Targeting lymphomas using non-engineered, multi-antigen specific T cells

Introduction

Immunotherapy is emerging as a potent therapy for a range of hematologic malignancies including lymphomas. Indeed adoptive transfer of T cells genetically engineered to express the CD19 chimeric antigen receptor (CAR) has now received FDA approval for the treatment of patients with refractory diffuse large B cell lymphomas (DLBCL). We have developed a non-engineered T cell-based therapy to treat patients with all types of lymphomas: Hodgkin’s (HL) and non-Hodgkin’s lymphomas (NHL). The approach uses single T cell lines that simultaneously target a range of tumor-associated antigens (TAA) that are frequently expressed by these tumors, including PRAME, SSX2, MAGE4, NY-ESO-1 and Survivin (Table 1). The use of whole antigen should remove the HLA restriction imposed by the use of transgenic TCRs specific for single peptides, while targeting multiple antigens simultaneously would reduce the risk of tumor immune evasion.

Clinical Outcomes

We first treated patients on the antigen escalation scheme (4 in each arm). None of the infused patients experienced infusion related toxicities, so we then proceeded with the dose escalation phase of the study. Of 18 patients who were infused as adjuvant therapy all but 2 remain in remission (range 3-42 months post-infusion).

Table 2: Clinical outcomes of patients treated on group A (adjunctive)

<table>
<thead>
<tr>
<th>ID</th>
<th>Age/sex</th>
<th>Disease</th>
<th>Prior Therapies</th>
<th>Response to mTAA T cells (duration)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39/M</td>
<td>DLBCL</td>
<td>ABVD → RICE → ASCT</td>
<td>CR (&gt;3 years)</td>
</tr>
<tr>
<td>2</td>
<td>78/F</td>
<td>DLBCL</td>
<td>R-ICEP + mTAA T cells → R-bendamustine</td>
<td>CR (&gt;3 years)</td>
</tr>
<tr>
<td>3</td>
<td>41/M</td>
<td>HL</td>
<td>ABVD → Bortezomib + Nav</td>
<td>CR (&gt;3 years)</td>
</tr>
<tr>
<td>4</td>
<td>34/M</td>
<td>HL</td>
<td>ABVD → ICE + ASCT + XRT to Brentuximab</td>
<td>CR (&gt;3 years)</td>
</tr>
<tr>
<td>5</td>
<td>14/M</td>
<td>DLBCL</td>
<td>R-ICEP + R-bendamustine</td>
<td>CR (&gt;3 years)</td>
</tr>
<tr>
<td>6</td>
<td>62/M</td>
<td>DLBCL</td>
<td>R-ICEP + XRT to Brentuximab + DMM</td>
<td>CR (&gt;3 years)</td>
</tr>
<tr>
<td>7</td>
<td>41/F</td>
<td>HL</td>
<td>ABVD → R-bendamustine + ICE + XRT</td>
<td>CR (&gt;3 years)</td>
</tr>
<tr>
<td>8</td>
<td>62/M</td>
<td>T cell</td>
<td>CHOP + XRT + ASCT</td>
<td>CR (&gt;3 years)</td>
</tr>
<tr>
<td>9</td>
<td>53/M</td>
<td>Mantle</td>
<td>HyperCVP + R-bendamustine</td>
<td>CR (&gt;3 years)</td>
</tr>
<tr>
<td>10</td>
<td>35/M</td>
<td>HL</td>
<td>ABVD → Brentuximab + Bendamustine</td>
<td>CR (&gt;3 years)</td>
</tr>
<tr>
<td>11</td>
<td>75/F</td>
<td>DLBCL</td>
<td>R-ICEP + R-ESHAP + RIE</td>
<td>CR (&gt;3 years)</td>
</tr>
<tr>
<td>12</td>
<td>56/F</td>
<td>DLBCL</td>
<td>HyperCVP + ASCT</td>
<td>CR (&gt;3 years)</td>
</tr>
<tr>
<td>13</td>
<td>65/F</td>
<td>DDLBCL</td>
<td>CHOP + ASCT</td>
<td>CR (&gt;3 years)</td>
</tr>
<tr>
<td>14</td>
<td>32/P</td>
<td>T cell</td>
<td>CHOP + Brentuximab + Cetuximab</td>
<td>CR (&gt;3 years)</td>
</tr>
<tr>
<td>15</td>
<td>25/M</td>
<td>HL</td>
<td>ABVD → R-bendamustine + ICE + XRT</td>
<td>CR (&gt;3 years)</td>
</tr>
</tbody>
</table>

*Antigen escalation cohort

Fifteen patients have received multi-TAA-specific T cells to treat active disease, all of whom had failed a median of 4 lines of prior therapy. Of these, 5 had transient disease stabilization followed by disease progression, 4 have ongoing stable disease, 3-18 months post-multi-TAA-specific T cells while the remaining 6 (3 with HL and 3 with DLBCL) have all had complete and durable responses (4 to 41 months), as assessed by PET imaging (Table 3). None of the treated patients developed cytokine release syndrome, neurotoxicity or any other infusion related adverse events.

