Control of malignancies by the immune system

Kim Margolin, M.D.
UW/Hutch/SCCA
May 12, 2012
5 Basic principles

• Tumor antigens recognized by immune system
• T cells recognizing tumors can be detected in cancer patients...but they are inefficient
• Tumors evade immune system by loss of antigens and secretion of suppressive factors
• Vaccination against tumor antigens is possible..but many antigens perceived as 'self'
• Antibodies against tumor-associated Ags can be effective but not alone/unmodified
Historical insights—all partially true

Paul Ehrlich (1909) Cancer Immunosurveillance—cancer would occur at “incredible frequency” if host defenses did not prevent the outgrowth of continuously arising cancer cells.

Lewis Thomas (1957) “primary function of cellular immunity….is to protect from neoplastic disease”

Macfarland Burnet (1957) “It is by no means inconceivable that small accumulations of tumour cells may develop and because of their possession of new antigenic potentialities provide an effective immunological reaction with regression of this tumor and no clinical hint of its existence”
What we know now

• Cancers are not common in immunosuppressed unless there are additional elements
  - Virus (e.g. EBV, hepatitis, HHV8/KSHV, HPV)
  - Chronic inflammation, bacteria (e.g. H pylori)

• Different immunodeficiencies confer different tumor risks (immune system complex!!)
  - AIDS
  - Post-bone marrow transplant
  - Post-solid organ transplant
  - Immunosuppressive Rx for autoimmune diseases

• Immune-tumor interactions change over time
CNN (5/9, Landau) "One in six cancer cases worldwide are caused by infections, many of which are preventable or treatable, (Lancet Oncology)." ABC News (5/9, Moisse) "French researchers pooled data on 27 cancers from 184 countries…fraction of cases attributable to viral, bacterial and parasitic infections." … found that "human papillomavirus (HPV), hepatitis B and C, and the ulcer-inducing Helicobacter pylori caused 1.9 million cancers worldwide in 2008." Minneapolis Star Tribune (5/9, Stoxen) approximately "half of infection-linked cancers seen in women were…cervix uteri cancers." “…among men…., more than 80% of cancers tied to infection were liver and gastric cancer." USA Today (5/9, Robins) "In the past decade, oncologists have noted an increase in cancers at the back of the tongue, in the tonsils and into the throat, especially in healthy, nonsmoking men." “…more than 7,000 new cases of oropharyngeal cancers are diagnosed each year." The majority "are in men, and those who don't use tobacco often find another common cause: the human papillomavirus, better known as HPV."
B vs T, Innate vs Adaptive

• Innate immune system (NK, granulocytes, monocyte/macrophages)
  - Not Ag-specific, HLA-restricted, or longlived
  - Rapid response but weak protection, no memory

• B cells (adaptive, Ag-specific, memory)
  - Immunoglobulin effective vs infections, not tumor
  - May synergize with other adaptive responses

• T cells (adaptive, Ag-specific, HLA-restricted)
  - Most important/potent responses
  - Longlived, memory
  - Highly interactive subsets, plasticity
Anti-Tumor Effector Mechanisms-1

B cells—produce tumor-specific antibodies
   a) Activate complement system to kill cells
   b) Activate cell-mediated killing by innate cells
   c) Bind to tumor cells and activate programmed cell death pathways

Macrophage killing mechanisms
   - Destroy tumors after binding antibody, see above
   - Secrete cytokines like tumor necrosis factor that binds to receptor on tumor, activates death
   - Produce chemical toxins like oxygen radicals
Anti-Tumor Effector Mechanisms-2

CD4⁺ T cells, CD8⁺ CTL, and NK cells

- Produce perforin & granzyme, and cytokines like TNF family members to kill tumor

- Other cytokines (e.g. interferons, GM-CSF)
  a) inhibit angiogenesis
  b) recruit other cells, especially innate (macrophage, dendritic cell, granulocyte)
Immunotherapy Strategies

High-dose Interleukin-2—still on the menu, But how does it work, and who benefits>toxicity?

3) Tumor-targeted antibodies (e.g., Herceptin)

4) Adoptive transfer of tumor-specific T cells

5) Donor lymphocyte infusions after BMT/HSCT (allogeneic bone marrow or hematopoietic stem cell transplant)

6) Vaccination

“Passive” Adaptive System

“Active” Adaptive System
Some definitions

• **Adoptive** vs. **Adaptive**
  Makes a vaccine work better by providing signals to accessory cells

• **Adjuvant** vs. **Adjuvant**
  Given after a curative-intent therapy to improve outcome
Tumor Elimination - Equilibrium - Escape

Schreiber et al. Immunity 2004
Tumor Antigens

Tumor-specific
Expressed by tumors ONLY

Tumor-associated
Preferentially expressed by tumors

Oncofetal
Expressed by tumors in adult, but also expressed by nonmalignant fetal tissues
Similarly, cancer-testis Ags are expressed in tumor and testis, an immune “sanctuary”
## Types of Tumor Antigens Recognized by T cells

