Tipping The Immune System Balance In Favor Of Effective Cancer Immunotherapy

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Many Challenges For Developing Cancer Vaccines In The Clinics

- What are the “immune relevant” targets?
- What is the best vaccine approach?
- What are the best immune monitoring methods?
- What approaches will overcome immune tolerance and eradicate cancer?
- What approaches will prevent cancer?
Many Challenges For Developing Cancer Vaccines In The Clinics

- What are the “immune relevant” targets?
- What is the best vaccine approach?
- What are the best immune monitoring methods?
- **What approaches will overcome immune tolerance and eradicate cancer?**
- What approaches will prevent cancer?
Immune tolerance mechanisms are the major barriers for developing effective cancer vaccines

• Systemic
  – T regs
  – Ineffective T cell activation
  – Low avidity T cell availability

• Local at the tumor site
  – COX-2 pathways
  – T regs
  – T cell down regulatory signals (new B7 family members)
  – Down-regulatory cytokines
    • IL-10, TGF-beta, VEGF
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Her2/neu in neu Transgenic Mice Provide a Model of Immune Tolerance

Parental mice
Vaccination day 15

neu transgenic mice
Vaccination day 1

Tumor size mm² (mean ± SEM)

Days post tumor injection

Controls (n=5)
Vaccination 3T3 neu/GM (n=5)

Controls (n=6)
Vaccination 3T3 neu/GM (n=6)

* * * p < .05
CY Given Prior to Priming
Enhances The Anti-Tumor Effect Of The Vaccine

Tumor  Cy  Vaccine

Controls 3T3/GM (n=10)
Vaccination 3T3-neu/GM (n=14)
3T3/GM + Cyclophosphamide 100 mg/kg + Doxorubicin 5 mg/kg (n=11)
Vaccination 3T3-neu/GM + Cyclophosphamide 100 mg/kg + Doxorubicin 5 mg/kg (n=14)

Tumor Free Probability

\[
\text{p} = 0.02
\]
\[
\text{p} = 0.3
\]
Cy Enhances The Potency Of The Vaccine Through A Mechanism Distinct From Direct Tumor Lysis

Dependent on Her-2/neu specific CD4 and CD8 T cell responses
Hypothesis:

T regulatory cells suppress Cy plus vaccine induced HER-2/neu-specific immunity
CD4⁺CD25⁺, FoxP3+ but not CD4⁺CD25⁻, FoxP3⁻ T cells suppress CY+ vaccine induced anti-tumor immunity
Cyclophosphamide treatment transiently suppresses peripheral Tregs
Cy selectively deletes cycling T cells in tumor bearing mice

Cy on Day 1  BdrU on Day 2  Analyze splenic T cells for CD4CD25- and CD4CD25+ T cells

% of CD25+ that are BrdU+

% BrdU+

0 1 2 3 4 5 6 7 8

Cy+BrdU  Cy+PBS  PBS+BrdU  PBS+

% of CD25+ that are BrdU+
Dissecting the mechanisms of immune tolerance to HER-2/neu requires knowledge of HER-2/neu derived T cell epitopes
RNEU\textsubscript{420-429} is immunodominant in non-tolerized mice

H-2D\textsuperscript{q} MHC/RNEU\textsubscript{420-429} Tetramer
Can RNEU_{420-429} T cells be isolated directly from vaccinated mice that are cured of their tumor?

Day 0:
- Tumor burden

Day 2:
- Chemotherapy

Day 3:
- Vaccine
- Overnight incubation with T-2/DqRNEU_{420-429}
- Analyze by ICS

Day 50:
- Isolate CD8^+ T Cells
- Chemotherapy
RNEU_{420-429}-specific T cells can be isolated from mice treated with Cy + vaccine
% of CD8+ that are IFNg+

FVB/N vac.
neu-N vac. A
neu-N chemo+vac. A

Dq-RNEU420-429 Tetramer

CD8

FVBN vac.
neu-N vac. A
neu-N chemo+vac. A

neu-N vac. A
neu-N vac. A
neu-N chemo+vac. A
neu-N chemo+vac. A
neu-N chemo+vac. A
neu-N chemo+vac. A

