Clinical Background: B Cell Lymphoma

- 55,000 cases diagnosed/year
- Increasing incidence
- Most respond to treatment by many will relapse
- Transplant can cure about 50% of those who relapse who are still responsive to chemotherapy

Acute Lymphoblastic Leukemia

- Standard chemotherapy of adult ALL basically unchanged
- No new drugs have been developed until 2014
- Results are unchanged over 3 decades: 35% disease free survival
- Better understanding of the results based on cytogenetics, molecular genetics and minimal residual disease
**Genetically Modified T-cells**

- T-cells are part of the immune system that recognizes virus, bacteria and fungus.
- T-cells have receptors on them that recognize foreign tissue antigens (organ transplant).
- Tumors have antigens on surface too.
- Can T-cells be "educated" to recognize tumor antigen?

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**Virus and Tumor Antigen-Specific T Cells**

- Studies in Epstein-Barr Virus and Cytomegalovirus diseases have established the transfer of virus-specific T cells as an effective strategy for restoring selected T-cell responses in immunodeficient patients.
- Can we make tumor specific T-cells to react against the cancer as the immune system does naturally against viruses?

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**The Chimeric Antigen Receptor (CAR)**

- The tumor specific chimeric antigen receptor (CAR) in lenti-viral vector.

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Fielding AK et al, Blood, 2007
**CD19: An Ideal Target for Immunotherapy**

- CD19 has near universal expression on B-cell malignancies.
- Limited normal tissue expression restricted to B cells.
- Off-tumor targeting of B cells is well tolerated.
- Over 9 institutions with open INDs using CD19-specific CAR T cells.
- Remarkable clinical responses observed in a subset of patients in both ALL and CLL.

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**Chimeric Antigen Receptor (CAR) T Cell Therapy**

**Advantages of CARs**
- Modular design.
- HLA-independent antigen recognition.
- Functional in both CD8+ and CD4+ T cells.
- Significant numbers of tumor specific T cells can be readily generated.
- The potential to generate long-term antitumor immunity.

**Challenges**
- Single antigen specificity.
- Primarily restricted to extracellular antigens.
- On-target and off-target toxicities.

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**Phase I Clinical Trial Using First Generation CD19R-CAR Engineered Central Memory T cells**

**Patient Population and Clinical Design**
- Autologous CAR T cells infused on day +2 after auto-HSCT.
- Poor prognosis with transplant.
- Myeloablative conditioning to promote homeostatic expansion.
- Engraft cells as a component of the reconstituted immune system.

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**NHL1 Patient Summary**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/Sex</th>
<th>Diagnosis</th>
<th>Disease Status at SCT</th>
<th>Salvage Prior to SCT</th>
<th>CAR T Cell Dose</th>
<th>Patient Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>UPM068</td>
<td>68yr, F</td>
<td>Diffuse large B cell</td>
<td>2nd Remission</td>
<td>None</td>
<td>50M</td>
<td>Expired 2yr post T cells</td>
</tr>
<tr>
<td>UPM047</td>
<td>70yr, M</td>
<td>Diffuse large B cell</td>
<td>Partial Remission</td>
<td>2 cycles R-ICE</td>
<td>250M</td>
<td>NED @ 18 mo</td>
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<tr>
<td>UPM048</td>
<td>69yr, F</td>
<td>Diffuse large B cell</td>
<td>Partial Remission</td>
<td>2 cycles R-ICE</td>
<td>50M</td>
<td>NED @ 12 mo</td>
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<tr>
<td>UPM052</td>
<td>70yr, M</td>
<td>Mantle Cell NHL</td>
<td>1st Remission</td>
<td>4 cycles R-GEMAC</td>
<td>50M</td>
<td>NED @ 12 mo</td>
</tr>
<tr>
<td>UPM051</td>
<td>59yr, F</td>
<td>Diffuse large B cell</td>
<td>Partial Remission</td>
<td>3 cycles R-ICE, ATO</td>
<td>250M</td>
<td>NED @ 11 mo</td>
</tr>
<tr>
<td>UPM055</td>
<td>59yr, F</td>
<td>Diffuse large B cell</td>
<td>Partial Remission</td>
<td>2 cycles R-CE</td>
<td>100M</td>
<td>Expired 3 mo post T cell</td>
</tr>
<tr>
<td>UPM056</td>
<td>59yr, M</td>
<td>Diffuse large B cell</td>
<td>Post Remission</td>
<td>Stable Disease</td>
<td>100M</td>
<td>NED @ 4 mo</td>
</tr>
<tr>
<td>UPM058</td>
<td>59yr, M</td>
<td>Diffuse large B cell</td>
<td>Post Remission</td>
<td>Stable Disease</td>
<td>100M</td>
<td>NED @ 5 mo</td>
</tr>
</tbody>
</table>
NHL1 Lessons Learned…….

