TUMOR IMMUNOLOGY
(self-study)

Objectives: At the end of this activity, the student should be able to:

1. Describe how mutations and mutation rates promote the cellular alterations involved in cancer
2. Outline the basic steps involved in cancer development and metastasis
3. Define biomarker
4. Define tumor-specific antigen (TSA) and tumor-associated antigens (TAA) and be able to list at least 2 examples of each
5. Describe how TSAs and TAAs can be used clinically in cancer diagnosis, assessment of prognosis, and as a tool in assessing relapse
6. Describe the immune response elicited by tumors
7. Explain mechanisms by which tumors evade the immune system including loss of immunogenicity, tolerance of tumor, tumor-induced immune suppression, and immunoprivileged sites
8. Describe briefly the immunotherapy approaches in cancer treatment with a focus on the monoclonal antibody treatments (Rituximab, Herceptin, Ipilimumab), adoptive cellular therapies (LAK cells, TIL cells, graft versus tumor cells), enhancing tumor immunogenicity using antigen loaded dendritic cells, gene therapy up-regulating B7 or GM-CSF on/in tumor cells, and preventative vaccines (HPV16, HBV)

Reading (FYI only): Abbas. Cellular and Molecular Immunology 7th edition, Chapter 17 (pages 389-405)

I. BASIC CANCER FACTS
- There are >1.5 million new cases of cancer every year (~1,596,670 new cases in 2011, cancer.org).
- Nearly 12 million American have a personal history of cancer. Some of these individuals are cancer-free, while others still have evidence of cancer and may be undergoing treatment. (Data from 2007, cancer.org)
- Approximately 41% of individuals will develop cancer over their life-time, and approximately 21% of individuals will ultimately die of cancer (Review 1975-2008, seer.cancer.gov).
- Cancer is the second most common cause of death in the United States (Americans 567, 628 in 2010, CDC.gov).
- The NIH estimates overall costs of cancer in 2010 to be $263.8 billion ($102.8 billion for direct medical costs-all health expenditures and $20.9 billion for indirect morbidity costs-lost productivity due to illness)
- $140.1 billion for indirect mortality costs (lost productivity due to premature death)
- Significant psychological impact in part due to the high mortality rate of cancer, the effects of treatment, the impact that the disease has on families, cultural traditions/views, and that the cancer arose from within the body.

II. BASIC PRINCIPLES OF CANCER

Normally body cells undergo a regulated process of dividing, resting or dying as needed by the body. However, billions of mutations occur every day in these cells, and these can alter the regulatory circuits that govern cell growth or cell attrition leading to proliferation that is out of control and the generation of a tumor or neoplasm. As long as the cells remain clustered as a single mass, the tumor is said to be benign. A tumor cell is called malignant or cancer when it acquires the ability to invade surrounding tissues. Cancer cells are defined by their ability and the ability of their progeny to 1) reproduce in defiance of the normal restraints on cell division and 2) invade and colonize areas normally reserved for
Invasiveness is usually associated with the ability to break loose, enter the blood stream or lymphatic vessels, and form secondary tumors called **metastases**.

Generally, cancers are derived from a single abnormal cell and result from somatic mutations. These mutations are transmitted to their progeny (daughter cells); therefore, they are heritable. Evidence suggests that cancer causing mutations generally accumulate slowly over time. Tumor progression is thought to involve successive rounds of mutation and selection of cells with fewer constraints on their growth. Most human cells are genetically unstable. Genetic instability, including defects in repair and cell cycle regulation, lead to increased rates of point mutations and often have trouble maintaining the integrity of chromosomes. These changes can include many different underlying pathogenetic causes including single gene mutations leading to loss of gene function (tumor suppressor genes) or gain-of-function (oncogenes), chromosome mutations (translocations, deletions), genomic amplification (gaining multiple copies), and genome mutations (aneuploidy). Interestingly, recent advances in genome-wide technology have revealed the extensive involvement of dysregulated epigenetic components ([lay article - http://www.economist.com/node/21552168](http://www.economist.com/node/21552168)). While the consequences of genetic instability often decrease the fitness of the cell, it does increase the likelihood of additional mutations that confer a selective advantage. Clonal expansion and the acquisition of additional somatic mutations lead to tumor heterogeneity and the fully malignant phenotype.

