Tumor Immunology I & II

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Tumor Immunology I & II

Tumor Immunology I:

- Overview of cellular transformation and malignancy
- How the immune system reacts (or does not) to malignancy

Tumor Immunology II:

- Can the immune system be harnessed to control/eradicate tumors?
Tumor Immunology I:

- Overview of cellular transformation and malignancy

- How the immune system reacts (or does not) to malignancy
Cellular transformation and malignancy

CANCER:

Represents a condition that results in the uncontrolled division of cells into a mass ("tumor") that dysregulates local and systemic physiologic homeostasis and often results in metastasis and formation of secondary and tertiary tumors.
Cellular transformation and malignancy

General Causes:

FAILURE TO

- control cell division
- maintain orderly cell death
- regulate orderly differentiation of stem cells into more mature cells
- protect cells from DNA damage

FAILURE OF IMMUNE SURVEILLANCE

- innate immune mechanisms (inadequate immune effectors)
- evolution of “stealth” by malignant cells
Cellular transformation and malignancy

TUMORS are:

- **Benign**
  - growth is self-limiting and/or no metastatic potential

- **Malignant**
  - uncontrolled growth, non-invasive
    (dysregulated local tissue/organ homeostasis)
  - invasive, metastatic
    (dysregulated local tissue/organ homeostasis) \( \times n \) (sites)

**SYSTEMIC BREAKDOWN**
Why Does Cancer Matter?

As of 2012
In the United States:
1.7 million new cases per year.
600,000 Deaths
Figure 1. Trends in Age-adjusted Cancer Death Rates* by Site, Males, US, 1930-2012

*Per 100,000, age adjusted to the 2000 US standard population. †Mortality rates for pancreatic and liver cancers are increasing.

Note: Due to changes in ICD coding, numerator information has changed over time. Rates for cancers of the liver, lung and bronchus, and colon and rectum are affected by these coding changes.


©2016, American Cancer Society, Inc., Surveillance Research
- Improved detection (diagnostics)
  - Improved treatment options
  - Awareness
- Lifestyle changes (diet/smoking)
What causes cancer?

Mainly due to MUTATIONS
- newer definition: alteration in *chromatin structure* and *behavior* that predisposes a cell to a pathologic state

- physical chromosomal alterations (aneuploidy, translocations/breaks)
- changes in nucleotide sequence (single base change, multiple nucleotide changes: different amino acid(s))
- DNA sequence amplification
- DNA sequence deletions
- DNA epigenetic changes (methylation/acetylation)
  - histone acetylation/methylation/ubiquitination
- Changes in DNA expression (coding/non-coding)

Genomic Instability
*hallmark of most cancers
Molecular basis for cancer progression

1. DNA damage
2. Failure to repair damage
3. Failure to destroy cells with DNA damage
4. Mutation
5. Selection advantage

Diagram shows the cascade of events leading to cancer progression.
Progressive acquisition of neoplastic features
Alterations of Specific Cellular Functions in Cancer

DNA Repair

Tumor Suppressor Genes
- Inactivation

Oncogenes
- Activation

Differentiation
Apoptosis/Proliferation

CANCER
Specific Cellular Functions in Cancer: Genetic Alterations

Genetic Instability: RER Phenotype

DNA Repair

CANCER

Tumor Suppressor Genes

Oncogenes

Interstitial Deletion

Inactivating Mutation

Gene Amplification

Gene Overexpression

Hypermethylation

Activating Mutation
Cellular transformation and malignancy

At the cellular level, malignancy arises as a consequence of:

- Acquired (environmental) DNA damaging agents: chemicals, radiation, viruses
- Inherited mutations in: genes affecting DNA repair, genes affecting cell growth or apoptosis
- Failure of DNA repair
- Mutations in the genome of somatic cells:
  - Activation of growth-promoting oncogenes
  - Alterations of genes that regulate apoptosis
  - Inactivation of cancer suppressor genes
- Expression of altered gene products and loss of regulatory gene products:
  - Clonal expansion
  - Additional mutations (progression)
  - Heterogeneity
- Malignant neoplasm
Basic Biologic Features of Neoplasms

