PDL-1 is strongly Expressed on DC/Myeloma Fusions
PD-1 Expression is Increased on T cells isolated from Patients with Active Myeloma

PD1 blockade enhances immune response to DC/myeloma fusion vaccine in vitro

PD-1 blockade in conjunction with DC/myeloma fusion stimulation of T cells in vitro results in:
- increased IFN-gamma secretion
- decreased IL-10 secretion
- decreased expansion of Tregs
- enhanced tumor killing
PD-1 Blockade Results in Increased Lysis of Autologous Tumor by Fusion Stimulated T Cells

Control

+ anti-PD-1

23%

32%

Granzyme

FL1-H

FL2-H

FL1-H

FL2-H

Tumor
• CT-011 (3 mg/kg) intravenously q 6 weeks X 3 doses

• Monthly follow up x 6 months

• Peripheral blood samples for correlative science prior to each dose of CT-011 and at 1,3, 6 months following final infusion of CT-011
Study Schema
Cohort 2

- Monthly follow up x 6 months
- Peripheral blood samples for correlative science prior to each dose of CT-011 and at 1, 3, 6 months following final infusion of CT-011
Patient Characteristics

Cohort 1

• Patients Enrolled To Cohort 1: 27 patients

• 65% Male, 35% Female

• Median Age: 58 years (range 30-70 years)

• Median % Plasma Cells in Bone Marrow: 56%

• Pre-Transplant Treatment Regimens: 5 RVD, 3 RD, 6 VCD, 6 VD

• 13 patients initiated CT-011
  - 7 completed follow up
  - 5 currently in follow up
  - 1 currently receiving treatment
<table>
<thead>
<tr>
<th>Adverse Events</th>
<th># of Episodes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Grade 3</strong></td>
<td></td>
</tr>
<tr>
<td>Neutropenia (lasting 48 hrs)</td>
<td>1</td>
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<tr>
<td><strong>Grade 2</strong></td>
<td></td>
</tr>
<tr>
<td>Neutropenia</td>
<td>2</td>
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<tr>
<td>Fatigue</td>
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</tr>
<tr>
<td><strong>Grade 1</strong></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>15</td>
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<tr>
<td>Leukopenia</td>
<td>8</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>4</td>
</tr>
<tr>
<td>TSH</td>
<td>2</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>2</td>
</tr>
<tr>
<td>Night Sweats</td>
<td>1</td>
</tr>
<tr>
<td>Fatigue</td>
<td>1</td>
</tr>
<tr>
<td>Rash</td>
<td>1</td>
</tr>
<tr>
<td>Weakness</td>
<td>1</td>
</tr>
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<td>Periorbital Edema</td>
<td>1</td>
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<tr>
<td>Nausea</td>
<td>1</td>
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<tr>
<td>Allergic Rhinitis</td>
<td>1</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>1</td>
</tr>
<tr>
<td>Eosinophilia</td>
<td>1</td>
</tr>
</tbody>
</table>
Circulating Myeloma Reactive T cells

IFNγ

Pre-Mob  Pre-infusion #1  Pre-infusion #2  Pre-infusion #3  1 month  3 month  6 month

CD8

Pre-infusion

21.2

0.13

0.59

2.18

2.67

0.82

0.91

1.94

15.8

16.5

16.8

23.7

18.3

18.8
Circulating Myeloma Reactive T cells

CD4/IFNγ

% CD4+ T cells expressing IFNγ

(n=5) (n=5) (n=5)

Pre-Mob Pre-CT-011 #1 Peak

CD8/IFNγ

% CD8+ T cells expressing IFNγ

(n=5) (n=5) (n=5)

Pre-Mob Pre-CT-011#1 Peak
MUC1 Specific T cells

% CD8+ T cells expressing MUC1

Pre-Mob  | 1 mth  | 3 mth  | 6 mth

Pre-Infusion time points

CD8/MUC1

PM03    | PM05

Months post 3rd Infusion time point
Expansion of tumor reactive T cell populations in the bone marrow following treatment with CT-011.
Expansion of antigen specific T cells

PM03

<table>
<thead>
<tr>
<th></th>
<th>MUC1</th>
<th>NY-ESO</th>
<th>PRAME</th>
<th>Survivin</th>
<th>WT1</th>
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</thead>
<tbody>
<tr>
<td>Pre-Immunotherapy</td>
<td>2%</td>
<td>1.5%</td>
<td>3%</td>
<td>2%</td>
<td>1.5%</td>
</tr>
<tr>
<td>3 Month f/u</td>
<td>5%</td>
<td>7%</td>
<td>14%</td>
<td>9%</td>
<td>7.8%</td>
</tr>
<tr>
<td>6 Month f/u</td>
<td>8.5%</td>
<td>8.8%</td>
<td>12.5%</td>
<td>12%</td>
<td>9.4%</td>
</tr>
</tbody>
</table>
AML
Case Presentation