Table 3: Clinical outcomes of patients treated on group B (active)

<table>
<thead>
<tr>
<th>ID</th>
<th>Age/sex</th>
<th>Disease</th>
<th>Prior Therapies</th>
<th>Response to mTAA T cells (duration)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31/P</td>
<td>ABVD → RICE → ASCT + mTAA T cells</td>
<td>Stable disease (5 months) → OFF study (4 months)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>55/P</td>
<td>HL</td>
<td>R-ICEP + XRT to ICE + ASCT</td>
<td>CR (&gt;3 years)</td>
</tr>
<tr>
<td>3</td>
<td>38/P</td>
<td>HL</td>
<td>ABVD → ICE to ICE + ESHAP</td>
<td>CR (&gt;2 years ongoing)</td>
</tr>
<tr>
<td>4</td>
<td>44/F</td>
<td>HL</td>
<td>ABVD → ICE + ICE to Brentuximab</td>
<td>CR (&gt;3 years)</td>
</tr>
<tr>
<td>5</td>
<td>46/F</td>
<td>HL</td>
<td>ABVD → ICE + ICE to Bendamustine</td>
<td>CR (&gt;3 years)</td>
</tr>
<tr>
<td>6</td>
<td>46/F</td>
<td>HL</td>
<td>R-ICEP + ICE to ASCT</td>
<td>CR (&gt;3 years)</td>
</tr>
<tr>
<td>7</td>
<td>31/P</td>
<td>HL</td>
<td>ABVD → ICE to ICE + Nav + ASCT</td>
<td>Stable disease (5 months) → PD1</td>
</tr>
<tr>
<td>8</td>
<td>69/H</td>
<td>NY-ESO-1</td>
<td>R-ICEP + mTAA T cells to ASCT</td>
<td>Stable disease (&gt;3 years)</td>
</tr>
<tr>
<td>9</td>
<td>54/M</td>
<td>DLBCL</td>
<td>R-ICEP + 3ICE to ICE + ASCT</td>
<td>Stable disease (6 months) → PD1</td>
</tr>
<tr>
<td>10</td>
<td>18/P</td>
<td>HL</td>
<td>ABVD → ICE to FIVlor + Bendamustine</td>
<td>Stable disease (9 months) → PD1</td>
</tr>
<tr>
<td>11</td>
<td>18/P</td>
<td>HL</td>
<td>R-ICEP + R-ICEP + R-ICEP to ICE + ASCT</td>
<td>CR (&gt;1 year)</td>
</tr>
<tr>
<td>12</td>
<td>49/M</td>
<td>HL</td>
<td>ABVD → ICE + ICE + ICE to ICE + XRT to ICE + XRT + Bendamustine</td>
<td>PD (3 months)</td>
</tr>
<tr>
<td>13</td>
<td>54/M</td>
<td>DLBCL</td>
<td>R-ICEP + R-ICEP + R-ICEP + ASCT</td>
<td>Stable disease (6 months) → CR1</td>
</tr>
<tr>
<td>14</td>
<td>64/M</td>
<td>DLBCL</td>
<td>E-CHIP + E-CHIP + E-CHIP + ICE + ICE + ICE</td>
<td>PD (9 months)</td>
</tr>
<tr>
<td>15</td>
<td>68/M</td>
<td>DLBCL</td>
<td>R-ICEP + R-ICEP + R-ICEP + ASCT</td>
<td>Stable disease (4 months) to OD1</td>
</tr>
</tbody>
</table>

*Antigen escalation cohort

Responding patients

Six of 15 patients entered a durable CR, which correlated in the in vivo expansion of mTAA directed T cells. Shown in Figure 7 is an example of a patient with Hodgkin lymphoma with residual mediastinal disease despite undergoing an autologous hematopoietic stem cell transplant (ASCT). Eight weeks post-infusion, this patient enters a CR concomitant with an increase in the circulating frequency of targeted as well as non-targeted tumor antigen-specific T cells (Figure 7).