Transformed cells have seven essential alterations in cell physiology caused by specific mutations as compared to "normal" cells. These alterations are:

1. **self-sufficiency in growth factors**;
2. **insensitivity to growth-inhibitory (antigrowth) factors**;
3. **evasion of programmed cell death (apoptosis)**;
4. **limitless replicative potential (immortalization)**;
5. **sustained angiogenesis**;
6. **tissue invasion and metastasis**;
7. **evasion of the immune system**

### Cancer Development and Metastasis

Other points to note:

**Angiogenesis** is very important in establishment of tumor foci. Unrestrained growth does not, by itself, cause invasion and metastasis. This will require additional steps. Invasion and metastasis can be facilitated by proteins which stimulate tumor cell attachment to host cellular or extracellular matrix determinants, tumor cell proteolysis of host barriers (e.g., basement membrane), tumor cell locomotion, and tumor cell colony formation in the target organ for metastasis.

During this process, the tumor needs to escape from immunity. Some tumor cell attributes help a tumor to **escape immune destruction** (discussed later).
III. IMMUNE SURVEILLANCE

Immune Surveillance is a theory stating that one function of the immune system is to search out and destroy abnormal and transformed cells before they grow into tumors. While the importance of immune surveillance is controversial, there is evidence that immune system plays a role in protecting the body against tumors and cancer. A few examples include:

- Immunosuppressed and immunodeficient individuals and animals are at increased risk of cancer.
- Infiltration of tumors (melanoma, carcinomas of the colon, breast cancer) by lymphocytes, macrophages, NK cells, and eosinophils suggest a more positive prognosis and improved survival rates.
- Lymphocyte proliferation is noted in the draining lymph nodes in certain cancers.
- Regression of metastases has been noted after the removal of primary tumor (pulmonary metastases from renal carcinoma).
- Case reports have documented that spontaneous regression has been noted after immune stimulation (melanoma, lymphoma).

Experimental data suggest that tumors stimulate a protective specific, adaptive immune response and that this protective response could be adaptively transferred from one animal to another through CD8+ T cells. However, empiric evidence suggests that in some cases immune responses are unable to prevent tumor growth and that the immune response may be associated with tumor inhibition but not elimination. Researchers speculate that this suboptimal response is due to 1) the weak immunogenicity of the tumor due to its host origins, 2) the rapid growth of the tumor could overwhelm the immune system, and 3) the selective pressures on tumor cells promote the development of mechanisms for evading host immune responses.

IV. TUMOR ANTIGENS

The ability of the immune system to recognize and target tumors and malignancies suggest that these abnormal cells express antigens that can trigger and be recognized by the immune system. These antigens are generally broken down into tumor-specific antigens and tumor associated antigens.

A. Tumor-Specific Antigens (TSAs) are antigens that are uniquely expressed by tumor cells but not by normal cells. They are sometimes referred to as neoantigens. Various biochemical and molecular methods have been developed that allow the identification of TSAs in various tumors. Hypothetically, any of these antigens could be recognized by the immune system. Examples of tumor-specific antigens included:

1. Mutated host genes associated with the development of the malignant phenotype
   a. Altered proteins – resulting from gene mutations that give rise to new peptides (example: the conversion of a proto-oncogene to an oncogene)
   b. Fusion proteins – recombinations or deletions within genes (example: Bcr/ABL in CML)
2. Mutated host genes unrelated to the malignant phenotype
3. Viral proteins – component proteins or new enzymes that are induced in the cell to aid in the replication of the virus. Viral “fingerprints” found in tumors form a major part of the evidence that links viruses with human malignancy (e.g., HPV E6 and E7).

<table>
<thead>
<tr>
<th>Viruses Associated with Human Cancers</th>
<th>Associated Tumors</th>
<th>Areas of High Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DNA Viruses</strong></td>
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<tr>
<td>Papillomavirus (many distinct strains)</td>
<td>Warts (benign), Carcinoma of uterine cervix</td>
<td>Worldwide</td>
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<tr>
<td>Hepatitis B virus</td>
<td>Liver cancer (hepatocellular carcinoma)</td>
<td>Southeast Asia &amp; Tropical Africa</td>
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<td>Epstein-Barr virus</td>
<td>Burkitt’s lymphoma Nasopharyngeal carcinoma B cell lymphoproliferative disease</td>
<td>West Africa, Papua New Guinea, Southern China, Greenland (Inuit), Immunosuppressed or immunodeficient patients</td>
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<td><strong>RNA Viruses</strong></td>
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<tr>
<td>Human T cell leukemia virus type 1 (HTLV-1)</td>
<td>Adult T cell leukemia/lymphoma</td>
<td>Japan (Kyushu) West Indies</td>
</tr>
<tr>
<td>Human immunodeficiency virus (HIV) Human herpes virus 8 (HHV8)</td>
<td>Kaposi’s sarcoma</td>
<td>Central Africa</td>
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</tbody>
</table>
B. **Tumor-Associated Antigens (TAAs)** are normal cellular antigens that are expressed at higher levels on tumors as compared to normal cells or expressed at different stages of development or differentiation.

