Immunotherapy combinations: From mice to man

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Department of Immunology
I have research collaborations with Bristol Myers Squibb, and am a consultant for Jounce and BD.

I receive royalties from the patent “Methods and Compositions for Localized Secretion of anti-CTLA-4 Antibodies”.

I will be talking about investigational therapeutics.
Why does the immune system fail to eliminate cancer?

Antigenic Cancer Cells Grow Progressively in Immune Hosts without Evidence for T Cell Exhaustion or Systemic Anergy

By Maresa Wick,* Purnima Dubey,* Hartmut Koeppen,* Christopher T. Siegel,† Patrick E. Fields,* Lieping Chen,* Jeffrey A. Bluestone,* and Hans Schreiber*
Like pathogens, tumors deploy multigenic immune evasion programs

With < 9.8 kB of genome space HIV, like many other viruses devotes a large percentage of its genome to immune evasion.

Can access the entire $3 \times 10^9$ base genome for evolutionary as well as adaptive immune evasion.
Agonist Antibody

Blocking Antibody

Dendritic Cell Co-Stimulatory Receptors

CD40
4-1BB

T-cell

CD28
ICOS
4-1BB
OX40
GITR
CD27
HVEM
TIM-1

PD-L1
CTLA-4
PD-1
BTLA
TIM-3
VISTA
LAG-3

APC
T cells are activated in two steps: T cell receptor ligation and co-stimulation.
CTLA-4, a negative regulator of T cell activity, limits the lifespan of activated T-cells.
Which T-cells are affected by Ipilimumab (αCTLA-4)?

The greater the percentage of active T-cells in a patient targeting the tumor when αCTLA-4 is initiated, the greater the efficacy and selectivity should be.
Why choose to block the PD-1 and CTLA-4 pathways in combination?

Blocking one co-inhibitory receptor leads to reciprocal upregulation of the other

CD4 Teff - %CTLA-4+ TIL

CD4 Teff - %PD-1+ TIL

CTLA-4 and PD-1 inhibitory signals are non-redundant

CTLA-4-/ Mice

2-3 Weeks

Lethal Lymphoproliferation

PD-1-/ Mice

5 Months (Balb) >14 Months (B6)

Cardiomyopathy (Balb) Lupus (B6)
Evidence of CTLA-4 induction and subsequent progression?

Figure 1. Activity of Anti–PD-L1 Antibody in Patients with Advanced Melanoma and Non–Small-Cell Lung Cancer.
PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors

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Contributed by James P. Allison, January 19, 2010 (sent for review December 17, 2009)
<table>
<thead>
<tr>
<th></th>
<th>B7-1</th>
<th>CTLA-4</th>
<th>PD-L1</th>
<th>PD-1</th>
<th>B7-1</th>
<th>PD-L1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inhibits T cell proliferation</strong></td>
<td>++++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td><strong>Reduces cytokine production</strong></td>
<td>+</td>
<td>++++</td>
<td>++</td>
<td>++</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td><strong>Reduces cytotoxicity</strong></td>
<td>+</td>
<td>++++</td>
<td></td>
<td>?</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Reduces APC co-stimulation</strong></td>
<td>++</td>
<td>--</td>
<td>--</td>
<td>--</td>
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<td></td>
</tr>
<tr>
<td><strong>Induces T cell apoptosis</strong></td>
<td>*/+</td>
<td>++</td>
<td></td>
<td>?</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ligand expressed on tumor</strong></td>
<td>--</td>
<td>++</td>
<td></td>
<td>+/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ligand in microenvironment</strong></td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Supports Treg suppression</strong></td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Supports Teff to Treg conversion</strong></td>
<td>++++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Conversion of the tumor micro-environment from suppressive to inflammatory
Risk/Benefit: αPD-1 monotherapy IrAE were less severe but largely overlapping with αCTLA-4
Phase I study: Concurrent and sequenced nivolumab and ipilimumab in melanoma