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neu-N chemo+vac. A
neu-N chemo+vac. A
neu-N chemo+vac. A

Dq-RNEU420-429 Tetramer

CD8
Adoptively Transferred Tregs from Tolerized Mice Suppress RNEU\textsubscript{420-429}-Specific T Cells in Vaccinated Non-Tolerized Mice

Tregs transferred on day 0
Vaccinated on day 1
Assayed by ICS day 14

% of CD8+ that are IFNg+

No Transfer
CD4\textsuperscript{+}CD25\textsuperscript{+} T cells
Current working model of CD8+ peripheral tolerance in *neu* mice

Positively Selected T Cells Leave Thymus

THYMUS

High Affinity

Low Affinity

T Regulatory Cell Suppression

Encounter with Self-antigen

Vaccine

Little to no anti-tumor response

Down regulation
Current Working Model of CD8^+ Peripheral Tolerance

Positively Selected T Cells Leave Thymus

THYMUS

High Affinity

Low Affinity

Encounter with Self-antigen

T Regulatory Cell Suppression

Immune Modulation

Vaccine

Tumor Regression

?
Summary of Mouse Data

• High avidity RNEU$_{420-429}$ T cells are suppressed rather than deleted in neu mice

• Inhibition of Tregs allows for the recruitment of high avidity T cells specific for the immuno-dominant epitope RNEU$_{420-429}$ to the immune response

Ercolini et al., J Exp Med, 2005
Pancreas Cancer by Stage

Survival

- Resectable Stage I and II
- Unresectable Stage III
- Stage IV
- All Stages

1 yr Survival
5 yr Survival
Pancreatic Cancer Therapy

Stage 1, 2, or 3 (Locoregional)
• Surgery
• Adjuvant chemoradiation
• 70-80% recurrence at 1 yr

Stage 4 (Metastatic)
• Gemzar +/- other
• Experimental therapy
• Palliation
Pancreas Cancer Team at Hopkins

- **Surgery**
  - John Cameron
  - Charles Yeo
  - Steven Leach
  - Kurt Campbell

- **Pathology**
  - Ralph Hruban
  - Scott Kern
  - Christine Iacobuzio Donahue
  - Anirban Maitra

- **Gastroenterology**
  - Marcia Canto
  - Sanjay Jaganneth
  - Michael Goggins

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  - Manuel Hidalgo
  - Dan Laheru
  - Wells Messersmith

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  - Deborah Frassica
  - Fariba Asrari
Design of Protocol J9617: A Phase I Study of an Allogeneic GM-CSF Vaccine

Surgical Resection
First Vaccine

Dose Escalation
$10^7$, $5 \times 10^7$, $10 \times 10^7$, $50 \times 10^7$
GM-CSF secretion
120 ng/10$^6$ cells/24 hours

Second Vaccine
Third Vaccine
Fourth Vaccine

Adjuvant Chemoradiation

Weeks
0 4 8 12 16 20 24 28 32 36 40 44 48

TSH, Amylase, Lipase
DTH Testing
Common Recall Testing
CAT Scan, CA 19-9

TSH, Amylase, Lipase
DTH Testing
Common Recall Testing
CAT Scan, CA 19-9
Correlation of Post-Vaccination DTH with Disease-Free Survival

![Graph showing correlation between DTH and DFS](image-url)
Functional Genomic Approach

Tumor

Differential Gene Analysis

Normal Cell

Candidate Genes

Predict antigen epitopes that will bind to MHC I

APC

CD8

T-2 cells

IFN-gamma

ELISPOT Readout
Experimental Methods

- Three day ELISPOT procedure

- **Day 1**: Coat plate with primary Ab
- **Day 2**: Pulse T2 cells with peptide and add freshly thawed and enriched CD8+ T cells
- **Day 3**: Add secondary Ab and develop plate

- Developed plates are read using KS ELISPOT
Summary of Mesothelin Responses for 14 Patients

