>42 (35)</td>
<td>2028.7 (1100)</td>
<td>12 (11)</td>
<td>19529 (14723)</td>
<td>45.8 (19.7)</td>
<td>26 (8)</td>
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<tr>
<td>Early loss of BCA</td>
<td>16.37*(0.7)</td>
<td>1462.6 (895)</td>
<td>12.5 (11)</td>
<td>18533.1 (15501)</td>
<td>14.5 (8.7)</td>
<td>11.2 (5.2)</td>
</tr>
<tr>
<td>No loss of BCA</td>
<td>24(0.45)</td>
<td>2899.4 (1400)</td>
<td>12 (10)</td>
<td>26248 (17936)</td>
<td>54.9 (19.7)</td>
<td>22.1 (10.4)</td>
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</tbody>
</table>

**Supplementary table 1.** Proportion of bone marrow involvement and ALC parameters stratified by eventual response and B cell aplasia (BCA). Mean and median values (in parentheses) are shown.