1. **Oncospermatogonal antigens** (cancer-testis antigens): expressed in spermatocytes and cancer cells, but not normal cells of tissue of origin of tumor (non-lineage specific expression). Example: MAGE-1 (melanoma antigen-encoding gene) a normal testicular protein is expressed by many melanoma cells.

2. **Differentiation antigens**: Antigens expressed on a tumor that are also expressed at least at some stage of differentiation of non-malignant cells of the tumor’s cell lineage (lineage-specific antigens). Example: Melanocyte differentiation antigens (MART-1): antigens expressed by melanomas, but also normal melanocytes, Prostate Differentiation Antigen (PSA) in prostate.

3. **Overexpression of a normal protein/antigen**
   Example: HER2 (human epidermal growth factor-2/Neu) amplification in tumor results 100X higher expression of the receptor, relative to normal cells on some breast cancer cells.

4. **Clonal antigens**: Expressed by a small clonal subpopulation of normal cells
   Example: idiotypes on B cells

<table>
<thead>
<tr>
<th>Cancer/Testis (CT) Antigens</th>
<th>CT Antigen Family</th>
<th># of Genes</th>
<th>Chromosome</th>
<th>Cancer Patients</th>
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<tr>
<td>MAGE-A</td>
<td>15</td>
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<td>C &amp; H</td>
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<tr>
<td>MAGE-B</td>
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<td>Xp21</td>
<td>C</td>
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<td>4, 13</td>
<td>C</td>
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<td>Xp11.2</td>
<td>C &amp; H</td>
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<td>C9/BRDT</td>
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<td>NK</td>
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<td>Xq27</td>
<td>NK</td>
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<td>Xq28</td>
<td>NK</td>
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<tr>
<td>CAGE</td>
<td>1</td>
<td>Xp22</td>
<td>NK</td>
<td></td>
</tr>
<tr>
<td>Unmanipulated Immunity</td>
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5. **Tissue specific antigens**: Expressed only on normal cells of the same origin
   Example: CD20 on B cell lineage tumors

6. **Carcinoembryonic antigens or oncofetal antigens**. Embryonic or fetal antigens that are expressed in tumor cells, but are not normally expressed by non-malignant adult cells.
   Examples:
   - Carcinoembryonic antigen (CEA) is a fetal antigen restricted to fetal tissues during first two trimesters of gestation that is also expressed by colon carcinoma cells.
   - Alpha-fetoprotein (AFP) is an oncofetal protein expressed by fetal liver and yolk sac cells that is also expressed by tumors of the liver and testis.
   - See table (Kuby 21-3 above) to see how AFP and CEA levels are often elevated in certain cancers but rarely in diseases of the same organ or other cancer types.

7. **Glycolipid and Glycoprotein antigens**: many cancers express an elevated level or abnormal forms of surface glycolipids and glycoproteins. These changes might be associated with the malignant cell’s ability to invade tissue and undergo metastasis. Gangliosides (e.g., GM₂
GD2 and GD3 are associated with neuroblastomas) and mucins (e.g., CA-125, CA-19-9 associated with ovarian cancer and MUC-1 in breast cancer) are examples of this type of antigen that has been the target of clinical diagnostic and therapeutic investigation.

C. **Biomarkers for cancer**

*Biomarker* is a term often used to describe a protein or other component in the body (often blood or tissue) that can be used to yield indirect information about a disease. A cancer biomarker is a biological factor which can be quantitatively measured to yield cancer-related patient information including cancer predisposition, early detection, monitoring cancerous growth, selection of treatment, or overall prognosis. TAAs can be biomarkers and are often assessed to see if they could play a clinically significant role in detection, monitoring, or prognosis. A blood test for CA-125 is a clinically useful test for tracking patients, but it does not appear sensitive enough to be used in early diagnostic testing.