- Feasibility of engineering and expanding CAR+ CD8+ Tcm, even post salvage chemotherapy.
- Safety of CD19-specific CAR T cells when administered in conjunction with auto HSCT, with no delay in hematopoietic reconstitution and no observed cytokine release syndrome in this MRD setting.
- Very low levels of CAR T cells detected in peripheral blood for a subset of patients.
- Subset of patients display long-term B cell aplasia.
- CAR T cell persistence and B cell aplasia does not appear to be dose dependent.

Evidence for CD19R-CAR T cell Persistence

Persistence of Transferred T cells Correlates with Clinical Success

Strategies to Improve T cell Persistence
- Incorporate lymphodepletion regimens prior to ACT (Dudley et al. JCO. 2008; 26: 5233).
- Reduce transgene immunogenicity
- Engineer T cell subsets with the propensity for long-term persistence (i.e. T memory cells)
- Optimize CAR design to include co-stimulatory signaling

Chimeric Antigen Receptor T Cells for Sustained Remissions in Leukemia

Probability of Event-free and Overall Survival at 6 Months
Cytokine release syndrome

- A constellation of inflammatory symptoms from cytokine elevations.
- Association with T cell activation and proliferation in T cell-engaging therapies.
- Association with clinical benefit.
- CRS-related death reported after Blinatumomab treatment.

CRP is a good biomarker for CRS syndrome

Fig. 4. CRP levels in patients infused with 19-28z CAR T cells.

Summary of NHL3 Phase I Clinical Trials To Initiate in 2014

<table>
<thead>
<tr>
<th>Trial ID</th>
<th>PI</th>
<th>Patient Population</th>
<th>Agent</th>
<th>T cell dose</th>
<th>Enrollment</th>
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</thead>
<tbody>
<tr>
<td>13277</td>
<td>Dr. Popplewell</td>
<td>High-Risk NHL Patients with HSCT</td>
<td>NHL3 Autologous CD19R(EQ)28ζ/EGFRt+ Bulk TCM</td>
<td>50 to &lt;800x10⁶ CAR+ cells</td>
<td>18</td>
</tr>
<tr>
<td>13447</td>
<td>Dr. Khaled</td>
<td>Relapsed/Refractory ALL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13351</td>
<td>Dr. Siddiqi</td>
<td>Relapsed/Refractory CD19+ B Cell Neoplasms</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CAR Transduced T Cell Therapy for ALL

- Treatment of relapse as bridge to transplant
- Treatment of relapse after allogeneic transplant (donor derived DLI) ??
- Planned infusion of T cells as part of transplant regimens
  - Autologous transplant for B cell lymphoma
  - Allogeneic transplant for ALL
- Vaccine + T cells (viral bi-specific T cells)

Engineering CMV-specific CAR T cells to express tumor-specific CARs

- Expansion/persistence of CAR T cells in response to CMV reactivation (pre-emptive DLI after alloHCT) and/or CMV vaccine
- Improve safety of allogeneic adoptive T cell therapy w/o GVHD
- Control CMV reactivation/disease
- Applicable to any malignancy by substituting a CAR specific to the individual tumor type
Engineering CD123-specific CAR T cells for the Treatment of Acute Myeloid Leukemia

Research Team
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Regulatory Team
Jamie Wagner
Anita Kurien
Julie Ostberg
Sandra Thomas

Clinical Team
Elizabeth Budde
Stephen Forman

Acute Myeloid Leukemia

- The most common acute leukemia in adults and has the highest mortality rate
- Cure rate for primary AML is 35% and decreases with age
- 5-year OS for AML patients post-1st relapse: ~10%
- AlloSCT is the preferred treatment route following a 2nd CR
- However less than half of patients are able to proceed to transplant due to treatment resistance

AML – second CR

- Ideally, AlloSCT candidates should be in remission, to improve post-transplant outcomes
- 2nd CR is not easily achieved
  - < 20% in patients with early relapse (<1yr in remission)
  - ~40% in patients with late relapse (≥1yr in remission)
- We need new treatments for relapsed AML as a potential bridge to transplant.

