Drug Discovery in Tumor Immunotherapy: novel approaches from new insights

Tyler J. Curiel
curielt@uthscsa.edu
San Antonio Cancer Institute
UTHSCSA
Outline

• History of tumor immunology
• Our work that is changing the paradigm
• Translational predictions of our work
• Using our data for translational approaches
• Drug discovery and strategies
• Future directions
First immunotherapy for cancer


Louis Pasteur 1822-1895

Germ theory of immunity 1878

First demonstration of acquired immunity 1880
Increased cancer in immunosuppressed hosts

Spontaneous cancer remissions, especially in renal cell carcinoma and melanoma

Demonstration of tumor-specific immunity

*Tumor* 1957;18:769

Tumors express antigens

*Nature* 304, 165-7 (1983)
Current tumor immunotherapy paradigms build on infectious disease principles that may not apply to cancer

One answer: give more T cells


Dendritic cells: On the move from bench to bedside

Since their original discovery in 1973, dendritic cells have progressed from subset curiosity to preferred adjuvant for cancer immunotherapy. Dendritic cells are located strategically at the interface of potential pathogen entry sites and take up antigen, move into secondary lymphoid tissues and activate both helper and cytotoxic T cells. They also interact with B cells and probably natural killer cells and thus direct the character of the immune response. Apart from pathogen protection, dendritic cells might also have roles in central and peripheral tolerance and probably in anti-cancer immune responses. Pilot dendritic-cell vaccination studies have induced specific anticancer responses and chemokine-receptor expression. Important dendritic-cell inducers of maturation are pathogen-derived products (for example, lipopolysaccharide (LPS), viral dsRNA and CpG DNA), T-cell derived stimuli (for example, CD40L), mechanical stress and a variety of secreted pro-inflammatory stimuli (for example, interleukin (IL)-1 and -2, and tumor necrosis factor (TNF)-α) (Table 1). Dendritic cells matured in vitro correspond to their potent immunostimulatory counterpart in lymph nodes, whereas immature dendritic cells are the counterpart of the phagocytic but less stimulatory peripheral-tissue dendritic cells.

F. Hsu, et al.
B-cell lymphoma, autologous antigen-pulsed dendritic cells

F. Nestle, et al.
Melanoma, peptide- or tumor lysate-pulsed dendritic cells
The six fundamental hallmarks of cancer


- growth signal self-sufficiency
- antigrowth signal insensitivity
- apoptosis evasion
- limitless growth potential
- sustained angiogenesis
- tissue invasion including metastasis
The seventh fundamental hallmark of cancer

Lack of immune-mediated tumor rejection


Human ovarian cancers

Ovarian cancer is the 4th leading cause of female death in the USA.

Little improvement in the treatment of advanced-stage ovarian cancer over the past 30 years.

70% of patients are diagnosed with peritoneal dissemination and the formation of a large volume of ascites fluid: tumor cells, immune cells, soluble factors
Tumors reprogram dendritic cells to defeat host immunity, not the tumor

Current tumor immunotherapy paradigms build on infectious disease principles that may not apply to cancer
Tumor plasmacytoid DC generate IL-10$^+$ T cells


[\textsuperscript{3}H]thymidine incorporation (cpm x 10$^3$)

- Control
- + tumor PDC generated T cells
  - + anti-IL-10R

Levels:
- Control: 45,000 cpm
- + tumor PDC generated T cells: 10,000 cpm ($^*$)
- + tumor PDC generated T cells + anti-IL-10R: 30,000 cpm ($^{**}$)
Tumor myeloid DC induce IL-10+ T cells through B7-H1 signals


VEGF and IL-10 from the tumor induce B7-H1 expression
Immune recognition of tumor antigens as self is a significant problem.

**Infection:** rapidly dividing cells of external origin.

**Cancer:** rapidly dividing cells of internal origin. The tumor is a part of you (as opposed to an invading pathogen).
The new immunologic you

At puberty, the body makes a whole host of new proteins: breasts, breast milk, gonads, etc.

Your immune system accepts these.
**Thymus**

**Negative selection**

**Central tolerance**

- Naïve thymocytes
- Self-reactive

**Normal repertoire**

**Blood, LN, BM, spleen**

**Peripheral tolerance**

- CD4+CD25+
- Treg
Prior studies demonstrated elevated CD4$^+$CD25$^+$Foxp3$^+$ Tregs in blood of cancer patients. Are there CD4$^+$CD25$^+$Foxp3$^+$ Tregs in the tumor mass and draining lymph nodes?
FOXP3⁺ Tregs in tumors

Tumor Tregs allow tumor growth despite otherwise sufficient numbers of functional anti-tumor effectors.
Tumor Tregs allow tumor growth despite otherwise sufficient numbers of functional anti-tumor effectors.
Depleting CD4<sup>+</sup>CD25<sup>+</sup> Tregs enhances tumor immunity

