Biomarkers for Immuno-Oncology and Combination Development

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Antitumor Immunity Cycle and Mechanisms of Escape

Tumor Escape from Immune Recognition
- Antigen loss leads to tumor cell variant growth
- Low MHC/Peptide presentation
- Tumor-induced physical barrier – T cells can’t get in
- Tumor treated as ‘Self’ due to T cell tolerization
- Immune suppression by tumor and other cells

Antitumor Activity can be Promoted by Targeting Critical Phases in the Immune Cycle

**Antigen Presentation:**
Making tumors visible to the immune system

**Tumor Microenvironment (TME):**
Breaking down the protective shield around tumor cells

**T-Cell Activation:**
Turning resting/inactive immune cells into killer cells

**T Cell (Re)Activators**
- Checkpoint Inhibitors
- Agonists of CoStimulators

**AP Increases**
- Oncolytic virus; Small Molecule
- Radiation; Chemotherapy
- NK cell activators

**TME Modifiers**
- Treg and MDSC Depleters

**Oncolytic virus; Small Molecule Radiation; Chemotherapy**
**APC Co-stimulation vs. Co-inhibition of T cells**

- By targeting co-stimulatory or -inhibitory pathways we may amplify immune activity.

  Block inhibitory signals = ‘Release the brakes’
  Pathway agonism = ‘Press the Gas’

- Many current combination strategies focus on blockade of multiple inhibitory pathways (e.g. anti-CTLA-4 / PD-1) or mixed targeting of stimulatory and inhibitory pathways (e.g. anti-4-1BB / PD-1)

- Biomarker strategies will focus on understanding expression of these proteins

Goals of Pre-Clinical and Clinical Biomarker Studies

Biomarker studies aim to understand:
- Change over time
- Baseline predictive value
- Change with response

Immunotherapy (IMT) studies have sought:
- T cell proliferation
- Cytokine production
- Cytotoxic function

Preclinical Strategy for IMT vs Traditional Oncology

**Historic Oncology**

- **Xenograft Models**
  - Numerous
  - Well characterised
  - Derived from human disease
  - Immunocompromised

- **Tumor Cell Lines**
  - Numerous and high throughput
  - Well characterised
  - Derived from human disease
  - Can be carried in vivo

**IMT-C**

- **Syngeneic Models**
  - Intact immune system

- **GEMMs**
  - Immunocompetent
  - Increased disease relevance vs syngeneic

- **Primary Immune Cells**
  - Many established systems
  - Human derived
Model Systems Have Established Basic Principles of Antitumor Immune Activity

Syngeneic Models
Intact immune system

Primary Immune Cells
Many established systems
Human derived

Associated with Therapy-Induced Tumor Rejection

Acute, peripheral T cell activation and proliferation
Ki67+ T cells expressing activation markers (e.g. ICOS)

Increases in plasma / serum cytokines
Critical are Th1-biased responses -
Produce IFNγ, IL-2 and TNF-beta, evoking cell-mediated immunity and phagocyte-dependent inflammation

Chemokines expressed in the TME (Attract T cells to site)
IFNγ-induced chemokines CXCL9 and CXCL10 may be critical as they recruit effector T cells

Migration of CD8+ Cytotoxic T cells (that can overcome immunosuppressive signals in the TME)
However Model Systems Have Limitations

<table>
<thead>
<tr>
<th>Limitations of Syngeneic Models</th>
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<tr>
<td>Primarily models of acute inflammation and tumour initiation</td>
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<td>Variability in response within groups</td>
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<td>Limited window for intervention</td>
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<td>Rely on mouse cross reactivity or surrogate reagents</td>
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<td>Direct correlation to human disease may be limited</td>
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Combination studies may be challenging in Syngeneics and GEMMs may be limiting

- Insights from pre-clinical models are only useful if they relate to human disease
- *Essential* to build an understanding of the human tumor microenvironment
Cancer immunoediting concepts predict that many tumors will be characterized by immunosuppressive cells and/or by a lack of cytotoxic T cells.

Assessed by tumor tissue IHC to determine Prognostic roles of different resident cell types.