D. **Tolerance and TAA**

Interestingly, cancer patients can mount responses against these "normal" TAAs. For example, patients with melanomas sometimes have CD4+ and CD8+ T cells that recognize tryosinase, an enzyme normally expressed in melanocytes during melanin biosynthesis. Immunologists speculate that patients are not tolerant to this normal self-antigen because the protein is produced at such low levels in so few cells; therefore, tolerance is not induced.

V. **IMMUNE RESPONSE TO TUMORS**

The interaction between the host and a tumor that it harbors is extremely complex. The major immune responses that have been described in human patients and animal models involve both adaptive and innate immune responses.

A. **Adaptive Immune Response:**

1. **Specific cytotoxic CD8+ T cells** (CTLs) recognize tumor antigens and lyse tumor cells. These cells are thought to constitute the major immunologic barrier against tumors. If the tumor antigen isn’t cleared as a pathogen would be, the tumor reactive cells would undergo many, many rounds of proliferation leading to CTLA-4 upregulation and down regulation of the CTL response.

2. **Tumor-specific CD4+ T cells** are thought to play an important role in helping induce CD8+ T cells and secreting cytokines which increase MHC expression, activate macrophages, etc…

3. **B-cell formation** of specific antibodies to tumor antigens might mediate:
   a. Antibody Dependent Cellular Cytotoxicity (ADCC) killing by NK cells and Macrophages
   b. Complement-mediated tissue damage and inflammation (lysis?)

B. **Innate Immune Response:**

1. **Natural killer (NK) cells**

   Many tumors down-regulate class I MHC to evade tumor antigen specific CTL-mediated killing. Because NK cell activation is controlled by a balance of activating and inhibitory receptors, the loss of class I MHC, a ligand for an inhibitory receptor, can result in tumor killing and cytokine production which can increase the immune response. NK cells can also kill tumor cells by **ADCC**. NK cell mediated killing can be enhanced through IFN-γ, IL-12, and IL-15 (not discussed in course).

   **Clinical application:** Interestingly, upregulating an NK-stimulatory ligand (NKG2D) induces short-term rejection of tumors and a somewhat protective adaptive immune response. IL-2 activated NK cells are called lymphokine-activated killer (LAK) cells.

2. **Tumor-Associated Macrophages**

   Tumor-associated macrophages are thought to be able to recognize the abnormal cells through ADCC or recognize phospholipids that are expressed ectopically in the membrane of a malignant cell. Tumor-antigen specific CD4+ T cells help to control macrophage function. Th1 cells produce IFN-γ and TNF which help to activate the macrophages so that they promote defense against tumor growth though ROI generation, NOI generation, the release of
lysosomal components, and cytokine release (e.g., TNF-α). These can lead to thrombosis in tumor blood vessels. In contrast, Th2 cells produce IL-10, TGF-β, and vascular endothelial growth factor (VEGF) which down-regulate the immune response and promote tumor angiogenesis. These effects could contribute to tumor progression.

C. Tumor microenvironment: the unique cellular composition surrounding the tumor can create a pro-tumor or anti-tumor environment. While the anti-tumor immune response was outlined above, pro-tumor immune cells can generate an immunosuppressive environment.

1. **Tregs** (CD4+CD25+CTLA-4+FoxP3+): limit CTL self-reactivity; produce soluble factors that promote tumor cell proliferation and dissemination

2. **Alternatively activated macrophages**: lack cytotoxic activity, block CTL proliferation/infiltration; produce soluble factors that promote neoplasia

3. **Immunosuppressive monocytes**: aka myeloid-derived suppressor cells; suppress T and NK cell proliferation and promote Tregs

D. **Putting it all together**

1. Tumor progression results in the remodeling of surrounding stroma, local damage (DAMPs and proinflammatory cytokines), and the overexpression/release of heat shock proteins ("stress proteins") leading to an immune response.