Improves endogenous immunity

Improves actively-induced immunity

**FIGURE 3.** Induction of tumor immunity by administering anti-CD25 mAb to normal mice. Eight-week-old BALB/c or B6 mice were i.v. injected with 1 mg each of purified PC61 on 4 and 2 days (filled arrows) before s.c. inoculation of $1 \times 10^5$ RL<sup>31</sup> or B16 cells (open arrow), and tumor growth was monitored for individual mice (five mice per group).
Elevated tumor CD4$^+$/CD25$^+$ T cells predict poor survival in ovarian cancer

Proposed mechanisms of Treg-induced tolerance to cancer

Zou Nature Reviews Immunology 6, 295–307 2006
CD4+CD25+ CTCL cell  CD4+CD25+ Treg
Hypothesis:
Ontak will kill CD4^{+}CD25^{+} Tregs, and reverse immunosuppression

- Phase 0/I with immune end point
- Patients with advanced-stage cancer
- Single IV infusion of Ontak
- Test immunity before and after
- No clinical endpoints planned
<table>
<thead>
<tr>
<th>Patient</th>
<th>Ontak μg/kg</th>
<th>Age in years</th>
<th>Gender</th>
<th>Tumor type</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>59</td>
<td>F</td>
<td>ovarian</td>
<td>S, C</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>41</td>
<td>F</td>
<td>breast</td>
<td>HT, C</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>50</td>
<td>M</td>
<td>lung</td>
<td>C, RT</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>53</td>
<td>F</td>
<td>ovarian</td>
<td>C, RT, S</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>31</td>
<td>F</td>
<td>ovarian</td>
<td>C, S</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>36</td>
<td>F</td>
<td>ovarian</td>
<td>C, S</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>72</td>
<td>M</td>
<td>pancreatic</td>
<td>C, HT, S</td>
</tr>
</tbody>
</table>
Ontak reduces blood CD4⁺CD25⁺ T cell concentration
Ontak increases blood IFN-γ⁺CD3⁺ T cell concentration
Thymidine (10^4 c.p.m.)

Before

3 days after

23 days after

FoxP3
Patient 4

- Resistant stage IV (metastatic) disease.
- First recipient of the dose-escalated 12 µg/kg, with significant immune response.
- Because she had measurable disease, she received six additional Ontak doses to test clinical efficacy (IRB-approved).
Patient 4 was treated with weekly Ontak at 12 µg/kg x 6 weeks
Ontak reduces metastatic tumor burden in treatment-refractory ovarian cancer.
Corroborating trials

Summary

• Tregs can be depleted in blood of human cancer patients for up to 30 days after a single Ontak administration (9 or 12 μg/kg).

• Treg depletion is associated with improvements in immunity and some good clinical responses.

• Relationship between clinical and immune changes and Treg depletion are postulated, but not proven to be a mechanism in humans (some evidence in a mouse model (JI 177:84-91 2006).

• Tregs are a reasonable target for overcoming tolerance in cancer, and Ontak is one agent for this purpose.

• Ontak also kills anti-tumor CTL
Development of a second generation Treg depletion agent

12-mer expressed on surface of M13 phage

CD122-targeting 12-mer

$E.\ coli$-derived extracellular domain of mouse CD122 coated on surface of microtiter well

CD122 is IL-2 receptor $\beta$-chain. We found that it is expressed on the surface of many epithelial carcinomas.
Screen for phage binding mouse Tregs

Percent binding

M13 library  no phage

3.3 3.9 4.5 3.4 4.15 3.6 4.4 3.7 4.3

PELMAGSWDIA NPANYHKALEHI CNGKCCGVHY DYPHFLTIHC FKHMTWHSCHF MGEDMMWCDFFR MGEDMMWCDFFR MCDMMHFGCGHW MCDMMECGFH MYNMDMYFNIWF
Challenge with ID8 tumor

Treatment of tumor bearing mice with CD122-binding/toxin conjugate increases survival

Percent survival vs. survival (weeks)
In tumors, many pathways lead to Tregs

Our drug discovery strategy

• Use simple approaches
• Target highly relevant pathways
• Think about patient populations and trial design
• Use reagents with known clinical track records:
  – Peptides and DT have good safety records
  – Peptides and DT are simple and cheap to manufacture
• Gear lab work directly towards translation:
  – We ignored many obvious scientific questions to get straight into the clinic. These questions are now being addressed through additional grants and laboratory work.
• Identify clinical and manufacturing partners early
Current and future plans

- Detailed studies of CD4\(^+\)CD25\(^+\) Tregs
- Studies of other regulatory T cells
- Phase II trial of Ontak in ovarian cancer
- Further develop novel Treg-depleting agents
- Combine Treg depletion with vaccines
• Tulane
  W. Zou, B. Barnett, J. Rueter, J. Cheng, M. Burow, S. Wei, Ben Daniel, Leann Myers, I. Kryczek, M. Brumlik

• University of Alabama
  D. Curiel

• University of PA
  G. Coukos

• Fred Hutchinson
  N. Disis, K. Knutson

• Johns Hopkins
  L. Chen

• INSERM, Paris
  D. Emilie, V. Machélon

• Art/Graphical
  A. Curiel, M. Curiel