**Concurrent Cohorts**

<table>
<thead>
<tr>
<th>Nivolumab</th>
<th>Ipilimumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>once every 3 weeks (8 doses)</td>
<td>once every 12 weeks (8 doses)</td>
</tr>
</tbody>
</table>

- **Ipilimumab** once every 3 weeks (4 doses)
- **Nivolumab** once every 3 weeks (8 doses)
- **Ipilimumab** once every 12 weeks (8 doses)
- **Nivolumab** once every 12 weeks (8 doses)

**Sequenced Cohorts**

<table>
<thead>
<tr>
<th>Nivolumab</th>
<th>Ipilimumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>once every 2 weeks (up to 48 doses)</td>
<td>every 3 weeks (4 doses)</td>
</tr>
</tbody>
</table>

- **Nivolumab** once every 2 weeks (up to 48 doses)
- **Ipilimumab** every 3 weeks (4 doses)

- Tumor assessments by mWHO and immune-related mWHO criteria
- Data as of Feb 2013 for 86 patients are reported for the ongoing study
Clinical activity: combination of nivolumab and ipilimumab therapy

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>ORR</th>
<th>Patients with ≥80% Tumor Reduction at 12 Wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ipilimumab (3 mg/kg)</td>
<td>137</td>
<td>11%</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Nivolumab (3 mg/kg)</td>
<td>17</td>
<td>41%</td>
<td>&lt;3</td>
</tr>
<tr>
<td>Concurrent therapy (3 mg/kg ipilimumab + 1 mg/kg nivolumab)</td>
<td>17</td>
<td>53%</td>
<td>41%</td>
</tr>
</tbody>
</table>

Patient 011 (MSKCC) – Dramatic Response

Pre-treatment

10 cm gastric mass

12 weeks

3.7 cm gastric mass

5 cm peripancreatic mass

3.2 cm peripancreatic mass
Using what we know from the murine studies, what potential biomarkers should we monitor?

Ipilimumab / Nivolumab Combination Monitoring Panel

<table>
<thead>
<tr>
<th>T-cell Gating:</th>
<th>Inhibitory:</th>
<th>Activation:</th>
<th>PD-1 Monitoring:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live/Dead</td>
<td>CTLA-4</td>
<td>Icos</td>
<td>αhIgG4 (detects α PD-1)</td>
</tr>
<tr>
<td>CD3, CD8</td>
<td>Tim-3</td>
<td>Ki-67</td>
<td>αPD-1 MIH4 (total surface)</td>
</tr>
<tr>
<td>CD4, FoxP3</td>
<td>LAG-3</td>
<td>Granzyme B</td>
<td>αPD-1 EH12 (total unblocked)</td>
</tr>
</tbody>
</table>
In the mouse, accumulation of CTLA-4 / PD-1 double positives in TIL correlates with tumor rejection.

%CTLA4/PD1+ %CTLA4/PD1+  
CD8 T-cells     CD4 T-eff  
80%             62%  
77%             51%  
70%             41%  
54%             38%  
54%             36%  

32%/25%
Increase in circulating CTLA-4+/PD-1+ CD4 T\textsuperscript{eff} following treatment
In the mouse combination co-inhibitory blockade leads to increased proliferation of TIL but offers little benefit over αCTLA-4 alone.
Durable increases in CD4 T\textsuperscript{eff} proliferation following treatment
Increased frequency of activated \((\text{ki67}^+)\) CD4 and CD8 T cells with concurrent nivolumab + ipilimumab

**Concurrent**

**Sequenced**

**Percent change in ki67\(^{+}\) CD8\(^{+}\) T cells**

**Percent change in ki67\(^{+}\) CD4\(^{+}\) T cells**

**Weeks on Treatment**
Christian suggests that this slide be deleted and only the ICOS data on the next slide be presented.
Or show CD4 data for ICOS and ki67 and at the bottom of the slide state
"A similar effect was seen for ICOS + and ki67+ CD8 T cells"

Leinbach, Susan, 5/14/2013
In the mouse, increased Icos expression on CD4 T-cells, especially Tregs, correlates with response to αCTLA-4/αPD-1 blockade.