2. Cells within the tumor die due to a lack of perfusion or lethal mutations associated with genome instability.

3. Tumor antigens from the necrotic cells could be phagocytosed by dendritic cells.

4. The immunostimulatory signals within the tumor deliver the “danger” signal and prompt the DC to migrate to the lymph node. During the movement, the DC would undergo processing and presentation of tumor antigens onto class I and class II MHC and prompt the upregulation of B7 on its surface.
5. Naïve T cells are activated by 1) the tumor antigen in the context of MHC and 2) the B7 co-stimulatory signal.
   a. CD8+ T cells recognize tumor antigen on class I MHC
   b. CD4+ T cells recognize tumor antigen on class II MHC

6. Activated CD4+ and CD8+ T cells proliferate and differentiate into effector cells.
   a. Activated T cells express IL-2 and the high affinity IL-2 receptor (α, β, and γ subchains).
   b. This leads to the clonal proliferation of tumor specific T cells.

7. Effector T cells leave the lymph node via the lymphatics and enter the blood.

8. Effector T cells are recruited to the site of the tumor.
   a. CD8+ T cells kill tumor cells via perforin/granzyme, Fas:FasL, cytokine production (TNF)
   b. CD4+ T cells
      - Th1 cells enhance immune function through the generation of IL-2, IFN-γ, and TNF-α. These cytokines promote CTL, NK and macrophage function.
      - Th2 cells inhibit immune function and might promote tumor cell growth through increased angiogenesis.

VI. TUMOR ESCAPE FROM IMMUNE CONTROL

Tumor cell attributes often allow a tumor to escape immune destruction. While pathogens often express one or more PAMPs, tumor cells, which are derived from host cells, do not express these molecules. This could attenuate the anti-tumor innate immune response and ultimately the adaptive immune response. In addition, tumor cells are constantly undergoing high rates of heritable somatic changes due to their high mitotic index and their genome instability. If a mutation decreases the immunogenicity of the tumor cells, this variant and its progeny will have a selective advantage and will be more likely to survive.

A. Decreased immunogenicity
   1. Antigen loss
      - Mutations in tumor cells can result in the down-regulation or loss of tumor antigens or immuno-dominant epitopes. This could impact both T and B cell responses.
      - Antigenic modulation occurs when antibodies bind to tumor antigen on the cell surface receptor. This leads to receptor mediated endocytosis and degradation of the antigen. This process is similar to the loss of the acetylcholine receptor seen in patients with Myasthenia Gravis.
   2. Low levels of MHC class I expression
      - The expression of class I MHC varies between different locations. Low initial expression can decrease tumor recognition by CTL.
- Up to 50% of tumors down-regulate class I MHC to decrease killing by CTLs. This occurs through the loss of TAP, β₂m, microglobulin, HLA class I, components of the proteasome, necessary transcription factors, alterations in glycosylation and transport.

3. **Decreased expression of adhesion molecules** as CTLs require LFA-1 and ICAM-1 binding for effective antigen recognition and killing.

4. **Proteolytic shedding of MIC - MIC**, a stress-induced alert molecule, can bind to the NK cell activating receptor, NKG2D. Without this activating receptor, NK cells fail to get their stimulatory signal so that the lack of class I MHC will no longer trigger killing.

5. **Blocking antibodies** – In some cases, anti-tumor antibodies bound to tumor antigens can inhibit CTL function and possibly ADCC by NK cells (neuroblastoma).

**B. Tumor antigens are treated as a self-antigen**

Mechanisms exist in the body to induce peripheral tolerance to self-antigens. Failure to induce a robust “danger signal”/poor innate response or prolonged antigenic exposure can result in a tolerogenic state.

1. The “danger signal” results in
   - The up-regulation of **B7** and increased levels of class I and II MHC on the APC/DC. This allows the effective stimulation and activation of naïve T cells. If naïve T cells encounter tumor antigens without co-stimulation, the T cell could be anergized as part of peripheral tolerance.
   - Without a “danger signal” **Treg cells** could be generated towards tumor antigens.

2. If tumor cells are not promptly eliminated, tumor antigen specific T cells can be down regulated by AICD (activation induced cell death) and CTLA-4 up regulation.

   **Clinical application:** Therapeutic blockade of CTLA-4 binding has been able to induce tumor regression in some cases.

   ***Note: the down regulatory effects of CTLA-4 will be / are discussed in the context of autoimmunity.***

**E. Tumor–induced immune suppression** – Tumors can express or secrete factors that alter the immune response.

1. **TGF-β** is produced by many tumors. It inhibits the immune functions of T cells and inflammatory macrophage functions while promoting macrophages to secrete substance that increase angiogenesis.

2. **IL-10** is produced by some tumors. It is likely to decrease Th1 function.