- Differentiation
- Abnormal Proliferation
- Angiogenesis
- Invasion
- Apoptosis
- Senescence

Oncogenic Lesion (e.g. RAS, MYC, E2F Activation)
**Hayflick Limit:**
All cells have a finite number of cell divisions EXCEPT STEM CELLS
Telomeres and Cell Senescence

- Telomeric DNA repeats, (TTAGGG)n
- Metaphase chromosome

Elongation

- Telomerase
- CAAAACC

Translocation

- 3'
- Telomerase reverse transcriptase

Repeated elongation

- 3'
- Telomerase RNA

Normal cells

- No telomerase
- Cells stop dividing
- Senescence

Cancer cells

- Cells keep dividing
- Immortality
- Telomerase activity
Cancer incidence varies among tissues
- Common in EPITHELIA
  - sheets of cells the form the upper layer of SKIN and that line the WALLS OF CAVITIES AND TUBES

CARCINOMAS approx. 80% of cancers

QUESTION: WHY?
Q: Why are 80% of human cancers of epithelial origin?

- epithelial cells are exposed to the environment (skin, lungs, mouth, esophagus, stomach, intestine, urinary tract, cervix)
  - chemical mutagens
  - radiation (DNA damage)
  - biological (viral insertional mutagenesis)

- HIGH REPLACEMENT RATE OF EPITHELIAL CELLS
  - rapidly-dividing/differentiating stem cells
  - rapid genome replication can in and of itself result in mutations, and overall genetic instability
Non-epithelial tumors

- **SARCOMAS**: tumors of the connective tissues (1%)

- **LEUKEMIAS/LYMPHOMAS**: tumors of the blood cells (17%)

- **CENTRAL & PERIPHERAL NERVOUS SYSTEM**
  - neurectodermal tumors (2.5%)
Viruses and tumors

- Can cause cancer through the “collateral damage” induced during anti-viral inflammatory responses
  - e.g. reactive oxygen species generated by immune cells damage DNA of infected cells as well as adjacent cells

- RETROVIRUSES: viral oncogene, viral anti-apoptotic gene; and insertional mutagenesis
Human papilloma virus

Cervical Cancer

The Nobel Committee for Physiology or Medicine 2008 Illustration: Annika Röhl

HPV developed several mechanisms to escape the host immune system:

- suppression of immune responses via the modulation of pro-inflammatory cytokines, type I interferon (IFNa, IFNβ) and adhesion molecules expression.

- Inhibition of Th1 response in the presence of a switch from Th1 to Th2.

- The capsid of HPV particles may influence the recruitment, stimulation and trafficking of Langerhans cells (LC) and dendritic cells (DC).

- The pre-neoplastic cells infected by HPV exhibit a reduced expression of major histocompatibility complex class I (MHC1), transporters of antigenic peptides (TAP) and low-molecular proteins (LMP), thereby preventing cytotoxic T cell induction.
Control of cell growth

Proliferation Factors (growth factors)

Receptors (Tyr/Ser/Thr Kinases)

Intracellular signal transducers (amplify initial kinase signal and/or integrate multiple signals)

Chromatin modifiers/DNA polymerase
Accessory protein complexes/DNA quality control proteins

Receptor Accessory Signal transducers and/or regulators (phosphatases)

Activation of DNA synthesis

MITOSIS

Is chromatin intact before, during and after DNA replication?

-Cell attempts to repair chromatin
-If irreparable, it undergoes apoptosis

CELL DIVIDES
Oncogenes and Tumor Suppressor Genes

Oncogenes:
- Increase cell cycle kinetics
- Translocation or transposition
- Gene amplification
  - within a control element
  - within the gene

Normal growth-stimulating protein in excess

Proto-oncogene DNA

Tumor-suppressor gene:
- Decrease/Regulate cell cycle kinetics
- Tumor-suppressor gene
- Normal growth-inhibiting protein
- Cell division under control

Mutated tumor-suppressor gene:
- Defective, nonfunctioning protein
- Cell division not under control

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What would be the expected oncogenes/suppressor genes?