%Icos+
CD4 Teff

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percent Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>αCTLA-4/αPD-1</td>
<td>41%</td>
</tr>
<tr>
<td>αPD-L1</td>
<td></td>
</tr>
<tr>
<td>αCTLA-4/αPD-1</td>
<td>37%</td>
</tr>
<tr>
<td>αPD-1</td>
<td></td>
</tr>
<tr>
<td>αCTLA-4</td>
<td>30%</td>
</tr>
<tr>
<td>αPD-L1</td>
<td>24%</td>
</tr>
</tbody>
</table>
Increased frequency of activated (ICOS+) CD4 and CD8 T cells with concurrent nivolumab + ipilimumab

Concurrent

Sequenced

Percent change in ICOS+ CD8+ T cells

Percent change in ICOS+ CD4+ T cells

Weeks on Treatment
In some patients Icos upregulation correlates with clinical response
Agonist Antibody

Blocking Antibody

T-cell
Co-Stimulatory Receptors

Agonist Antibody

T-cell
Co-Inhibitory Receptors

Blocking Antibody

Dendritic Cell
Co-Stimulatory Receptors

CD40

4-1BB

APC

CD28

ICOS

OX40

GITR

CD27

HVEM

TIM-1

PD-L1

CTLA-4

PD-1

BTLA

TIM-3

VISTA

LAG-3
Tumor infiltrating T-cells from α4-1BB treated mice upregulated KLRG1 on most CD8s and ~50% of CD4s
We have termed this CD4+ T-cell phenotype ThEO and the corresponding CD8 phenotype TcEO.
Using what we know from the murine studies, what potential biomarkers should we monitor?

Urelumab (α4-1BB/αCD137) Patient Monitoring Panel

<table>
<thead>
<tr>
<th>Population Gating:</th>
<th>ThEO Phenotype:</th>
<th>Activation:</th>
<th>Inhibitory:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live/Dead, CD3</td>
<td>Eomes, KLRG1</td>
<td>Icos</td>
<td>CTLA-4</td>
</tr>
<tr>
<td>CD8, CD4, FoxP3</td>
<td>Granzyme A, B, K</td>
<td>Ki-67</td>
<td>PD-1</td>
</tr>
<tr>
<td>CD16, CD56, CD11c</td>
<td></td>
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</tbody>
</table>
Preliminary data suggests αCD137 treatment evokes Eomes upregulation in patient PBMC

1) Is Eomes upregulation in PBMC a marker of pharmacologic response to the antibody?

2) Does Eomes (and KLRG1) upregulation on PBMC correlate with clinical response?
What is the root of 4-1BB induced liver inflammation and how is it ameliorated by αCTLA-4?

4-1BB agonist and CTLA-4 blocking antibodies were able to mutually ameliorate each others’ side affects in the mouse.
Why this trial should happen.

1. Therapeutic synergy between Ipilimumab and Urelumab (αCD137) in multiple tumor models
2. Mutual amelioration of each agents IrAE by the other
3. Potential to expand the pool of patients eligible to receive and remain on Ipilimumab
Seeking combinations outside of immunotherapy

- Immune recognition of tumor and mobilization of anti-tumor effectors
  - Vaccines
  - Pharmacologic killing
    - Chemotherapy
    - Targeted therapies
  - Radiotherapy
  - ACT (CARs, TCR xfer)

  - Co-inhibitory blockade
  - Co-stimulatory activation
  - Activation of APCs
  - Innate immune recognition

- Removal of physical and stochastic barriers to immune rejection.
  - Tumor vascular resistance
  - Desmoplastic stroma
  - Extreme hypoproliferation
  - Hypoxic microenvironments
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-MSKCC

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(α 4-1BB)
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