<table>
<thead>
<tr>
<th>Normal host cell displaying multiple MHC-associated self antigens</th>
<th>Not recognized by T cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor cells</td>
<td></td>
</tr>
<tr>
<td>Mutated self protein</td>
<td>CD8+ CTL</td>
</tr>
<tr>
<td>Over-expressed or aberrantly expressed self protein</td>
<td>CD8+ CTL</td>
</tr>
<tr>
<td>Oncogenic virus</td>
<td>Virus antigen-specific CD8+ CTL</td>
</tr>
<tr>
<td>Class of antigen</td>
<td>Antigen</td>
</tr>
<tr>
<td>------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Tumor-specific mutated oncogene or tumor-suppressor</td>
<td>Cyclin-dependent kinase 4</td>
</tr>
<tr>
<td></td>
<td>β-Catenin</td>
</tr>
<tr>
<td></td>
<td>Caspase-8</td>
</tr>
<tr>
<td>Germ cell</td>
<td>MAGE-1</td>
</tr>
<tr>
<td></td>
<td>MAGE-3</td>
</tr>
<tr>
<td>Differentiation</td>
<td>Tyrosinase</td>
</tr>
<tr>
<td></td>
<td>Surface Ig</td>
</tr>
<tr>
<td>Abnormal gene expression</td>
<td>HER-2/ neu</td>
</tr>
<tr>
<td>Abnormal post-translational modification</td>
<td>MUC-1</td>
</tr>
<tr>
<td>Oncoviral protein</td>
<td>HPV type 16, E6 and E7 proteins</td>
</tr>
</tbody>
</table>
Use of Human Tumor Ag-Specific Cloned CTL for Identification of Tumor Antigens

Generation of tumor-specific CTL clones

A

Melanoma
Purify mononuclear cells from tumor site

Surgically resect tumor

Tumor cells

Patient's mononuclear cells

Coculture mononuclear cells and melanoma cells

Melanoma cell line

Isolate and clone activated CD8+ CTLs
Identification of tumor antigens recognized by tumor-specific CTLs

1. Tumor cDNA library
2. Coculture with CTL clone
3. Transfect into class I MHC+ target cell line
4. Isolate transfected DNA and sequence
5. Gene encoding tumor antigen recognized by melanoma-specific CTL

TNFα Production
- - - +
FDA-approved monoclonal antibodies for treatment of malignancies

<table>
<thead>
<tr>
<th>MAb Name</th>
<th>Trade Name</th>
<th>Used to Treat:</th>
<th>Approved in:</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rituximab</td>
<td>Rituxan</td>
<td>Non-Hodgkin lymphoma</td>
<td>1997</td>
</tr>
<tr>
<td>Her2</td>
<td>Trastuzumab</td>
<td>Breast cancer</td>
<td>1998</td>
</tr>
<tr>
<td>CD33</td>
<td>Gemtuzumab</td>
<td>Acute myelogenous leukemia (AML)</td>
<td>2000</td>
</tr>
<tr>
<td>CD33 ozogamicin*</td>
<td>Mylotarg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD52</td>
<td>Alemtuzumab</td>
<td>Chronic lymphocytic leukemia (CLL)</td>
<td>2001</td>
</tr>
<tr>
<td>CD20</td>
<td>Ibritumomab</td>
<td>Non-Hodgkin lymphoma</td>
<td>2002</td>
</tr>
<tr>
<td>CD20</td>
<td>Tiuxetan*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD20</td>
<td>Tositumomab*</td>
<td>Non-Hodgkin lymphoma</td>
<td>2003</td>
</tr>
<tr>
<td>CD20</td>
<td>Cetuximab</td>
<td>Colorectal cancer</td>
<td>2004</td>
</tr>
<tr>
<td>CD20</td>
<td></td>
<td>Head &amp; neck cancers</td>
<td>2006</td>
</tr>
<tr>
<td>EGF-R</td>
<td>Bevacizumab</td>
<td>Colorectal cancer</td>
<td>2004</td>
</tr>
<tr>
<td>VEGF</td>
<td>Avastin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Modifications of monoclonal antibodies for therapy

Tumor-specific antibody

Antibodies bind to the tumor cell

NK cells with Fc receptors (CD16) are activated to kill the tumor cells

Tumor-specific antibody conjugated to toxin

Antibody-toxin conjugates bind to the tumor cell

Conjugates are internalized, killing the cell

Tumor-specific antibody conjugated to radionuclide

Radioactive antibody binds to the tumor cell

Radiation kills the tumor cell and neighboring tumor cells
Passive Immunotherapy with a twist (the antigen is not immunogenic)

Inhibitory Receptor Blockade with Monoclonal Antibodies

(e.g. CTLA-4, PD-1)
Skin and hair de-pigmentation by treatment of B16 melanoma with anti-CTLA-4 and GM-CSF-producing vaccines
CTLA-4 Blockade: 
Anti-tumor immunity, Autoimmunity

The good news....

The bad news....

Phan et al. PNAS (2003) 100:8372
Ipilimumab + DTIC improved survival over DTIC
Hazard ratio 0.72,
Median survival 11 vs 9 months, p=0.0009

First-line therapy Study 024, ASCO 2011
Ipilimumab survival data
1 year 43%
2 years 24%
3 years 20%
Successful active vaccination against virus-induced malignancies (primary prevention)

Vaccine to feline leukemia virus for cats

Hepatitis B vax prevents human liver cancer

Vaccination against human papilloma viruses prevents cervical cancer
Active Immunization - Tumor cells or Ags

- Tumor cells or extracts (Melacine)
- Tumor peptide + adjuvant vaccine
- Tumor protein/peptide loaded on dendritic cell—this is the principle of Dendreon’s Sip-T/Provenge for PrCA
- Tumor antigen cDNA vaccination
- Tumor antigen in recombinant virus
- Feeding dendritic cells dead tumors
- Feeding dendritic cells tumor RNA
Or use fusion protein that stimulates dendritic cells and provides tumor antigen

Sipuleucel-T

Isolate dendritic cells

Mix fusion protein with dendritic cells from patient

Return dendritic cells and tumor antigens to patient
Tumor-infiltrating lymphocytes and lymphodepletion strategies

- Combines the power of strategies designed to grow T cells for adoptive cell Rx
- Homeostatic cytokines are stimulated to enhance expansion of T cells
- Interleukin-2, a T cell growth factor, stimulates persistence, function of T cells
- Checkpoint blockade further re-activates cytotoxic T cells
- Additional modifications under investigation