3. **FasL** is expressed on some tumors. FasL on the tumor can bind to the Fas on leukocytes recruited to the tumor. The FasL on the tumor cell can induce the apoptotic death of the attacking cells.

**F. Immunoprivileged sites** – Immunoprivilege sites have little to no immune surveillance. They are usually maintained behind a barrier. This barrier either inhibits the movement of immune cells into and out of the site or involves cell surface glycocalyx molecules that hide/cover tumor antigens.

Classic anatomical immunoprivileged sites include the central nervous system (CNS), the anterior chamber of the eye, the testis, the placenta, and the fetus. That being said, some people are adding acquired immunoprivilege to the definition. This means that healthy tissues may act as de facto privileged sites because they lack pro-inflammatory or ‘danger’ signals.
VII. IMMUNOTHERAPY FOR TUMORS  ***Note this field is rapidly advancing focus on concepts and learning objectives***

While current cancer therapy methods generally focus on surgery, chemotherapy, and hormonal therapy, immunotherapy is an expanding area of interest. Immunotherapy is a form of biologic therapy or biotherapy that uses certain parts of the immune system to fight diseases. Cancer treatment has been a major target for immunotherapy.

<table>
<thead>
<tr>
<th>Monoclonal antibodies used to treat cancer</th>
<th>MAbs name</th>
<th>Trade name</th>
<th>Used to treat (yr approved)</th>
</tr>
</thead>
<tbody>
<tr>
<td>gemtuzumab ozogamicin*</td>
<td>Mylotarg®</td>
<td>acute myelogenous leukemia (AML, 2000**)</td>
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<tr>
<td>alemtuzumab</td>
<td>Campath®</td>
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<td>panitumumab</td>
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<tr>
<td>ipilimumab</td>
<td>Yervoy™</td>
<td>Melanoma (2011)</td>
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<tr>
<td>brentuximab vedotin*</td>
<td>Adcetris™</td>
<td>Hodgkin lymphoma (2011), anaplastic large cell lymphoma</td>
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</tbody>
</table>

F. **Monoclonal antibodies** (mAb) to tumor surface antigens - mAbs can be “naked” or conjugated to a chemotherapy drug, toxin, or radionucleotides. Please see supplement on mAbs regarding nomenclature.
1. Proposed mechanisms for *naked mAb*

   a. *mAbs can prompt tumor cells for destruction/depletion* through apoptosis and/or immune mediated function.  
      *Clinical Example:* Rituximab (Rituxan) treatment of patients with relapsed or refractory, low-grade or follicular, CD20-positive, B-cell non-Hodgkin’s lymphoma (NHL). The mAb binds the CD20 antigen on the surface of normal and malignant B cells and leads to destruction of the cells. Early B-cell progenitors lack CD20 allowing healthy B cells to regenerate after treatment.

   b. *mAbs can block or down regulate a receptor needed for growth of the cell.*  
      *Clinical Example:* Herceptin is a humanized antibody used in the treatment of HER2/neu (human epidermal growth factor-2) positive metastatic breast and stomach cancer.  
      *Clinical Example:* Bevacizumab (Avastin) targets the VEGF which is secreted by tumor cells to prompt the development of new blood vessels. The mAb blocks blood vessel formation and can be combined with traditional chemotherapy to treat some colorectal, lung, breast, and kidney cancers, as well as glioblastomas.

   c. *mAbs might modify the immune response*  
      *Clinical Example:* Ipilimumab (Yervoy) is a fully human monoclonal antibody that binds to CTLA-4 on both Treg cells and cytotoxic T cells. Some speculate that it prevents the inhibition of anti-tumor CTL function. In addition, it could inhibit the inhibitory function of Treg cells. Both actions could lead to a more robust anti-tumor immune response. In a recent phase III study, ipilimumab was used in conjunction with a peptide vaccine and nearly doubled the 12 and 24 month survival rates in patients with advanced metastatic melanoma.

