CANCER IMMUNOTHERAPY-OPPORTUNITIES AND BARRIERS

Darwinian selection and Newtonian physics wrapped up in systems biology
IMMUNOSURVEILLANCE
**Immunosurveillance**

- Concept published in 1957* by Macfarland Burnet (1960 Nobel Laureate for the theory of induced immune tolerance, leading to solid organ transplantation)

- Changes take place on the surface of cancer cells

- These changes can be used by immune cells to identify and eliminate neoplastic cells (similar to transplant rejection)

- *Immunosurveillance = spontaneous host immune response to cancer*

Survival is associated with the ability of T cells to infiltrate metastases

Patient Survival by Immunotype (n = 147)

- No immune cell infiltrate
- Diffuse immune cell infiltrate
- Perivascular cuffing

LO 1,2,3
## Immunodeficiency States and Cancer Incidence

<table>
<thead>
<tr>
<th>Cause of Immunodeficiency</th>
<th>Common tumor types</th>
<th>Virus involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inherited</td>
<td>Lymphoma</td>
<td>EBV</td>
</tr>
<tr>
<td>Induced</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• organ transplant</td>
<td>Kaposi’s sarcoma</td>
<td>Human herpes B</td>
</tr>
<tr>
<td>• HIV/AIDS</td>
<td>Cervical cancer</td>
<td>Human papilloma</td>
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<tr>
<td></td>
<td>Liver cancer</td>
<td>Hepatitis B</td>
</tr>
<tr>
<td>Malaria</td>
<td>Burkitt’s lymphoma</td>
<td>EBV</td>
</tr>
<tr>
<td>Autoimmunity</td>
<td>Lymphoma</td>
<td>EBV</td>
</tr>
</tbody>
</table>
Separate yet interlinking responsibilities
Different qualities of innate and adaptive immunity

<table>
<thead>
<tr>
<th></th>
<th>innate</th>
<th>adaptive</th>
</tr>
</thead>
<tbody>
<tr>
<td>self / non-self</td>
<td>present, reaction is against foreign</td>
<td>present, reaction is against foreign</td>
</tr>
<tr>
<td>discrimination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lag phase</td>
<td>absent, response is immediate</td>
<td>present, response takes at least a few days</td>
</tr>
<tr>
<td>specificity</td>
<td>limited, the same response is mounted to a wide variety of agents</td>
<td>high, the response is directed only to the agents that initiated it.</td>
</tr>
<tr>
<td>diversity</td>
<td>limited, hence limited specificity</td>
<td>extensive, and resulting in a wide range of antigen receptors.</td>
</tr>
<tr>
<td>memory</td>
<td>absent, subsequent exposures to agent generate the same response</td>
<td>present, subsequent exposures to the same agent induce amplified responses</td>
</tr>
</tbody>
</table>
Acute Innate sensing of pathogen components activates inflammatory pathway and cellular recruitment

Nonspecific Inflammatory Response

1. When skin is cut, bacteria penetrates the wound.
2. The presence of bacteria to local white blood cells, called mast cells, trigger a release of histamine granules to surrounding tissue.
3. Histamine granules are chemicals that increase permeability to local blood vessels & stimulate other cells of immunity.
4. Since local blood vessels are dilated from histamine, plasma flow to area is increased, causing redness, swelling, and pain.
5. White blood cells, called neutrophils, come to site of infection from local blood vessels & engulf bacteria & damaged cells.
Sensing innate signals mobilizes DC to the lymph node

- Antigen capture by dendritic cells (DC)
- Inflammatory cytokines
- Loss of DC adhesiveness
- Immature DC in epidermis (Langerhans cell)
- Maturation of migrating DC
- Afferent lymphatic vessel
- Antigen presentation
- Lymph node
- Mature dendritic cell presenting antigen to naive T cell
Antigen acquired in the periphery by DC is processed and presented on MHC molecules to T cells in the lymph node.

Distinct from antibodies
Antigen presentation, in the appropriate context, leads to the activation of T cells.
Diverse inflammatory signals lead to the maturation of DC with promotes T cell immunity over tolerance.

**TLR/NLR agonists**

**Cellular components**
- Innate responses
- Pathogens

**Inflammation**
- Interferons
- NK cells
- Activated CD4+ T cells

**Antigen uptake**
- Antigen processing

**Immunity**
- Antigen presentation
- Costimulation
- T cell activation

**Tolerance**
Effector T cells

- T cells expand and differentiate in response to extrinsic factors often derived from inflammation.

- They traffic to sites of infection following chemokine trails, and extravaste using adhesion molecules.

- They perform their effector functions upon TCR engagement in the periphery; killing cells and secreting cytokines.

