Role of Co-Stimulatory Molecules and Immune Cell Checkpoints in Cancer Suppression

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Disclosures

*Intellectual Property related to the PD-1 / PD-1 Ligand pathway licensed non-exclusively to:*
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- Merck-Serono
- Boehringer-Ingelheim
- Amplimmune/AstraZeneca
- Novartis

*Intellectual Property related to TIM-3 licensed to Novartis*

Consultant: Novartis, BMS, Roche, Lilly, Seattle Genetics, Bethyl Labs

Immunology has offered hope for curing cancer for 100 years

What is different now?

New Strategy

Blockade of pathways used by tumors to inhibit anti-tumor immunity

Checkpoint blockade
T cells are white blood cells that can kill cancer cells: more is better; T cell clonal expansion

- There are positive and negative second signals

The PD-1 Pathway Inhibits T Cell Activation

Costimulation regulates T cell response to antigen dose
**PD-1 = Programmed Death-1**

- Cloned from a CD3-activated T cell hybridoma undergoing activation-induced cell death (Honjo lab)
  - Does not directly activate caspases and cause cell death or apoptosis; not like CD95 (Fas)
  - Indirect effect on cell death by reduced cytokines, survival factors (less Bcl-xL, more BIM)

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**Why have negative signals like PD-1?**

1. Tune down the immune response after elimination of disease
2. Prevent too strong an immune response damaging tissues
3. Maintain immune tolerance

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**Identify the target: block PD-1/PD-L1**

Engagement of the PD-1 Immunoinhibitory Receptor by a Novel B7 Family Member Leads to Negative Regulation of Lymphocyte Activation


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**PD-1 or PD-L1 Blockade Stimulates anti-tumor T cell response**

- **Increased cytokines**
  - IFN-γ
- **Increased killing**
  - CD8+ CTL
- **antibody drug**
  - PD-1
  - PD-L1
- **Tumor cell**
Discovery may shed light on cancer's shield against the immune system

PD-L2 is a second ligand for PD-1 and inhibits T cell activation

**PD-L1 on Breast cancer cell lines**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>MDA-231</th>
<th>SKBR-3</th>
<th>MCF-7</th>
<th>BT474</th>
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</thead>
<tbody>
<tr>
<td>PD-L1</td>
<td>Log fluorescence intensity</td>
<td></td>
<td></td>
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</table>

• Expressed on cell surface of ~30% solid tumors and selected hematologic malignancies
• Inhibits anti-tumor immune responses

**PD-L1 in Cancer**

- Kidney tumor
- Non-small cell lung cancer

Brown = PD-L1  Rodig, Signoretti, McDermott; BWH & DFCI

**Where does checkpoint blockade function?**

CTLA-4 in the lymph node  PD-1 in the tumor

**Why doesn’t directly stimulating the immune response cure cancer?**
Once the tumor gets ahead and expresses PD-L1, immuno-inhibition is dominant and maintained by a feedback loop.

Taube et al: Adaptive resistance

Hypoxia regulates the immune response


Hypoxia Response Element in the PD-L1 promoter

Chen et al, Annals Onc. 27:409 (2016)

PD-1+ T cells at a PD-L1 tumor interface in melanoma

PD-L1+ melanoma  PD-1+ T cells

George Murphy, Scott Rodig, Gordon Freeman, BWH & DFCI
Hypoxia upregulates PD-L1 expression


1. ccRCC with bi-allelic VHL inactivation had more PD-L1 expression compared to those with one VHL WT allele.

2. HIF-2 directly bound to a hypoxia response element in the PD-L1 promoter and was the major regulator of PD-L1 expression.

Messai, … Chouaib, European Urology (2016)

Agents in Clinical Trials

- Anti-PD-1
  - Nivolumab (BMS)
  - Pembrolizumab (Merck)
  - Pidilizumab (Curetech)
  - MEDI-0680 (Medimmune-AZ)
  - PDR001 (Novartis)
  - REGN2810 (Regeneron)

- Anti-PD-L1
  - Atezolizumab (MPDL3280, GNE)
  - Durvalumab (MEDI-4736 Medimmune-AZ)
  - Avelumab (MSB0010718C EMD Serono)
  - MDX-1105 (BMS)

Multiple other agents in development

VHL loss upregulates PD-L1

Phase I clinical trial of anti-PD-1 antibody Nivolumab:
Kidney Cancer cohort (34 patients)