Immune Contexture is Prognostic

- CD8+ cells and Th1-biased immune responses tend to be associated with good prognosis

- Treg, Th17, and Th2-biased immune responses tend to be associated with poor prognosis

This underscores the role of the immune system in cancer and suggests the possibility that similar measures may be predictive of response to IMT

Response to IMT in the Clinic: CD8+ Cells in the TME

Pre-Treat melanoma

Post-Treat

Tumor CD8 Density (cells/mm²)

Responders ($n = 13$)

Progressors ($n = 12$)

PD-L1: Lead Predictive Marker for Anti-PD-1 Pathway Agents

- Baseline, tumor PD-L1 expression predicts likelihood of response / benefit
  - Tumors use PD-L1 to evade T cell activity
  - PD-L1 expression is a beacon for ongoing, cellular immune responses that may be reinvigorated

nivolumab was approved in all-comers because of improved safety vs. docetaxel

Anti-CTLA-4 / PD-L1: Responses in PD-L1- NSCLC

Suggests PD-L1 status may be used also to ID patients for combo regimens

Emerging Data - Mutation (and MSI)

- Mutation load appears to be proportional to neoantigen formation
- Such tumors may be most ‘visible’ to the immune system

MMR-Deficient CRC

Demonstrates clinical activity is greater in MMR-deficient vs. proficient CRC
Selection of IMT for the Right Patients May Rely on Multiple Biomarker Measures

- Current Biomarker Landscape:

  Individual positive selection measures that enrich for response to PD-1 monotherapy and may include –

  - PD-L1: Companion Dx currently on the market
  - MSI-H: Routinely measured clinically (for CRC)

  However, negative PD-L1 status may not preclude response / benefit and MSI is found only in a limited percentage of patients

Is there a set of factors that together identify patients for receiving monotherapy versus receiving combination therapy by default?
One example of a combined approach – PD-L1 / IFNγ

Biology of IFNγ and relationship with tumoral PD-L1

- IFNγ is a Th1 and NK cytokine that promotes cancer cell cytotoxicity:
  - Recruits tumor-infiltrating macrophages
  - Induces nitric oxide production
  - Increases cytotoxic T-cell proliferation
- IFNγ can induce expression of PD-L1
- High IFNγ mRNA expression might indicate immune active tumor microenvironment
  - Potentially identifies responders to durvalumab

Suggests multiple measures may improve both positive and negative selection
Composite Biomarker Status to Inform Combo Decisions?

- Measures like CD8, IFNγ/PD-L1, and MSI are converging as means to identify *inflamed tumors* which may respond to current IMT; however, how these measures provide predictive value together is not fully understood.

- Depending on the TME contexture, additional agents may be necessary for current IMT agents to be effective – likely to rely on:
  i) Treg and/or MDSC depletion
  ii) Blockade of other co-inhibitory pathways
  iii) Agonism of co-stimulatory pathways

  AND Methods to detect the presence of immuno-modulating pathways

- If tumors are not T cell-inflamed, agents that kick-start the immune reaction will likely be necessary (e.g. agents that increase *Antigen Presentation*).
What may be monitored to ID patients for Therapy?

**T-Cell Activation**
- PD-L1
- Co-activators, Co-repressors
  - e.g. Tim3, Lag3, TNFRSF Proteins
- Measures of T cell activity – Gene Signatures

**Tumor Microenvironment**
- Treg (FoxP3+)
- Myeloid Derived Suppressor Cells (MDSC)
- Pathologic features -
  - Digital Imaging (with other T cell measures above)

**Antigen Presentation**
- Mutation / MSI (and neoantigens)
- Hallmarks of DNA damage repair deficiency
- MHC and peptide antigens
- Antigen-directed T cell measures
- Markers of ‘immunogenic cell death’
Efforts to phenotype the tumor microenvironment before and after treatment are providing a better understanding of immune processes that lead to tumor killing.

In parallel to the initial IMT approvals, the first wave of prognostic and selective marker(s) are being established.

As new combination regimens are evaluated, TME phenotyping (and peripheral measures) may identify additional markers or sets of markers associated with patients likely (or unlikely) to receive benefit.

Though classic Companion Dx strategies have typically relied on a single measure to identify patients for therapy, IMT may rely on composite biomarker information and/or a ‘Biomarker Decision Tree’ to aid in choice of therapy or therapies.