Cytokines induce transcription factors that drive the polarization of T cells.
Phases of a T cell response

Antigen recognition

Lymphocyte activation

Antigen elimination

Contraction (homeostasis)

Memory

Antigen presenting cell

Antibody-producing cell

Effector T lymphocyte

Naive T lymphocyte

Naive B lymphocyte

Differentiation

Clonal expansion

Elimination of antigens

Humoral immunity

Cell-mediated immunity

Apoptosis

Surviving memory cells

Days after antigen exposure

0
7
14
21

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II. Barriers to tumor immunity

- T cell activation
- The healing wound hypothesis
- Extrinsic regulators of immune function
- Intrinsic regulators of immune function
Weaknesses in tumor specific immunity that constrain responses

Extrinsic regulators

- Regulatory T cells (Treg)
- Myeloid derived suppressor cells (MDSC)
- Tumor associated macrophages (M2)

Intrinsic regulators

- Tolerance; weak TCR
- Anergy: weak activation (DAMPs/PAMPS)
- Inhibitory molecules
- cAMP

Tumor-derived regulators

- Antigen loss
- Antigen processing defect
- Inhibitory molecule expression
- Suppressive cytokine secretion
- Vasculature
Activation of T cell responses to tumors

HSPs

NK ligands
1) Missing self: mutation-induced alteration/loss in MHC molecules is sensed by NK cells

2) Stress-induced expression of NK-ligands also can make them a target for NK mediated lysis
Many of the anti-inflammatory and immunosuppressive aspects of a healing wound are found in the tumor microenvironment.
Tumors express chemo-attractants such as the CCL22 chemokine, which decorates blood vessels, and is followed by CCR4-expressing regulatory T cells to the tumor.

Once in the tumor environment, regulatory T cells can prevent the activation and attenuate the function of effector T cells.
Immature myeloid cells are commonly mobilized in cancer patients. They secrete cytokines and enzymes that can suppress effector T cell and NK cell function and promote the development and activity of regulatory T cells.
A large array of molecules are expressed on the surface of activated T and NK cells in response to chronic stimulation. These molecules serve to prevent immunopathology; genetic knockouts often develop autoimmunity or over-exuberant immune responses.

Antibodies blocking checkpoint molecules have shown promising efficacy in melanoma, NSCLC and Hodgkins lymphoma and others.
CTLA-4 is induced acutely on T cells by initial TCR engagement and prevents activation of naïve T cells.

PD-1 is upregulated over a period of 2-3 days and constrains effector T cell activity against PD-L1 expressing cells in the periphery.
Clinical activity of antibodies that block checkpoint molecules

Immunosurveillance sculpts the tumor

- Elimination
- Equilibrium
- Escape
### IMMUNE THERAPY: NEXT-GEN IMPROVEMENTS

<table>
<thead>
<tr>
<th>Approach</th>
<th>Example</th>
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<tbody>
<tr>
<td>Genetic modification of lymphocytes to introduce new recognition specificities</td>
<td>Tumor-Ag-specific TCR</td>
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<tr>
<td>Genetic modification of lymphocytes to alter function functions of T cells</td>
<td>Costimulatory molecules (CD8, 41BB)</td>
</tr>
<tr>
<td></td>
<td>Cytokines (IL-2, IL-12, IL-15)</td>
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<td></td>
<td>Homing molecules (CD62L, CCR7)</td>
</tr>
<tr>
<td></td>
<td>Prevention of apoptosis (Bcl-2)</td>
</tr>
<tr>
<td>Modify host lymphodepletion</td>
<td>Selective depletion of CD4+ cells or T regulatory cells</td>
</tr>
<tr>
<td>Block inhibitory signals on reactive lymphocytes</td>
<td>Antibodies to CTLA-4 or PD-1</td>
</tr>
<tr>
<td>Administer vaccines to stimulate transferred cells</td>
<td>Recombinant virus, peptides, dendritic cells</td>
</tr>
<tr>
<td>Administer alternative cytokines to support cell growth</td>
<td>IL-15, IL-21, IL-12</td>
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<tr>
<td>Stimulate antigen presenting cells</td>
<td>Toll-like receptor agonists</td>
</tr>
<tr>
<td>Overcome antigen escape variants</td>
<td>NK cells</td>
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III. OPPORTUNITIES FOR FOCUSED ULTRASOUND

- monotherapy
- Combinatorial therapy
Focused ultrasound and tumor immunity

- HIFU treatment of experimental neuroblastoma induced immunological memory against subsequent tumor challenge.
- HIFU can lead to reduced inhibitory signaling pathways (cytokines?) and increased DC function in TME.
- Cellular debris generated from mechanical disruption (compared to coagulation) induces DC activation (ICD;HSPs?).
Increased antigen availability

Direct tumor destruction (auto vaccination)
- HIFU vs LOFU (temp; sonoporation)
- coagulation vs mechanical
- ICD?

Drug delivery (microbubbles)
- immunostimulation (DC)
- inhibit the inhibitors?
- transfection/knockdown
- targeting

- stress ligands
- MHC molecules

- Antibody access
- T cell trafficking
- abscopal effect?

What cellular responses will be elicited by FUS in humans? NK; myeloid?

What cytokines will be induced? Pro-inflammatory or inhibitory? (IFN; IDO; TGFβ)

What happens to the expression of adhesion molecule ligands on tumor vasculature after FUS?

Will tumors/stroma express inhibitory molecules after FUS?

How can tumor penetration by MB be increased?

Will systemic responses be generated?