Figure 7: Complete responses in a patient with Hodgkin lymphoma correlates with expansion of infused mTAA-T cells along with antigen spreading.

Conclusions

Thus, infusion of autologous mTAA-targeted T cells directed to PRAME, SSX2, MAGE4, NY-ESO-1 and Survivin has been safe and provided durable clinical benefit to patients with lymphomas. Responses in all six patients who entered a CR were durable and associated with an expansion of infused T cells as well as the induction of antigen spreading.

Figure 8: Durable CR in a patient with DLBCL of the mesentery that was refractory to high dose chemo/ASCT correlates with in vivo expansion of mTAA-T cells and antigen spreading.

Three of the six CR patients had treatment refractory diffuse large B cell lymphoma. In one of these cases the patient initially developed a “tumor flare”, 3 months post-infusion which coincided with increasing levels of TA-A directed T cell in the circulation. Without additional therapies, the patient entered a complete response, 9 months post-infusion at which time point not only was there a robust increase in targeted TAA-specific T cells, but also non-targeted MAGE1-specific T cells indicating antigen spreading.

Figure 9: No CR in a patient with DLBCL despite expansion of infused T cells.
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

• Leukemic 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}s 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 over expressed 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

WT1, Survivin, Prame

Pepmix loaded DC

WT1, Survivin, Prame

Cytokine DC

7 days

16 days

23 days

Characterization of Multi TAA specific T-cells

Phenotype

Specificity

• Designed a Phase 1 study for patients with high-risk ALL who undergo allogeneic HSCT.
• Donor-derived mTAA 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 mTAA 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

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>Dose level</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5/F</td>
<td>Ph-ALL</td>
<td>1</td>
<td>Not evaluable*</td>
</tr>
<tr>
<td>2</td>
<td>18/F</td>
<td>HR-ALL</td>
<td>1</td>
<td>CR with mixed chimerism for 6 months—Relapse</td>
</tr>
<tr>
<td>3</td>
<td>18/F</td>
<td>Ph-ALL</td>
<td>1</td>
<td>Remains in CR (16 months)</td>
</tr>
<tr>
<td>4</td>
<td>41/M</td>
<td>HR-ALL</td>
<td>1</td>
<td>Remains in CR (22.4 months)</td>
</tr>
<tr>
<td>5</td>
<td>6/M</td>
<td>Ph-ALL</td>
<td>1</td>
<td>Remains in CR (8 months)</td>
</tr>
<tr>
<td>6</td>
<td>48/F</td>
<td>HR-ALL</td>
<td>2</td>
<td>Not evaluable</td>
</tr>
<tr>
<td>7</td>
<td>12/F</td>
<td>T-cell ALL</td>
<td>2</td>
<td>Remains in CR (11 months)</td>
</tr>
<tr>
<td>8</td>
<td>18/M</td>
<td>HR-ALL</td>
<td>2</td>
<td>Remains in CR (9 months)</td>
</tr>
<tr>
<td>9</td>
<td>12/F</td>
<td>MPAL</td>
<td>3</td>
<td>Remains in CR—recent infusion</td>
</tr>
<tr>
<td>10</td>
<td>16/M</td>
<td>Ph-ALL</td>
<td>4</td>
<td>Remains in CR—recent infusion</td>
</tr>
</tbody>
</table>

CLINICAL TRANSLATION

Study outline

Preliminary data

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

<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</td>
<td>MRD SCT</td>
</tr>
<tr>
<td>2</td>
<td>18/F</td>
<td>HR-ALL</td>
<td>Completed therapy for HR-ALL</td>
<td>MRD SCT</td>
</tr>
<tr>
<td>3</td>
<td>18/F</td>
<td>Ph-ALL</td>
<td>Completed therapy for Ph-ALL</td>
<td>MRD SCT</td>
</tr>
<tr>
<td>4</td>
<td>41/M</td>
<td>HR-ALL</td>
<td>HypoCVID + Damonubicel &amp; Ty Cycles</td>
<td>MRD SCT</td>
</tr>
<tr>
<td>5</td>
<td>6/M</td>
<td>Ph-ALL</td>
<td>Induction chemo</td>
<td>MRD SCT</td>
</tr>
<tr>
<td>6</td>
<td>48/F</td>
<td>HR-ALL</td>
<td>Induction chemo</td>
<td>MRD SCT</td>
</tr>
<tr>
<td>7</td>
<td>12/F</td>
<td>T-cell ALL</td>
<td>Induction chemo for T-ALL</td>
<td>MRD SCT</td>
</tr>
<tr>
<td>8</td>
<td>18/M</td>
<td>HR-ALL</td>
<td>Induction chemo</td>
<td>MRD SCT</td>
</tr>
<tr>
<td>9</td>
<td>12/F</td>
<td>MPAL</td>
<td>Induction chemo</td>
<td>MRD SCT</td>
</tr>
<tr>
<td>10</td>
<td>16/M</td>
<td>Ph-ALL</td>
<td>Relapsed on therapy for Ph-ALL</td>
<td>MRD SCT</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
Safety and efficacy of multi-TAA-T cells for Myeloma