2. **Conjugated mAbs** are used to target a drug, toxin, or radioactive substance directly to the cancer cells. Ideally, it allows the concentration of the substance to be high at the site of the malignancy while sparing the rest of the body.

   a. **Treatment**
      - **Immunotoxins** are made by attaching mAbs to bacterial toxins (e.g., ricin A, pseudomonas toxin, diphtheria toxin). While gemtuzumab ozogamicin (Mylotarg) was approved to treat some people with acute myelogenous leukemia, its approval was withdrawn due to lack of efficacy and no other immunotoxins are approved to treat cancer at this time.
      - **Chemotherapeutics** can be conjugated to mAb. For example, brentuximab vedotin (Adcetris) can be used to treat anaplastic large cell lymphoma.
      - **Radionuclides** can be conjugated to mAb to allow the delivery of radiation directly to the cancer cells over an extended period of time. Protocols have been approved in non-Hodgkin’s lymphoma (Ibritumomab Tiuxetan / Zevalin).

   b. **Diagnostic/tracking imaging** - mAbs labeled with radionuclides that emit gamma rays or nanodots, target tumors, permitting detection of tumors or metastatic disease and in the future could help surgeons identify malignant tissue.

   **Quantum dot nanoparticles conjugated to a monoclonal antibody recognizing a tumor surface antigen.** Quantum dots emit light when excited by a laser beam. They emit light for a long period.
G. **Adoptive Cellular Therapy** – involves the transfer of anti-tumor cells into the cancer patient

1. Anti-tumor cells are isolated from the patient, cultured in vitro (often in IL-2), and returned to the patient in a more activated manner.
   a. Lymphokine activated killer cells (LAK cells) are taken from the patient’s peripheral blood and stimulated with IL-2. LAK cells are mostly activated CD16+ CD3- cells (NK cells).
   b. Tumor Infiltrating Lymphocytes (TILs) are T cells isolated from the tumor.

2. **Graft-versus-tumor/leukemia** - Individuals with leukemia receive an infusion of alloreactive T cells during complete or partial (non-ablative) hematopoietic stem cell transplants. Similar to graft-versus-host reactions, the allo-response could be directed against residual tumor cells and contribute to eradication of the tumor (graft-versus-tumor). This mechanism might in part explain why certain hematopoietic cancers have lower relapse rates after allogeneic transplants than after autografts.

H. **Block of Treg function** – Experimental models are looking for ways to decrease anti-tumor Treg cells.

I. **Cytokine therapies**

1. **IFNα** might work by increasing MHC class I expression and tumor antigen presentation. It also can play a role in activating T cells, B cells, DCs and macrophages.
2. **TNFα and IFNγ** are effective anti-tumor agents in animal models; however, their use in patients is limited by serious toxic side effects.
3. **IL-2** has been used to promote T cell differentiation, proliferation and activation of CD4+ T cells, CD8+ T cells and increased NK cell function.
4. **IL-12** has been introduced in some cancer cell vaccines to enhance development of cell-mediated immune responses.

J. **Vaccine strategies to enhance tumor immunogenicity**

1. Therapeutic vaccines
   - Protein or peptide loaded autologous dendritic cells
   - Recombinant viral vectors encoding tumor antigens or peptides
   - DNA vaccines encoding tumor antigens or peptides together with cytokines or costimulation molecules
   - Immunization with autologous tumor cells that express costimulation molecules (e.g., B7)
   - Immunization with cytokine secreting autologous tumor cells (e.g., GM-CSF)
   - Immunization with tumor-derived heat shock proteins
2. Preventive vaccines
   - Vaccination against viruses that are known to be oncogenic can prevent future cancer (e.g., Human papilloma virus type 16 or Hepatitis B virus)
**Supplemental information (FYI only)**

**Nomenclature of monoclonal antibodies (mAbs)**

mAbs are given a generic (nonproprietary) name in addition to proprietary name. The generic name includes information about which species from which the antibody is derived.

- **-omab** derived from a mouse
- **-ximab** (chimeric mAb) where the murine mAb constant regions are replaced with human sequences
- **-zumab** (humanized mAb) where all but the complementary determining regions of the murine mAb are replaced with human sequences
- **-umab** created by “human” B cells
1. What role do somatic mutation and genome instability play in the development of cancer?

2. A unique tumor-specific antigen (TSA) is found in only a single tumor and is not present in any other tumor, whether it is of the same or different histologic type. How can this be explained?

3. Oncofetal antigens such as alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) are normal components of developing fetal tissues that may appear later in life as tumor-specific antigens. As tumor-associated antigens (TAAs), they may be excreted or may be components of the tumor itself. Can elevated AFP levels in the serum be used to diagnose liver cancer? Can elevated serum levels of CEA serve as a prognostic indicator of colorectal carcinoma relapse following surgical intervention? Explain.