- Generally tolerable: fatigue, rash, pruritus, diarrhea
  - Each line follows growth or shrinkage of tumor in one patient
  - 29% objective responses

Drake ASCO 2013
PD-1 Cancer Immunotherapy is different from chemotherapy

- Well tolerated: This is not chemotherapy or a cell poison! Some nausea, no hair loss, no blood count decline.
- Good safety profile
- Most serious adverse events are autoimmune-mediated, like pneumonitis, colitis. Less than 10% of patients
- Physicians will have to learn to manage a different spectrum of adverse events than those seen in chemotherapy
- This can be community hospital medicine: half-hour intravenous drug infusion.

PD-1 is better than chemo in melanoma

PD-1 antibodies pembrolizumab and nivolumab are now FDA approved for advanced melanoma, lung cancer, and renal cancer

Better Quality of life: Squamous NSCLC: EQ-5D Utility Index

Mean Scores Over Time While on Treatment

PD-1

Chemo

Mean EQ-5D Utility Index Score

Nivolumab (n = 97)

Docetaxel (n = 89)

Population Norm*

Lung Cancer Norm (UK-based): 0.67b

Higher scores indicate better health status.

Only time points that had PRO data available for ≥5 patients in either treatment arm are plotted on the graph.

Checkpoint works equally well in the aged

Meta-analysis of 6 Phase III PD-1 and CTLA-4 trials
2,078 younger patients < 65-70 years
1,224 older patients >65-70 years

Younger: Hazard Ratio, 0.73; P<0.001
Older: Hazard Ratio, 0.72; P=0.004

T Funakoshi et al., SITC 2015

Better Quality of Life

• Martin Reck said “suggests prolonged survival occurs with a resumption of normal life”

90 year old with metastatic melanoma and 4 brain metastases:
Treated with PD-1 mab pembrolizumab
Predictive biomarkers are essential for getting the right treatment to the right patient.

A new era in PD-L1 immunohistochemistry
Now at least 5 good PD-L1 IHC mAbs available

extracellular
SP142  Roche - Spring
eHL3N  CST
9A11  Freeman - CST

intracellular
5H1  Chen
22C3  Merck - Dako/Quest
28-8  BMS - Dako/Quest

PD-L1 expression in tumor increases the likelihood of response to PD-1/PD-L1 blockade

<table>
<thead>
<tr>
<th>Objective Response Rates</th>
<th>n= 42</th>
<th>44</th>
<th>34</th>
<th>113</th>
<th>129</th>
<th>55</th>
<th>411</th>
<th>94</th>
<th>30</th>
<th>53</th>
<th>65</th>
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<tbody>
<tr>
<td>unsanctioned</td>
<td>21%</td>
<td>32%</td>
<td>23%</td>
<td>40%</td>
<td>19%</td>
<td>18%</td>
<td>40%</td>
<td>21%</td>
<td>29%</td>
<td>23%</td>
<td>26%</td>
<td>11%</td>
</tr>
<tr>
<td>PD-L1+</td>
<td>36%</td>
<td>67%</td>
<td>44%</td>
<td>49%</td>
<td>37%</td>
<td>46%</td>
<td>49%</td>
<td>36%</td>
<td>27%</td>
<td>46%</td>
<td>43%</td>
<td>22%</td>
</tr>
<tr>
<td>PD-L1-</td>
<td>0%</td>
<td>15%</td>
<td>17%</td>
<td>13%</td>
<td>11%</td>
<td>11%</td>
<td>13%</td>
<td>13%</td>
<td>20%</td>
<td>15%</td>
<td>11%</td>
<td>4%</td>
</tr>
<tr>
<td>Treatment:</td>
<td>anti-PD-1 Antibody</td>
<td>anti-PD-L1 Antibody</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Assay:</td>
<td>Membranous pattern on tumor cells</td>
<td>Immune infiltrate NR</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Nivolumab</td>
<td>Pembrolizumab</td>
<td>MPDL3280A</td>
<td>MEDI 4736</td>
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</table>

Cytoplasmic tail
Extracellular domain

9A11  E1L3N  SP142  7G11  015

cHL
DLBCL
NPC
NSCLC
RCC
21% Discordancy between PD-L1 on Primary and Metastasis in RCC

<table>
<thead>
<tr>
<th>Primary</th>
<th>Metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
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</tbody>
</table>

- PD-L1 positivity was heterogeneous and almost exclusively detected in high nuclear grade areas ($P < 0.001$).
- Assessment as a predictive biomarker for PD-1 blockade may require analysis of metastatic lesions.
- Pathologists should select high grade tumor areas for PD-L1 IHC analysis to avoid false negatives.