# Problems with myeloma therapy

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexamethasone</td>
<td>Infections, osteoporosis</td>
</tr>
<tr>
<td>Melphalan</td>
<td>Immunosuppression, second cancers</td>
</tr>
<tr>
<td>Thalidomide</td>
<td>Neuropathy, Clots, anemia</td>
</tr>
<tr>
<td>Lenalidomide</td>
<td>Clots, anemia, second cancers</td>
</tr>
<tr>
<td>Bortezomib</td>
<td>Neuropathy, viral infections</td>
</tr>
<tr>
<td>ASCT</td>
<td>Immunosuppression, infections</td>
</tr>
</tbody>
</table>

New therapies needed
MultiTAA T cell therapy for MM

MAGEA4
PRAME
Survivin
NYESO1
SSX2

MultiTAA T cells
• Simultaneously target multiple TAAs
MultiTAA-T Cell Generation

Overlapping pepmixes

DC

PBMCs

T cells

Expansion
Profile of MultiTAA-T cells

Phenotype

% Positive cells

% Specific lysis

Safety

CD3+ CD4+ CD8+ DR+/

RO+/

62L+

RO+/

62L−

DR+/

CD83+
MultiTAA T cell specificity/polyclonal diversity

Clonal diversity (\(v\beta\) deep sequencing)

SFC/2x10^5

PRAME  SSX2  MA4  NYESO1  Survivin

n=10

mean = 4,597 clones
Clinical trial design
- Dose escalation (ARM A and B)

PRAME/SSX/MAGE/NYESO1/Survivin-specific T cells:
2-4 pts at each level, 2 infusions 14 days apart

Dose Level 1:
Day 0 and 14: $5 \times 10^6$ cells/m$^2$

Dose Level 2:
Day 0 and 14: $1 \times 10^7$ cells/m$^2$

Dose Level 3:
Day 0 and 14: $2 \times 10^7$ cells/m$^2$
Clinical Trial - Eligibility

- Any patient $\geq 18$ yrs with myeloma diagnosis (post completion of at least 1 treatment regimen)

<table>
<thead>
<tr>
<th>Group A:</th>
<th>$&gt;90$ days post autologous or syngeneic transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group B:</td>
<td>$&lt;90$ days post autologous or syngeneic transplant</td>
</tr>
</tbody>
</table>

- No lymphodepletion
### Group A:

<table>
<thead>
<tr>
<th>ID</th>
<th>Age/G</th>
<th>Disease</th>
<th>DL</th>
<th>Prior Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>53/M</td>
<td>IgG-kappa</td>
<td>1</td>
<td>Bor/Dex → ASCT</td>
</tr>
<tr>
<td>6</td>
<td>61/M</td>
<td>IgG-kappa</td>
<td>1</td>
<td>RVD → ASCT</td>
</tr>
<tr>
<td>7</td>
<td>44/M</td>
<td>IgG-kappa</td>
<td>1</td>
<td>CyBorD → ASCT</td>
</tr>
<tr>
<td>14</td>
<td>47/M</td>
<td>IgG-kappa</td>
<td>2</td>
<td>RVD → ASCT</td>
</tr>
<tr>
<td>3*</td>
<td>65/F</td>
<td>IgG-kappa</td>
<td>1</td>
<td>RVD → ASCT → CyBorD → Carf/D → ASCT</td>
</tr>
<tr>
<td>13</td>
<td>31/F</td>
<td>IgG-kappa</td>
<td>2</td>
<td>VD</td>
</tr>
<tr>
<td>10</td>
<td>69/F</td>
<td>IgG-kappa</td>
<td>2</td>
<td>VD → ASCT → R → Pom/Carf/D</td>
</tr>
<tr>
<td>15</td>
<td>70/M</td>
<td>IgA-kappa</td>
<td>3</td>
<td>RVD → ASCT → R-vidaza → Pom/D → ibrutinib/Carf → dinaciclib/VD → CyBorD → Daratumumab → RD-Elot → Ixa/RD</td>
</tr>
<tr>
<td>2*</td>
<td>40/M</td>
<td>Free lambda</td>
<td>2</td>
<td>RVD → ASCT → Pom/Carf/D → ASCT → mTAA T cells</td>
</tr>
<tr>
<td>18</td>
<td>50/F</td>
<td>Free Kappa</td>
<td>3</td>
<td>VD → ASCT → Dara/VD → XRT → ASCT</td>
</tr>
<tr>
<td>20</td>
<td>57/M</td>
<td>IgG-lambda</td>
<td>3</td>
<td>RVD → ASCT → R → VD → Pom/D → KPD → ASCT → Ixa → Dara/D</td>
</tr>
</tbody>
</table>
### Patients Infused