Proliferation Factors (growth factors)

Receptors (Tyr/Ser/Thr Kinases)

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What would be the expected oncogenes/suppressor genes?

Proliferation Factors (growth factors)
ONCOGENES
Receptors (Tyr/Ser/Thr Kinases)
ONCOGENES
Intracellular signal transducers (amplify initial kinase signal and/or integrate multiple signals)
ONCOGENES; Phosphatases: SUPPRESSORS
Chromatin modifiers/DNA polymerase
Accessory protein complexes/
DNA quality control proteins
ONCOGENES and SUPPRESSORS

Receptor Accessory Signal transducers (ONCOGENES) and/or regulators (phosphatases); SUPPRESSORS
### Oncogenes and tumor suppressor genes (some examples)

<table>
<thead>
<tr>
<th>Oncogene</th>
<th>Chromosome Location</th>
<th>Mutation</th>
<th>Function</th>
<th>Tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABL</td>
<td>9q34.1</td>
<td>translocation</td>
<td>signal transduction (tyrosine kinase)</td>
<td>CML, other leukemia</td>
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<td>BCL-2</td>
<td>18q21.3</td>
<td>translocation</td>
<td>anti-apoptosis</td>
<td>B-cell lymphoma</td>
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<tr>
<td>CDK4*</td>
<td>12q14</td>
<td>amplification, point mutation</td>
<td>cell cycle regulation</td>
<td>sarcomas, familial melanoma</td>
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<tr>
<td>cyclin D1</td>
<td>11q13</td>
<td>amplification, translocation</td>
<td>cell cycle regulation</td>
<td>breast- and other carcinomas, B-cell lymphoma</td>
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<tr>
<td>C-MYC</td>
<td>8q24</td>
<td>amplification, translocation</td>
<td>transcription factor</td>
<td>lymphomas, carcinomas</td>
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<tr>
<td>ERB B</td>
<td></td>
<td>amplification</td>
<td>growth factor (EGF) receptor</td>
<td>glioma, squamous cell carcinoma, carcinomas</td>
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<tr>
<td>FOS</td>
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<td>AP-1 transcription factor</td>
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<td>Ha-RAS</td>
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<td>signal transduction (C-protein)</td>
<td>bladder carcinomas, thyroid cancer</td>
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<tr>
<td>HST</td>
<td>11q13.3</td>
<td>amplification</td>
<td>growth factor (FGF-like)</td>
<td>stomach</td>
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<tr>
<td>INH2</td>
<td>11p13</td>
<td>amplification, mutation</td>
<td>growth factor family (FGF-like)</td>
<td>esophageo, breast, glioblastoma</td>
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<tr>
<td>Ki-RAS</td>
<td>12p12.1</td>
<td>point mutation</td>
<td>signal transduction (C-protein)</td>
<td>pancreas, colon, lung, adenocarcinomas, endometrium, other carcinomas, melanoma</td>
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<tr>
<td>KIT*</td>
<td>4q11-21</td>
<td>constitutive activation, point mutation</td>
<td>receptor tyrosine kinase (stem cell factor receptor)</td>
<td>(hereditary) GIST, ANLL, testis</td>
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<tr>
<td>MET*</td>
<td>7q31</td>
<td>point mutation</td>
<td>receptor tyrosine kinase</td>
<td>(hereditary) papillary kidney tumour</td>
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<td>NEU/ERBB2</td>
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<td>growth factor (EGF) receptor</td>
<td>breast, ovary, stomach, other carcinomas</td>
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<td>signal transduction (C-protein)</td>
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<td>PIM-1</td>
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<td>RET*</td>
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<td>receptor tyrosine kinase</td>
<td>thyroid cancer (MEN2)</td>
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<td>SKC</td>
<td>20q12.13</td>
<td>Point mutation</td>
<td>signal transduction (tyrosine kinase)</td>
<td>colon, stomach, squamous cell cancer</td>
</tr>
</tbody>
</table>
Cancer as viewed by the immune system

Immune surveillance:
- theory that proposes that the immune cells can recognize aberrations in cell phenotype that predispose to malignancy

- cells that are on pathway towards malignancy are recognized as “different” and the immune system can regulate their growth, mobility, and or eradicate them
How could a tumor cell appear to the immune system?

