Basic Concepts and Future Horizons in Cancer Immunotherapy

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The amplitude of immune responses is determined by a balance of positive signals (antigen+costimulation) and negative forces (immune checkpoints).

**Immune Checkpoints = Brakes**

- Stat3, ~20 receptor/ligand, Treg, MDSC

**Immune Response**

**Immunization = Accelerator**

- Signal 1 – Antigen
- Dendritic Cells
- Signal 2 - Costimulation

Why do some vaccines induce tumor-specific immunity but rarely induce tumor regression?
The Immunologic Synapse

Antigen Presenting Cell or Tumor Cell

- PD-L1/PD-L2
- B7.1/B7.2
- B7RP-1
- B7H3
- HVEM
- MHC/pep
- TCR

T Cell

- PD-1
- CD28
- ICOS
- CTLA-4
- CD137L
- CD137
- OX40L
- OX40
- LIGHT
- LIGHT-R
- PS/galectin9
- Tim1/Tim3
- CD200R
- CD200
- CD40
- CD40L

Cytokines (IL-1, IL-6, IL-10, IL-12, IL-18)
The PD-1 Pathway is a Master Checkpoint within the Tumor Immune Microenvironment

Activation (cytokines, lysis, prolif., migration)

Inhibition (anergy, exhaustion, death)

APC

B7.1

CD28

TCR Signal 1

MHC-Ag

T cell

Tumor

PD-1

PD-L1

Anti-PD-1

Tumor
Multiple Cell Types within the Tumor Immune Microenvironment Express PD-1 and PD-1L

- Tumor
  - IDO
  - TTO
  - PD-L1
  - B7-H3
  - B7-H4

- MDSC
  - IDO

- DC/MΦ
  - IDO
  - maturation
  - PD-L1
  - PD-L1/2

- CD4 T cell
  - PD-1
  - A2aR
  - B7-H3
  - B7-H4
  - IL10, TGFβ

- Anti-tumor CD8 killer cell
  - PD-1
  - LAG-3
  - Tim3
  - BTLA

- Treg
  - PD-1
  - LAG-3

Inhibitory cytokines (VEGF, IL-10, TGFβ)
Adenosine
2 Mechanisms for PD-L1 upregulation in tumors

**Innate (tumor cell intrinsic) Resistance**

Constitutive tumor signaling induces PD-L1 on tumor cells.

**Adaptive Resistance**

T cell-induced PD-L1 up-regulation.
2 Mechanisms for PD-L1 upregulation in tumors

**Innate (tumor cell intrinsic) Resistance**

Constitutive tumor signaling induces PD-L1 on tumor cells.

Oncogenic Pathway (Stat3, Akt)

**Adaptive Resistance**

Tumor-induced PD-1 up-regulation
TGF-β enhances TCR-dependent transcriptional induction of PD-1 in T cells – this effect is mitigated by a TGF-βRI inhibitor.
TGF-β dependent induction of PD-1 is dependent on SMAD3 binding to SBE in the PD-1 promoter.

<table>
<thead>
<tr>
<th>SBE#1</th>
<th>SBE#2</th>
<th>NFATc1</th>
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<tbody>
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<td>5’-GTCTG-</td>
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<td>-TTTTTCC-3'</td>
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<tr>
<td>-CAGAC-</td>
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</tr>
<tr>
<td>WT</td>
<td>Mut</td>
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</table>

Human PD-1 promoter:
-1.2kb -1.0kb +1

\[pdcd1\]

CD4cre x SMAD2-flox
- αCD3
- αCD3 + TGF-β1

CD4cre x SMAD3-flox
- αCD3
- αCD3 + TGF-β1

FL2-H::PD1 PE

Histograms showing MFI values:
- MFI 343
- MFI 951
- MFI 248
- MFI 338

Relative Luciferase Unit
- WT
- WT
- SBE#1
- SBE#2
- SBE#1/2
- Medium
- αCD3/28
- αCD3/28 + TGF-β1

* NS

+ TGFβRI/RII
Effect of TGF-β signaling in T cells on tumor growth and PD-1 expression on TIL

Growth of B16 melanoma in wildtype mice vs T cell-specific TGF-βRI KO mice

TGF-βRI KO TIL have much lower PD-1 expression
High PD-1 expression on tumor specific TIL is Smad3 dependent
Can a tumor’s genetics affect it’s microenvironment and, as follows, its response to immunotherapy?
Mutational density among cancers sorted by histology
Does higher mutational load = anti-PD-1 responsiveness?

- MSI$^{hi}$
- POLE/POLD1
- HPV+$^{+}$
Cancer Genotype linked to Immune Microenvironment – the case of Colorectal Cancer

- Lymphocyte infiltration into CRC an independent predictor of clinical outcome (Gallon et al)
- Major genetic classification of CRC based on loss of key mismatch repair genes (Microsatellite instable vs stable)
- Even though CRC does not typically respond to anti-PD-1 as monotherapy, one CRC CR was observed – this patient was MSI$^\text{hi}$ (Lipson et al, CRC 2013)
Analysis of the immune microenvironment of CRC reveals a subset (~20%) of tumors that are “inflammed”.