#### Group A:

<table>
<thead>
<tr>
<th>ID</th>
<th>Age/G</th>
<th>Disease</th>
<th>Marrow</th>
<th>Prior Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>53/M</td>
<td>Active</td>
<td>10%</td>
<td>Bor/Dex → ASCT</td>
</tr>
<tr>
<td>6</td>
<td>61/M</td>
<td>In remission</td>
<td>0%</td>
<td>RVD → ASCT</td>
</tr>
<tr>
<td>7</td>
<td>44/M</td>
<td>In remission</td>
<td>0%</td>
<td>CyBorD → ASCT</td>
</tr>
<tr>
<td>14</td>
<td>47/M</td>
<td>Active</td>
<td>0% (MRD+)</td>
<td>RVD → ASCT</td>
</tr>
<tr>
<td>3</td>
<td>65/F</td>
<td>Active</td>
<td>90%</td>
<td>RVD → ASCT → CyBorD → Carf/D → ASCT</td>
</tr>
<tr>
<td>13</td>
<td>31/F</td>
<td>Active</td>
<td>4%</td>
<td>VD → ASCT → R → Pom/Carf/D</td>
</tr>
<tr>
<td>10</td>
<td>69/F</td>
<td>Active</td>
<td>10%</td>
<td>VD → ASCT → R-vidaza → Pom/D → ibrutinib/Carf → dinaciclib/VD → CyBorD → Daratumumab → RD-Elot → Ixa/RD</td>
</tr>
<tr>
<td>15</td>
<td>70/M</td>
<td>Active</td>
<td>80%</td>
<td>RVD → ASCT → Pom/Carf/D → mTAA T cells</td>
</tr>
<tr>
<td>2</td>
<td>40/M</td>
<td>Active</td>
<td>15%</td>
<td>RVD → ASCT → Pom/Carf/D → ASCT → mTAA T cells</td>
</tr>
<tr>
<td>18</td>
<td>50/F</td>
<td>In remission</td>
<td>0%</td>
<td>VD → ASCT → Dara/VD → XRT → ASCT</td>
</tr>
<tr>
<td>20</td>
<td>57/M</td>
<td>Active</td>
<td>5%</td>
<td>RVD → ASCT → R → VD → Pom/D → KPD → ASCT → Ixa → Dara/D</td>
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<td>DL</td>
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<td>---------------</td>
<td>----</td>
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<tr>
<td>2</td>
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<td>Free lambda</td>
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<td>IgG-lambda</td>
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<tr>
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<td>Free lambda</td>
<td>2</td>
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<tr>
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<td>IgG-kappa</td>
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<td>Disease</td>
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<td>-------</td>
<td>-----------------</td>
<td>--------</td>
<td>------------------------------------------</td>
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<td>In remission</td>
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</tr>
<tr>
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<td>50/F</td>
<td>In remission</td>
<td>0%</td>
<td>RVD → ASCT</td>
</tr>
<tr>
<td>11</td>
<td>53/M</td>
<td>In remission</td>
<td>0%</td>
<td>VD → RVD → ASCT</td>
</tr>
<tr>
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<td>54/M</td>
<td>In remission</td>
<td>0%</td>
<td>RVD/rituximab → Rd → ASCT</td>
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<tr>
<td>17</td>
<td>44/F</td>
<td>Active</td>
<td>0% (MRD+)</td>
<td>VRD → KD → ASCT</td>
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<tr>
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<td>70/M</td>
<td>In remission</td>
<td>0%</td>
<td>XRT → VD → ASCT → R → VD → KPD → ASCT</td>
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## Clinical Outcomes