- 76 year old physician presents with fatigue and easy bruisability
- WBC of 30K Hct 25 Plts 40
- Peripheral smear demonstrates 60% blasts
- BM consistent with AML

- What is the role of therapy-to what end?
Treatment Dilemma

- 50% of patients achieve remission but most relapse
- Transplant is the only curative therapy for high risk disease
- Salvage rate for patients with poor prognosis is very limited
- No clear therapy for patients over age 60 with significant percentage of long term remission
  - Higher incidence of MDR phenotype
  - Lower response rate
  - Higher complex cytogenetics
Impact of Type of Graft and GVHD on Relapse

(Horowitz, Blood 1990)
Toxicity vs. Relapse
Patient Characteristics

- Patients Enrolled to Date: 33
- 55% Male, 45% Female
- Median Age: 64 years (range 21-81 years)
- Newly diagnosed 31 patients; first relapse 2 patients
- Median % Blasts in Bone Marrow: 62.5%
Patient Characteristics

TOTAL ENROLLED: 33
(Cohort 1)

Evaluable: 13
- 13 received 2 vaccines

Potential to be Evaluable: 5
- Receiving chemotherapy

Not Evaluable: 15
- 5 underwent allogeneic transplant in CR1
- 2 did not achieve remission
- 2 died during induction chemotherapy
- 1 did not collect sufficient tumor
- 2 relapsed
- 1 had ongoing chemotherapy related toxicity
- 1 patient chose to withdraw
Characteristics of patients who received vaccine

Induction Chemotherapy

Remission after 1 Cycle: 11 Patients
- 9 Patients: 7+3
- 1 Patient: MEC
- 1 Patient: 10 days of decitabine

Remission after 2 Cycles: 2 Patients
- 1 Patient: 7+3 followed by 5+2
- 1 Patient: 7+3 followed by MEC

Cytogenetics

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Cytogenetics</th>
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<tbody>
<tr>
<td>PA2</td>
<td>INV 16</td>
</tr>
<tr>
<td>PA3</td>
<td>Normal</td>
</tr>
<tr>
<td>PA5</td>
<td>Normal</td>
</tr>
<tr>
<td>PA8</td>
<td>INV 2</td>
</tr>
<tr>
<td>PA9</td>
<td>Normal</td>
</tr>
<tr>
<td>PA10</td>
<td>Normal</td>
</tr>
<tr>
<td>PA11</td>
<td>Complex</td>
</tr>
<tr>
<td>PA13</td>
<td>Complex</td>
</tr>
<tr>
<td>PA14</td>
<td>Normal</td>
</tr>
<tr>
<td>PA16</td>
<td>t(8,21) (-9)</td>
</tr>
<tr>
<td>PA18</td>
<td>Normal</td>
</tr>
<tr>
<td>PA24</td>
<td>Normal</td>
</tr>
<tr>
<td>PA23</td>
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## Adverse Events

<table>
<thead>
<tr>
<th>Adverse Events</th>
<th># of Episodes</th>
</tr>
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<tbody>
<tr>
<td>Vaccine site reaction (Grade 2)</td>
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<tr>
<td>Vaccine site reaction (Grade 1)</td>
<td>24</td>
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<tr>
<td>Pruritis (Grade 2)</td>
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</tr>
<tr>
<td>Pruritis (Grade 1)</td>
<td>2</td>
</tr>
<tr>
<td>Arthralgia (Grade 1)</td>
<td>1</td>
</tr>
<tr>
<td>Myalgia (Grade 1)</td>
<td>1</td>
</tr>
<tr>
<td>TSH (Grade 1)</td>
<td>3</td>
</tr>
<tr>
<td>Eosinophilia (Grade 1)</td>
<td>3</td>
</tr>
<tr>
<td>Increased Monocytes (Grade 1)</td>
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<tr>
<td>Leukopenia (Grade 1)</td>
<td>5</td>
</tr>
<tr>
<td>Thrombocytopenia (Grade 1)</td>
<td>3</td>
</tr>
<tr>
<td>Neutropenia (Grade 1)</td>
<td>2</td>
</tr>
</tbody>
</table>
Immunologic Response to Vaccination

Vaccination potently expands leukemia reactive T cells

IFNγ expressing CD8 T cells in study patient PA02
Immune Response to Vaccination
Expansion of Tumor Reactive T cells Following Vaccination

