**ADOPTIVE T-CELL THERAPY FOR ACUTE LEUKEMIA TARGETING MULTIPLE TUMOR ASSOCIATED ANTIGENS**


Center for Cell and Gene Therapy, Texas Children’s Hospital, Baylor College of Medicine, Houston, Texas.

**BACKGROUND**

- Leukemia relapse remains the major cause of treatment failure in hematopoietic stem cell transplant (HSCT) recipients
- Donor lymphocyte infusions (DLIs) are not always effective and are associated with the risk of life-threatening graft-versus-host disease (GVHD)
- The adoptive transfer of T cells, genetically modified to express CD19-specific chimeric antigen receptors (CARs), has shown potent anti-leukemia activity in HSCT recipients with recurrent disease
- However, CD19-CAR T cells carry the inherent risk of immune escape since a single antigen is targeted, and is limited to malignancies of B-cell lineage
- To overcome these limitations, we now propose to target multiple tumor-associated antigens (multiTAA) expressed in B- and T-cell ALL with donor-derived, multiTAA-specific T cells.

**DESIGN AND METHODS**

- **Choosing optimal TAA**
  - We choose the following tumor-associated antigens that are overexpressed on the surface of leukemic cells

<table>
<thead>
<tr>
<th>TAA</th>
<th>Function</th>
<th>Expression in leukemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT1</td>
<td>Zn finger transcription factor</td>
<td>ALL: 70-90%</td>
</tr>
<tr>
<td>Survivin</td>
<td>Inhibitor of apoptosis</td>
<td>ALL: 65-70%</td>
</tr>
<tr>
<td>PRAME</td>
<td>Repressor of retinoic acid receptor function</td>
<td>ALL: 40-45%</td>
</tr>
</tbody>
</table>

**Generating multi TAA specific T-cells**

©FMC DC

- Pepmix loaded with WT1, Survivin, Prame
- 7 days: Initiation
- 16 days: Expansion
- 23 days: Cytokine cocktail

**CLINICAL TRANSLATION**

**Study outline**

- Designed a Phase 1 study for patients with high-risk ALL who undergo allogeneic HSCT.
- Donor-derived multiTAA specific T-cells are infused after day +30 following allogeneic HSCT in 3 escalating dose levels: 1) DL1: 5x10^6 cells/m^2 2) DL2: 1x10^7 cells/m^2 3) DL3: 2x10^7 cells/m^2
- Eligible patients can receive up to 6 multiTAA specific T-cell infusions, 4-6 weeks apart
- There are 2 groups on study: 1) Group A: As Adjuvant therapy for patients in remission and 2) Group B: Patients with relapsed disease after transplant

**Preliminary data**

To date, we have enrolled 14 patients and infused 10 patients with multiTAA specific T-cell lines on Group A

**Characterization of Multi TAA specific T-cells**

- Phenotype
- Specificity

**Clinical outcome**

- All infusions were well tolerated without any adverse events
- To date, infused patients have not experienced any Dose Limiting Toxicities, Graft-versus-Host disease or CRS

<table>
<thead>
<tr>
<th>ID</th>
<th>Age/G</th>
<th>Disease</th>
<th>Prior Treatments</th>
<th>Dose level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5/F</td>
<td>Ph-ALL</td>
<td>Induction chemo + Primary induction failure + MRD SCT</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>18/F</td>
<td>HR-ALL</td>
<td>Complete therapy for HR-ALL + Relapse + MRD SCT</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>18/F</td>
<td>Ph-ALL</td>
<td>Complete therapy for Ph-ALL + Relapse + MRD SCT</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>41/M</td>
<td>HR-ALL</td>
<td>HyperCVAD + Fludarabine + Cyclophosphamide</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>6/M</td>
<td>Ph-ALL</td>
<td>Induction chemo + Primary induction failure</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>48/F</td>
<td>HR-ALL</td>
<td>Complete therapy for HR-ALL + Relapse + MRD SCT</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>12/F</td>
<td>T-cell ALL</td>
<td>Complete therapy for T-ALL + Relapse + MRD SCT</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>18/M</td>
<td>HR-ALL</td>
<td>Induction chemo + Primary induction failure + MRD SCT</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>12/F</td>
<td>MPAL</td>
<td>Induction chemotherapy + MRD SCT</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>18/M</td>
<td>Ph-ALL</td>
<td>Relapsed on therapy for Ph-ALL + MRD SCT</td>
<td>3</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**

- Safe to date and feasible for both B-cell and T-cell ALL
- In-vivo expansion of tumor-antigen associated T-cells directed to target antigens and evidence of antigen spreading which may contribute to disease control.
- Adoptive transfer of multi TAA-specific T cells may present a promising addition to current immunotherapeutic approaches for prophylaxis for leukemic relapse in HSCT recipients.

Fig.1. Dendritic cells loaded with pepmixes are used as APCs. In the presence of a Th1-polarizing/pro-proliferative cytokine cocktail T-cells are repeatedly stimulated to activate multi TAA specific T-cells.

Fig.2. Donor-derived multi TAA specific T cell lines are predominantly comprised of T-cells and have polyclonal repertoire as assessed by Flow Cytometry.

Fig.3. Donor-derived multi T AA specific T cell lines (n=11) show antigen specificity as assessed by Elispot assay.

Fig.4. Elispot assays show evidence of expansion of multi-TAA specific T-cell expansion by week 4 post-infusion in all patients that remained in CR. The one patient who relapsed had no evidence of multiTAA expansion, despite 3 additional infusions.

Fig.5. In-vivo antigen cascade. Elispot assays show evidence of antigen spreading, probably contributing to tumor control.