### Active Disease:

<table>
<thead>
<tr>
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<th>Disease</th>
<th>Marrow</th>
<th>Week 6</th>
<th>Wk 6</th>
<th>Mo12</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>53/M</td>
<td>Active</td>
<td>10%</td>
<td>Unknown</td>
<td>SD</td>
<td>PR</td>
</tr>
<tr>
<td>14</td>
<td>47/M</td>
<td>Active</td>
<td>0% (MRD+)</td>
<td>0% (MRD+)</td>
<td>SD</td>
<td>SD</td>
</tr>
<tr>
<td>3*</td>
<td>65/F</td>
<td>Active</td>
<td>90%</td>
<td>85%</td>
<td>SD</td>
<td>PD (2m)</td>
</tr>
<tr>
<td>13</td>
<td>31/F</td>
<td>Active</td>
<td>4%</td>
<td>0%</td>
<td>SD</td>
<td>SD</td>
</tr>
<tr>
<td>10</td>
<td>69/F</td>
<td>Active</td>
<td>10%</td>
<td>10%</td>
<td>SD</td>
<td>PD (7m)</td>
</tr>
<tr>
<td>15</td>
<td>70/M</td>
<td>Active</td>
<td>80%</td>
<td>80%</td>
<td>SD</td>
<td>PD (3m)</td>
</tr>
<tr>
<td>2*</td>
<td>40/M</td>
<td>Active</td>
<td>15%</td>
<td>15%</td>
<td>SD</td>
<td>SD (3m)</td>
</tr>
<tr>
<td>2*</td>
<td>40/M</td>
<td>Active</td>
<td>20%</td>
<td>0%</td>
<td>CR</td>
<td>CR</td>
</tr>
<tr>
<td>3*</td>
<td>65/F</td>
<td>Active</td>
<td>15%</td>
<td>10%</td>
<td>SD</td>
<td>PD (6m)</td>
</tr>
<tr>
<td>5</td>
<td>76/M</td>
<td>Active</td>
<td>20%</td>
<td>15%</td>
<td>SD</td>
<td>PR</td>
</tr>
<tr>
<td>17</td>
<td>45/F</td>
<td>Active</td>
<td>0% (0.4 g/dl)</td>
<td>0% (0.2 g/dl)</td>
<td>PR</td>
<td>PR (6m)</td>
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<tr>
<td>20</td>
<td>57/M</td>
<td>Active</td>
<td>5% (0.97 g/dl)</td>
<td>3% (0.53 g/dl)</td>
<td>SD</td>
<td>SD (3m)</td>
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In remission:

<table>
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<tr>
<th>ID</th>
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<th>Disease</th>
<th>Marrow</th>
<th>Week 6</th>
<th>Wk 6</th>
<th>Mo12</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>57/M</td>
<td>In remission</td>
<td>0%</td>
<td>0%</td>
<td>CCR</td>
<td>CCR</td>
</tr>
<tr>
<td>9</td>
<td>50/F</td>
<td>In remission</td>
<td>0%</td>
<td>0%</td>
<td>CCR</td>
<td>CCR</td>
</tr>
<tr>
<td>11</td>
<td>53/M</td>
<td>In remission</td>
<td>0%</td>
<td>0%</td>
<td>CCR</td>
<td>Relapse (7m)</td>
</tr>
<tr>
<td>12</td>
<td>54/M</td>
<td>In remission</td>
<td>0%</td>
<td>0%</td>
<td>CCR</td>
<td>CCR</td>
</tr>
<tr>
<td>6</td>
<td>61/M</td>
<td>In remission</td>
<td>0%</td>
<td>0%</td>
<td>CCR</td>
<td>CCR</td>
</tr>
<tr>
<td>7</td>
<td>44/M</td>
<td>In remission</td>
<td>0%</td>
<td>0%</td>
<td>CCR</td>
<td>CCR</td>
</tr>
<tr>
<td>19</td>
<td>70/M</td>
<td>In remission</td>
<td>0%</td>
<td>0%</td>
<td>CCR</td>
<td>CCR (6m)</td>
</tr>
<tr>
<td>18</td>
<td>50/F</td>
<td>In remission</td>
<td>0%</td>
<td>0%</td>
<td>CCR</td>
<td>CCR (8m)</td>
</tr>
</tbody>
</table>

Only one patient has relapsed at a median f/u of 21 months
Correlating clinical benefit with infused multiTAA T cells
How can we track non-gene-modified multiTAA T cells in vivo?

Rationale:

• In PBMCs (pre-infusion) tumor-specific T cell frequency v. low
  • below TCR \( \text{v}\beta \) deep sequencing detection threshold (1/100,000)

• Tumor-directed clones enriched in multiTAA T cells
  • Detectable by \( \text{v}\beta \) deep sequencing
How many “trackable” clones are present in our multiTAA T cells?