20 positive
33 negative in primary & met
53 cases

PD-L1 expression was heterogeneous even within individual RCC lesions

Low grade area
High grade area

- PD-L1 was almost exclusively detected in high nuclear grade areas ($P < 0.001$)

What does the immune system see in a tumor to attack?

The immune system recognizes protein coding changes in the tumor cell, called tumor neoantigens.

Tumors have multiple neoantigens that T cells can attack

Normal cell
Tumor cell

Driver mutation
Mutations
Neoantigens
Two evolutionary processes in cancer:

1. DNA mutation
   - Rare driver mutations
   - many passenger mutations
2. Immune evasion: PD-L1, IDO, TGF-b, IL-10, loss of MHC, others

Normal cell  Tumor cell  neoantigens

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Why the enthusiasm for immunotherapy?

Data from Steve Hodi, Jedd Wolchok & ECCO

Moderate percentage but long-term

High percentage but short-term

Understanding immunology and genetics has identified groups that respond well to PD-1/PD-L1 therapy

- Highly mutated tumors (MSI, defects in DNA repair) : 62%
- Genetically amplified PD-L1 and PD-L2 (Hodgkin) : 87%
- With Viral antigens (HPV, Head and neck, Merkel)
- What other cancer types might respond well ??

Clinical benefit with PD-1 blockade

- 18% in TN Bca
- 25% ORR
- 18% ORR
- 24-26% ORR
- 29-40% ORR


Mutation frequencies in protein coding regions from 3,083 tumor–normal pairs

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Prostate  Kidney  Bladder  Lung  Melanoma

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added - please delete it as needed

DFCI, 10/2/2013
Why did the T cells need PD-1 blockade to attack the tumor?

The anti-tumor immune response is a years long struggle.

The T cells had tried, failed, and become “exhausted”

PD-1 pathway mediates T cell exhaustion in Chronic Viral Infections

Dan Barber
John Wherry
Rafi Ahmed
2006

PD-1 is upregulated in both acute and chronic immune responses but stays high in chronic.

PD-1+ cells are “exhausted” and produce less cytokine

Tumor-Infiltrating T cells (TIL) behave like exhausted T cells

Dan Barber, Rafi Ahmed
Human Ovarian Tumor Infiltrating T cells (TIL) express high levels of PD-1

PD-1 blockade of ovarian TILs augments cytokine secretion by tumor antigen-specific (NY-ESO-1-peptide) CD8 TILs in vitro: TILs are exhausted T cells that respond to PD-1 blockade.

T cell exhaustion is more than PD-1

Exhausted Tumor infiltrating lymphocytes express multiple immunoinhibitory receptors:

These are druggable targets for tumor immunotherapy.
The Future is Combination Therapy

PD-1 + CTLA-4 is better than CTLA-4 alone

Could some chemotherapies synergize with PD-1 blockade?

The future of cancer therapy decisions

- **Tumor Immuno evasion Score:**
  - How much PD-L1, PD-L2, IDO, Galectin-1, Galectin-9, B7-H3, B7-H4, VISTA, HHLA2, Arginase, NKG2D-Ligands?
  - Choose best immunotherapy

- **Cancer Genome sequencing:**
  - Identify which oncogenes are drug targets?
  - Which mutations are immunogenic?
  - Choose best targeted therapy/vaccine
To be done

- How do we identify who will respond to PD-1 blockade?
- What are mechanisms of primary failure to respond?
  - Other immunoinhibitors?
  - Failure of immune cells to infiltrate tumor?
  - No good neoantigens?
- What are mechanisms of secondary failure to respond?
  - Expression of other immunoinhibitory receptors?
  - Loss of MHC?

It’s a great time to be an oncologist or researcher

- PD-1/PD-L1 works on a wide range of tumors with
  - moderate percentage of responders
  - good safety profile
- PD-1/PD-L1 gives us a foundation to build on
- With this success, human creativity has been unleashed and we’re learning to do better

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