4. What are the innate and adaptive immune responses that can attack malignant cells or tumors?
5. Mechanisms by which tumors evade host immunity are listed in the table below. For each tumor escape mechanism, indicate what the “effect” is on the host immune response.

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor is non-immunogenic</td>
<td></td>
</tr>
<tr>
<td>Tumor modulates its surface antigens</td>
<td></td>
</tr>
<tr>
<td>Loss of HLA class I molecules</td>
<td></td>
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<tr>
<td>Loss of adhesion molecules</td>
<td></td>
</tr>
<tr>
<td>Tumor secretes immunosuppressive cytokines</td>
<td></td>
</tr>
<tr>
<td>Tumor sheds the ligand for the NK cell</td>
<td></td>
</tr>
<tr>
<td>activating receptor</td>
<td></td>
</tr>
</tbody>
</table>

Please note that the answers are on the next page.
1. Cancers result from somatic mutation that usually accumulate slowly over time. Tumor progression is thought to involve successive rounds of mutation and selection of cells with fewer constraints on their growth. Genetic instability, including defects in repair and cell cycle regulation, lead to increased rates of point mutations and often trouble maintaining the integrity of chromosomes. These changes can include loss of gene function (tumor suppressor genes) or gain-of-function (oncogenes), chromosome mutations (translocations, deletions), genomic amplification (gaining multiple copies), genome mutations (aneuploidy), and dysregulated epigenetic control. Transformed cells have modified multiple essential components of cellular physiology.

2. A random mutation would result in a completely unique tumor-specific antigen. A virus – Virally induced tumors share antigens – that is, all tumors caused by a particular virus have very similar antigens. Derepression of a gene that codes for a protein during fetal development would not lead to a neoantigen. Immune surveillance controls the development of malignant neoplasms; it does not control the immunologic specificity of the tumor.

3. No. Oncofetal antigens cannot be used to diagnose malignancy because they are induced by a number of diverse factors (e.g., cigarette smoking). These antigens have little use in diagnosis – The number of false positive results is too great. Yes. The chief clinical value of AFP and CEA is as prognostic indicators of relapse following surgical or other therapeutic intervention (e.g., AFP levels in the serum directly correlate with tumor burden).

Note that the classification of tumor antigens is imperfect -- Some textbooks will refer to oncofetal antigens as “tumor-specific” because they are transcriptionally silenced after birth and not normally expressed by non-malignant adult cells, but may reappear later in life as “tumor-specific” markers. In fact, these molecules were among the earliest candidates for tumor-specific antigens. However, as you learned in lecture, oncofetal antigens are expressed by tumor cells and normal cells in the host which is why we classify them as “tumor associated antigens (TAAs)” – They are NOT neoantigens.

4. Anti-tumor/cancer immune responses

   Adaptive Immune Response
   - Specific cytotoxic CD8+ T cells (CTLs) ***IMPORTANT***
   - Tumor-specific CD4+ T cells **IMPORTANT**
   - B-cell formation of specific antibodies to tumor antigens might mediate:
     - Antibody Dependent Cellular Cytotoxicity (ADCC) killing by NK cells and Macrophages?
     - Complement-mediated tissue damage and inflammation (lysis?)

   Innate Immune Response:
   - Natural killer (NK) cells ***IMPORTANT***
   - Tumor-Associated Macrophages?
5. **Mechanism** | **Effect**  
--- | ---  
Tumor is non-immunogenic | No immune response occurs  
Tumor modulates its surface antigens | Avoids antibodies to tumor-specific antigens; Tumor changes antigens to avoid immune destruction  
Loss of HLA class I molecules | Tumor cannot be destroyed by MHC-restricted cytotoxic T cells  
Loss of adhesion molecules | Cytotoxic T cells cannot attach effectively to tumor membrane  
Tumor secretes immunosuppressive cytokines | Immune response is down-regulated  
Tumor sheds the ligand for the NK cell activating receptor | The activating receptor ligand (MIC) binds the NK cells activating receptor (KAR NKG2D) Without this ligand, NK cells cannot recognize the loss of class I MHC on the tumor cell allowing it to evade both CTLs (loss of class I MHC) and NK cells (loss of MIC)  
Tumor sheds MIC | PS. Do NOT memorize these ligands, but you should be able to apply your understanding of immunology to these situations.