Clonal diversity in multiTAA T cells

mean = 4,597 clones

n=10
What drives in vivo multiTAA expansion?

• Patients enrolled on different arms depending on proximity to transplant [> (Grp A) or < (Grp B) 90 days]
  • Does post-transplant lymphodepletion impact expansion?

• Patients with and without disease enrolled on study
  • Does presence of antigen influence in vivo expansion?
Antigen drives multiTAA expansion – TCR tracking

Peripheral blood

- **Active disease**
- **No disease**

Production frequency

Post infusion

- Pre
- Early
- Late
Antigen drives multiTAA expansion – ELIspot

**Peripheral blood**

- **Active disease**
- **No disease**

**Active disease**

- **Survivin**
- **NYESO1**
- **MAGEA4**
- **SSX2**
- **PRAME**

**No disease**

**SFC/5x10^5 PBMCs**

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>wk6</th>
<th>mo6</th>
<th>mo12</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Active disease</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>No disease</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
T cell kinetics in responders
Clinical Response – Pt#2

- **Diagnosis**: Pre 1st ASCT
- **ASCT +6m**: Pre-T cells (day+61)
- **ASCT +22m**: Post-T cells (day+113)
- **Pre 2nd ASCT +27m**: MultiTAA T cells

**Key Points**

- **1st line**: 1st ASCT
- **2nd line**: 2nd ASCT
- **3rd line**: 2nd ASCT

**Free lambda mg/L**

- **0**: Diagnosis
- **100**: Pre 1st ASCT
- **1000**: ASCT +6m
- **10000**: ASCT +22m
- **100000**: Pre 2nd ASCT +27m
- **1000000**: MultiTAA T cells
- **100000000**: Month 6

**Notable Observations**

- 20% Clonal plasma cells

**Diagram**

- Red line indicates clinical response over time.
Clinical Response – Pt#2

- Pt#2
- <1% Clonal plasma cells
- Cyclin D1 neg

Diagnosis Pre 1st ASCT ASCT +6m ASCT +22m Pre 2nd ASCT +27m

Pre-T cells (day+61) Post-T cells (day+113) Month 6

Free lambda mg/L

1st line

1st ASCT

2nd line

2nd ASCT

3rd line

MultiTAA T cells

Normal
Clinical Response – Pt#2

PBMC

Targeted antigens

SFC/5x10^5

Pre Wk4 Wk6 Mo3

Targeted antigens:
- Survivin
- NYESO-1
- MAGE A4
- SSX2
- PRAME
Clinical Response – Pt#2

MAGE-A4

Pre-infusion

8wk post T cells

Marrow

Survivin
NYESO-1
MAGE A4
SSX2
PRAME

Clinical Response

Pt#2

MAGE-A4

3+

SFC/5x10^5

1+

0 60 120 180

6 Wk Post
In vivo T cell tracking – Pt#2

PBMC
multiTAA-derived clones

Marrow
multiTAA-derived clones

% Production Frequency

Pre Wk4 Wk6 Mo3

% Production Frequency

Wk 6 Post

multiTAA-derived clones
Immune escape post multiTAA T cells
Clinical Course - Pt#3

- **Diagnosis**
- PD+3y
- Pre-1st HSCT
- HSCT +2y
- Pre-2nd HSCT

- **Clinical Course**
- **Pt#3**
- 1st line
- 2nd line
- 3rd line

- **IgG kappa M-spike (g/dl)**

- **1st HSCT**

- **2nd HSCT**

- **MultiTAA T cells**

- **Pre-T cells** (d+47)  
- **Post-T cells** (d+89)

- **15% Clonal plasma cells**
Clinical Course - Pt#3

Clinical Course - Pt#3

- **Diagnosis**: First-line therapy (PD+3y)
- **Pre First HSCT**: HSCT +2y
- **Pre-T cells**: Pre-T cells (d+47)
- **Post-T cells**: Post-T cells (d+89)

**IgG kappa M-spike (g/dl)**

- **1st line**: MultiTAA T cells
- **2nd line**: 15% Clonal plasma cells
- **3rd line**: 10% Clonal plasma cells

**Clinical Course**

- **1st line**: 15% Clonal plasma cells
- **2nd line**: 10% Clonal plasma cells
- **2nd HSCT**: 15% Clonal plasma cells
- **1st HSCT**: 10% Clonal plasma cells
- **MultiTAA T cells**: 15% Clonal plasma cells