CD123 expression on AML patients

<table>
<thead>
<tr>
<th>AML Sample ID</th>
<th>Age/Sex</th>
<th>Cytogenetics</th>
<th>Clinical Status</th>
<th>Sample Type</th>
<th>CD123 (RFI)</th>
<th>CD34+ (RFI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>179</td>
<td>74/M</td>
<td>Interphase telomerase-</td>
<td>Relapsed</td>
<td>PB</td>
<td>428.32</td>
<td>99.22</td>
</tr>
<tr>
<td>373</td>
<td>47/M</td>
<td>Remission, Complex abnormalities</td>
<td>Relapsed</td>
<td>PB</td>
<td>1052.83</td>
<td>99.66</td>
</tr>
<tr>
<td>455</td>
<td>50/F</td>
<td>Interphase telomerase-</td>
<td>Relapsed</td>
<td>PB</td>
<td>23.98</td>
<td>76.80</td>
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<tr>
<td>519</td>
<td>57/F</td>
<td>del(17)(q21), del(13)(q14); normal loss of 7q36/p15.1</td>
<td>Relapsed</td>
<td>PB</td>
<td>40.18</td>
<td>97.40</td>
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<tr>
<td>545</td>
<td>60/M</td>
<td>Interphase telomerase-</td>
<td>Induction failure</td>
<td>PB</td>
<td>37.19</td>
<td>90.93</td>
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<tr>
<td>559</td>
<td>55/M</td>
<td>SAML Complex abnormalities</td>
<td>Apheresis</td>
<td>Apheresis</td>
<td>5.30</td>
<td>95.0</td>
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<tr>
<td>656</td>
<td>55/M</td>
<td>Normal</td>
<td>Persistent</td>
<td>PB</td>
<td>23.03</td>
<td>98.9</td>
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<tr>
<td>732</td>
<td>22/M</td>
<td>Interphase telomerase-</td>
<td>Untreated</td>
<td>PB</td>
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<tr>
<td>913</td>
<td>45/F</td>
<td>Complex abnormalities</td>
<td>Untreated</td>
<td>PB</td>
<td>27.19</td>
<td>90.83</td>
</tr>
</tbody>
</table>

Background

- The interleukin-3 receptor α chain (CD123) is over-expressed on AML cells compared to normal adult bone marrow (Jordan CT et al Leukemia 2000)
- Two phase I trials (CSL360 and SL-401) have been completed (NCT00401739 and NCT00397579)

CD123-AML associated Antigen

- Over-expressed on AML cells compared to normal adult hematopoietic stem/progenitor cells
- Expressed at highest levels on plasmacytoid dendritic cells; at lower levels on basophils, monocytes, eosinophils, and myeloid dendritic cells
Therapeutic concept

HSC
Mutation(s)
Anti-CD123 therapy
LSC
Normal growth and differentiation
Developmental arrest
Leukemia blast cells
Normal blood cells

CD123 Clinical Experience

- Two phase I trials (CSL360 and SL-401) have been completed (NCT00401739 and NCT00397579)

<table>
<thead>
<tr>
<th>Trial</th>
<th>Treatment</th>
<th>#</th>
<th>SAE</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSL360</td>
<td>7G3mAb</td>
<td>23</td>
<td>1- fungal infection</td>
<td>1 CR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2-infusion rxn</td>
<td></td>
</tr>
<tr>
<td>SL-401</td>
<td>DT-IL3 q.o.d. x 6</td>
<td>45</td>
<td>8 - grade 3 AST/ALT</td>
<td>1 CR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 PR</td>
</tr>
</tbody>
</table>

CD123 CAR T cells

- Ag-specific activation
- Ag-driven proliferation
- Effective killing of autologous AML blasts

CD123 CAR T cells exhibit anti-leukemic activity in a xenogeneic model of AML

Primary AML samples are lysed by CD123-specific CAR T cells

CD123+ cell lines are lysed by CD123 CAR T cells

Primary AML samples are lysed by CD123-specific CAR T cells

CD123 CAR T cells

- Ag-specific activation
- Ag-driven proliferation
- Effective killing of autologous AML blasts

Primary AML samples are lysed by CD123-specific CAR T cells
Effect of CAR T cells on normal and leukemic progenitors

 normalized CFU-L

 Untreated CD19R 26292 32716

 150%

 100%

 50%

 0%

 200%

 p = 0.0472

 p = 0.0232

 p = 0.3778

 p = 0.2801

 Mardiros et al., Blood 2013

Adoptive Therapy for acute myeloid leukemia using CAR123-transduced T cells

- Specific Aim 1: Demonstrate the AML patient-derived CAR123-transduced T cells can be produced under current Good Manufacturing Practices at a clinical scale.
- Specific Aim 2: Functionally and phenotypically characterize GMP-produced AML patient-derived CAR123 T cells.
- Specific Aim 3: Initiate a first-in-human phase 1 clinical trial using autologous CAR123 T cells for the treatment of relapsed/refractory AML.