CD4+IFNγ

CD8+IFNγ

p<0.05

N=13

Pre-Vac1

Peak

Pre-Vac1

Peak

N=13
Expansion of Antigen Specific T cells Following Vaccination

CD8/MUC1+

CD8/WT-1+

CD8/Survivin+

N=4
T cells Infiltrating
The Bone Marrow Following Vaccination

n=2
Disease Response

Number Evaluable*: 13

9 patients remain in remission (69%)
  – Median time in remission: 30 months from CR

4 patients with disease progression

*Evaluable = received two or more vaccinations
MUC1 and Leukemia Stem Cells

• Leukemia stem cells are resistant to cytotoxic therapy, and capable of reconstituting disease
  – Functionally characterized by efficiency in establishing leukemia in immunodeficient mouse model
  – Previously described in the CD34+/lineage-/CD38- or CD34+/lineage-/CD38+ fractions

• Several MUC1 associated pathways differentiate LSCs from normal hematopoietic stem cells
  – NF-KB upregulated in LSCs but not normal HSCs
  – Wnt-β-catenin signaling important in maintaining LSC phenotype
Designing an Effective Cancer Vaccine

• Enhancing antigen presentation
  – Defining optimal antigenic targets
  – Effective antigen presentation to result in activation rather than tolerance

• Reversing the immunosuppressive milieu
  – Reversing effector cell dysfunction
  – Reduction in inhibitory cells

• Breaking tolerance establishing durable anti-tumor immunity
  – Downregulation of inhibitory pathways

• Targeting tumor heterogeneity
  – Defining critical antigens
  – Targeting the malignant stem cell
Regulatory Pathways in LSCs

Self-renewal pathways
- BMI-1
- Telomerase*
- Developmental pathways
- Notch
- Wnt/β-catenin*
- Shh
- Miscellaneous pathways
- FLT3*
- PI3K/AKT/mTOR*
- NFκB*
- Pgp and BCRP*
- Differentiation/Epigenetics*

A. Niche

B. LSC

Epigenetics

FLT3, PI3K/AKT/mTOR

AML

Bmi-1

Debulking

Durable remission

Cure

LSC-directed therapies

CCR Molecular Pathways
MUC1 is over-expressed on AML and not normal CD34+ cells
MUC1 expression in AML patients following sex mismatch Allogeneic transplantation

- MUC1 was highly expressed only in the recipient derived (XX) CD34+ cells, representing residual malignant stem cells
- MUC1 was not detected on donor derived (XY) CD34+ cells, representing transplanted normal hematopoietic stem cells
Engraftment of $\text{MUC1}^{\text{high}}$ and $\text{MUC1}^{\text{low}}$ cells in NSG mice

A. CD34+/lineage-

B. $\text{MUC1}^{\text{high}}$

C.

D. $\text{MUC1}^{\text{low}}$

E.
Effect of targeting MUC1 on AML engraftment in prevention model

A. PBS

B. GO-203

C. 

\[ p = 0.05 \]
AML treatment by inhibition of MUC1-C receptor subunit

Engraftment in BM of NSG mice inoculated with MUC1^{high} cells

Engraftment in BM of NSG mice inoculated with MUC1^{low} cells

Human CD45 (%)

\[ \begin{array}{cc}
\text{PBS} & \text{GO-203} \\
p=0.003 & p=0.3
\end{array} \]
Designing an Effective Cancer Vaccine

• Enhancing antigen presentation
  – Defining optimal antigenic targets
  – Effective antigen presentation to result in activation rather than tolerance

• Reversing the immunosuppressive milieu
  – Reversing effector cell dysfunction
  – Reduction in inhibitory cells

• Breaking tolerance establishing durable anti-tumor immunity
  – Downregulation of inhibitory pathways

• Targeting tumor heterogeneity
  – Defining critical antigens
  – Targeting the malignant stem cell
Hematological Malignancy Team

Clinical team
- David Avigan, Chief Section of Hematological Malignancy/BMT
- James Levine
- Robin Joyce
- Jon Arnason
- Vicky Boussiotis
- Jeff Zwicker
- Gosia Mcmasters
- Salvia Jain
- Nancy Giallombardo
- Jamie Mortellite
- Amy Corrao
- Tara Roy
- Aya Sato Dilorenzo
- Carol Delaney
- Carol Silver
- Emma Breault
- Tania Aziz
- Erika Nelson
- Tim Lens

- Cell Manipulation Facility
  - Poorvi Somaya

- Laboratory
  - David Vasir
  - Max Coll
  - Athalia Pyzzer
  - Kristen Palmer
  - Neha Srivastava
  - Katarina Luptakova
  - Dina Stroopinsky

DFCI
- Ken Anderson
- Don Kufe

Leukemia and Lymphoma Society
Multiple Myeloma Research Foundation
National Institutes of Health/ National Cancer Institute
Department of Defense/Gateway for Cancer Research