**Timeline**

- **Diagnosis**: Initial diagnosis
- **1st line**: First-line therapy
- **PD+3y**: Progression to disease
- **Pre 1st HSCT**: Preparatory phase for first HSCT
- **HSCT +2y**: Two years post-HSCT
- **Pre 2nd HSCT**: Preparatory phase for second HSCT
- **2nd HSCT**: Second HSCT
- **Post 2nd HSCT**: Post-second HSCT
- **MultiTAA T cells**: T cells targeting multiple tumor-associated antigens
Clinical Course - Pt#3 - ELIspot

PBMC

Targeted antigens

- NYESO-1
- MAGE A4
- SSX2
- PRAME

Non-targeted antigens

- WT1
- AFP
- MART1
- MAGE C1
- MAGE A3
- MAGE A2B
- MAGE A1

Marrow

- PRAME

SFC/5x10^5

SFC/2x10^5

Pre Wk4 Wk6 Mo3

6 wk POST
Clinical Course - Pt#3

**MultiTAA clones in Marrow**

**Targeted antigens**
- NYESO-1
- SSP2
- PRAME

**Non-targeted antigens**
- WT1
- AFP
- MART1
- MAGE C1
- MAGE A3
- MAGE A2B
- MAGE A1

**Production frequency**
- Pt#3 multiTAA clones in Wk6 post
Clinical Course - Pt#3

Diagnosis 1st line PD+3y Pre 1st HSCT HSCT +2y Pre 2nd HSCT Pre-T cells (d+47) Post-T cells (d+89) Month 6 Month 8

Clinical Course

- Pt#3

1st line

2nd line

3rd line

1st HSCT

2nd HSCT

MultiTAA T cells

IgG kappa M-spike (g/dl)

- 15% Clonal plasma cells
- 10% Clonal plasma cells

IgG kappa M-spike (g/dl)
Clinical Course - Pt#3

Diagnosis 1st line PD+3y Pre 1st HSCT HSCT +2y Pre 2nd HSCT Pre-T cells (d+47) Post-T cells (d+89) Month 6 Month 8

Clinical Course:

1st line
2nd line
3rd line

1st HSCT
2nd HSCT

15% Clonal plasma cells
10% Clonal plasma cells
90% Clonal plasma cells

IgG kappa M-spike (g/dl)

1st line
2nd line
3rd line

MultiTAA
T cells
Clinical Course - Pt#3

**PBMC**

![Graph showing SFC/5x10^5 for targeted antigens](image)

**Marrow**

![Graph showing SFC/5x10^5 for targeted antigens](image)

**Clinical Course**

- Pt#3
Mechanism of Escape

Survivin

MAGE-A4

PRAME

NYESO1

IHC Score

SFC/5x10^5
# Mechanism of Escape

**Pre Wk8 mth6**

<table>
<thead>
<tr>
<th>IHC Score</th>
<th>Pre</th>
<th>Wk8</th>
<th>mth6</th>
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<tbody>
<tr>
<td><strong>Survivin</strong></td>
<td>1+</td>
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<tr>
<td><strong>MAGE-A4</strong></td>
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<td>2+</td>
<td></td>
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<tr>
<td><strong>PRAME</strong></td>
<td></td>
<td>2+</td>
<td></td>
</tr>
<tr>
<td><strong>NYESO1</strong></td>
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<td>neg</td>
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**SFC/5x10^5**

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<th>mth6</th>
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<tr>
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**MAGE-A4, PRAME, NYESO1**

- **MAGE-A4**: 2+
- **PRAME**: 2+
- **NYESO1**: neg

**Mechanism of Escape**
Mechanism of Escape

**Survivin**

<table>
<thead>
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**MAGE-A4**

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**PRAME**

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**NYESO1**

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<tbody>
<tr>
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<td>5</td>
<td>0</td>
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</tbody>
</table>

**Targeted antigens**

- Survivin
- MAGE-A4
- PRAME
- NYESO-1
- PRAME

**Mechanism of Escape**
Mechanism of Escape

**Immune activating genes**

- MS4A1
- IL1B
- IL6
- CD86

**Immune inhibitory genes**

*Fulciniti M et al, Blood Cancer J, 2016*

*Mitchell JS et al, Nat commun, 2016*

*Linghua Wang, David Wheeler HGSC-BCM*
MultiTAA T cells for myeloma

- Safe to date (DL3 – Arm A & B)
- Feasible
- In vivo expansion of tumor-specific T cells directed to target antigens
- Antigen spreading
- Clinical benefit
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