Peptide Symbol Legend

- □ = Mesothelin A2(20-29)
- ○ = Mesothelin A2(530-539)
- ♦ = HIVGAGA
- △ = HIVNEF A3(9)
- ◊ = Mesothelin A24(435-444)
- ♣ = Mesothelin A3(83-92)
- ♦ = Tyrosinase A24(206-214)
Pre-clinical data driving the next clinical trials
Design of a Phase II study of an Allogeneic GM-CSF Secreting Tumor Vaccine (GVAX) Alone or in Sequence with Cyclophosphamide for Metastatic Pancreatic Cancer
Laferhu, et al and Cell Genesys

<table>
<thead>
<tr>
<th>Screen</th>
<th>Vaccinations 1-6</th>
<th>Monthly Follow-up visits 1-9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment Visits every 21 days</td>
<td>Every 28 days</td>
</tr>
<tr>
<td></td>
<td>Safety evaluations every 21 days</td>
<td></td>
</tr>
</tbody>
</table>

-4  0  3  6  9  12  15  18  19  23  27  31  35  39  43  51  Weeks

Cohort A treatment: 50x10^7 vaccine cells alone (30 patients)

Cohort B treatment: 250 mg/m^2 Cy given 1 day prior to vaccination with 50x10^7 vaccine cells (20 patients)
## SUMMARY

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Toxicity Grade 1/2 Local</th>
<th>Serum GM-CSF Levels</th>
<th>Stable Dz During Therapy (18 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine Only (30 Pts)</td>
<td>Tolerated well in Pts with ≥2 prior therapies</td>
<td>Peaked at 48 hours</td>
<td>16%</td>
</tr>
<tr>
<td>Cy (250 mg/m2) + Vaccine (20 Pts)</td>
<td>Tolerated well in Pts with ≥2 prior therapies</td>
<td>Peaked at 48 hours</td>
<td>40%</td>
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Mesothelin specific T cells observed in predominantly Cy + vaccine treated patients

![Graph showing INFg spots/10e5 CD8+ Tcells]

Solid line=Cy+vaccine
Dashed line=vaccine only
## Improved Survival Associated with Mesothelin-Specific T Cell Responses Following Vaccination

<table>
<thead>
<tr>
<th>Patient</th>
<th>HLA-A locus</th>
<th># vaccinations</th>
<th>Mesothelin Specific T cells/10e5 CD8 T cells</th>
<th>Survival (mo)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Pre</td>
<td>Vaccine 3</td>
<td>Vaccine 6</td>
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<tr>
<td>4.006</td>
<td>A2</td>
<td>2</td>
<td>10</td>
<td>NA</td>
</tr>
<tr>
<td>4.012</td>
<td>A2</td>
<td>1</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>4.018</td>
<td>A2</td>
<td>2 (+ Cy)</td>
<td>5</td>
<td>33</td>
</tr>
<tr>
<td>4.023</td>
<td>A2</td>
<td>3 (+ Cy)</td>
<td>153</td>
<td>108</td>
</tr>
<tr>
<td>4.024</td>
<td>A2</td>
<td>4 (+ Cy)</td>
<td>24</td>
<td>40</td>
</tr>
<tr>
<td>4.026</td>
<td>A3</td>
<td>6 (+ Cy)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4.028</td>
<td>A3</td>
<td>3 (+ Cy)</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>4.033</td>
<td>A2</td>
<td>6 (+Cy)</td>
<td>0</td>
<td>0</td>
</tr>
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</table>
Future Directions

• Assess T cell avidity differences in patients treated with Cy+vaccine versus vaccine alone

• Test combinations of vaccine with inhibitors of additional checkpoints
  – Systemic targets
  – Tumor micro-environment targets

• Test combinatorial immune based approaches at earlier stages of disease
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Cell Genesys
Conflict of Interest Statement

Under a licensing agreement between Cell Genesys and the Johns Hopkins University, the University is entitled to milestone payments and royalty on sales of the vaccine product described in this presentation. The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict of interest policies.