“Non-inflammed”
mRNA analysis from LCM reveals that MSI tumor microenvironment has enhanced Th1, CTL and checkpoint expression relative to MSS.
PD-L1 expression on myeloid cells inside MSI tumors
PD-1 & LAG-3 expression at TIL and stroma according to mismatch repair status

MSI

MSS

0.1mm

0.1mm
MSI^{hi} CRC TIL uniquely contain PD-1^{hi}/IFN\gamma^{hi} populations of CD4 and CD8 T cells
CEA changes in response to anti-PD-1 according to mismatch repair status

% CEA change from initiation of treatment

Weeks from initiation of therapy

MSS
MSI
Does genetics of colon cancer affect microenvironment? $\text{MSI}^{\text{hi}} \text{ vs MSS}$

- No $\text{POLE}$ or $\text{POLD2}$ mutation
- 49 total nonsynonymous exomic mutations (standard for MSS)
### 49 mutations – many potential neoantigens

<table>
<thead>
<tr>
<th>HLA allele</th>
<th>Gene</th>
<th>Peptide length</th>
<th>Normal peptide</th>
<th>Predicted logIC50</th>
<th>Tumor/neoantigen</th>
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<td>HLA-A*02:01</td>
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<td>FLVAILEGV</td>
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### Notes:
- **Gene**: Gene symbol for the protein involved.
- **Peptide length**: Length of the predicted peptide sequence.
- **Normal peptide**: Predicted peptide sequence from normal cells.
- **Predicted logIC50**: Predicted IC50 value for the normal peptide sequence.
- **Tumor/neoantigen**: Predicted peptide sequence from tumor or neoantigen cells.
- **Predicted logIC50**: Predicted IC50 value for the tumor or neoantigen peptide sequence.
Potential Neoantigens in MSS patient with 49 coding mutations

A  HLA-A201
   De Novo HLA binding

B  HLA-B1501
   New TCR contact in peptide with moderate – high HLA binding affinity

C  HLA-B3501
Therapeutic Implications for PD-1 Pathway Blockade of Adaptive Resistance Model

1. Inducer of Anti-tumor Immunity, ie vaccine
   - Weak Endogenous Anti-tumor Immune Response
     - No PD-L1 up-regulation on tumor/TAM
     - NO RESPONSE
   - PD-L1 expression on tumor/TAM
     - RESPONSE

2. Anti-PD-1
   - Weak Endogenous Anti-tumor Immune Response
     - Endogenous Anti-tumor Immune Response
     - RESPONSE
   - Strong Endogenous Anti-tumor Immune Response
     - PD-L1 up-regulation on tumor/TAM
     - RESPONSE
   - Single agent Anti-PD-1
     - RESPONSE
     - NO RESPONSE
Cyclic Dinucleotides (CDNs) and Intracellular DNA Activate Innate Immunity via STING

- Cyclic dinucleotides (CDNs) regulate gene expression in bacteria
- Intracellular bacteria secrete CDNs through MDRs into the cytosol of infected cells
- cGAS synthesizes c-GMP-AMP in response to binding cytosolic DNA
- CDNs are sensed by STING (Stimulator of Interferon Genes)
- STING activates innate immunity by signaling through the TBK-1/IRF-3 axis and NF-κB
- The potency of vaccines is significantly enhanced by co-formulation with CDNs
STING-CDN Crystal Structure Enables Rational Design of Molecules with 100x Potency Relative to Native Molecules – most potent known DC activators

[Diagram of CDN structure and c-di-GMP STING co-crystal]

- CDN composition & modifications:
  - Orange circle: Purine nucleotide base
  - Green circle: Phosphate bridge linkage
  - Red circle: Phosphodiesterase resistance
  - Blue circle: Formulation enhancement

STING–c-di-GMP complex
STINGVAX

1. Released Tumor Antigens
2. GM-CSF attracts Dendritic Cells
3. CDN Activate Dendritic Cells

Anti-tumor Immunity
STINGVAX vaccination results in enhanced IFNg production in tumors associated with increased PD-L1 induction and synergy with anti-PD-1.
Combination checkpoint blockade
Expression profiling of PD-1<sup>hi</sup> vs PD-1<sup>lo</sup> TIL reveals additional checkpoint pathways targetable with Ab: LAG-3

LAG-3 / PD-1 Double KO Mice Reject Poorly Immunogenic B16 Tumors
Epigenetic modulation of tumor antigenicity and the tumor microenvironment
Multiple immune genes induced by 5-aza treatment

Checkpoint receptors

Interferon receptors/Stats

Cancer Testes Ag

Antigen Processing/Presentation

Interferon pathway

IFN Regulatory Factors
Epigenetic silencing of genes in innate and adaptive immunity in NSSCL

Checkpoint ligand

Antigen processing & presentation

STATs/IFNγR

Interferon response genes

BATF family transcription factors

Interferon Regulatory factors

Epigenetic silencing of genes in innate and adaptive immunity in NSSCL

Adenocarcinoma

Squamous cell cancer
Response to anti-PD-1/PD-L1 after 5’aza-CR + HDACi in 5 pt with NSCLC
Improving Survival with Combination Therapy

% Survival vs Time

- Control
- Standard or Other Therapy
- Checkpoint Blockade
- Combination
Suzanne Topalian
Julie Brahmer
Charles Drake
Bill Sharfman
Bob Anders
Bert Vogelstein
Ken Kinzler
Nick Papadopolus
Steve Baylin

Janis Taube
Franck Housseau
Nico Llosa
Ben Park
Cindy Sears
Young Kim

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