- Lack molecular signatures that allow immune system to recognize them as "abnormal"
- Do not express PAMPs or non-self determinants
- Are largely SELF, thus they are ignored by the immune system
How could a tumor cell appear to the immune system?

- Immune cells do not enter a tumor site UNLESS RECRUITED by ACTIVATED INNATE IMMUNE CELLS; adaptive immunity is NOT initiated normally

- Even if APC enter the tumor, in the absence of INNATE INFLAMMATION, APC will NOT be triggered to “mature” and provide co-stimulation, secrete activating cytokines
How could a tumor cell appear to the immune system?

.....in other words,

The immune system is ordinarily MYOPIC to developing tumors in the absence of an inflammatory activation event;

TOLERANCE to the tumor is active
Immune surveillance is active.

Tumors become “stealthy” and escape from/suppress “immune surveillance”:

- Maintain a TH2-type environment (e.g. produce IL-10/TGF-beta)
- Convert innate immune cells like macrophages and neutrophils to suppressor-type cells
- Attract Tregs
An inflammatory state needs to be triggered in the tumor environment for immune system to react

HOWEVER,

This can also be unhelpful (paradoxically !!!)

- Activated innate immune cells produce ROS; further driving genomic instability in tumors by damaging DNA

- Adaptive responses to tumors eventually need the control of effector CD4+, CD8+ T-cells. Eventually, Ag-specific T-cells will be exhausted or rendered anergic

- While some malignant cells can be eradicated, those that accumulate additional mutations, or adapt by producing TH2 cytokines, will take over the tumor and escape (“Immune Editing”)
Tumor Antigens and Immune Surveillance

- If the immune system is to recognise a tumor, the following conditions should be operational:

  - Inflammation at site MUST exist
  - Tumor should express “neo-antigens”
  - Tumor should not express tolerogenic factors (soluble)/cell surface

  IF YES,

  - Macrophages and dendritic cells will acquire tumor fragments that include antigens and initiate an ADAPTIVE immune response inside the lymph nodes that drain the tumor

  - CD4+ T-cells, will then activate the humoral arm (B-cells producing tumor Ag-specific antibodies)
The special role and relevance of Natural Killer Cells (NK Cells) in immune surveillance

NK cells comprise 5-10% of peripheral blood lymphocytes in humans.

Express Fc-gamma receptors that bind antibodies involved in ADCC.

NK cell activity can also be intrinsic and not require interaction of Fc-gamma with IgG bound to antigen.

***NK cells will not kill cells that express class I MHC. All the cells in the body are therefore refractory to NK cell cytotoxicity.

HOWEVER, cells that do NOT express class I MHC WILL BE RECOGNIZED by NK cells and eradicated.
NK cells and cancer

**Figure 1** ADCC activity

When NK cells respond to the antibodies bound to cancer antigens on the surface of cancer cells, NK cells are activated and attack cancer cells.
NK cells and cancer

***the weak point of many tumors:

class I MHC expression is lost
NK cells and cancer
***the weak point of many tumors:

NK cells driving inflammation facilitate stress activation in tumor cells;

**EXPRESSION OF NEO-ANTIGENS** via dysregulated quality control processes in endoplasmic reticulum and Golgi

(e.g. changes in protein glycosylation, folding, other post-translational modifications

resulting in different "new", non-natural epitopes being produced
TUMOR ANTIGENS

• Tumor Specific Antigens (TSA)
  Expressed only in/on tumor cells and not on any normal cells and can be recognized by immune system.

• Tumor Associated Antigens (TAA)
  Not unique to tumors; are also expressed in normal cells***
Tumor Antigens

Tumor Specific Antigens (TSA): NOT NORMALLY EXPRESSED IN ADULT TISSUES

- Cancer testis antigen (MAGE-1; melanoma, bladder cancer)
- Mucin (altered glycosylation patterns; MUC-1)

- Oncofetal antigens (alpha-fetoprotein, carcinoembryonic antigen (CEA), SSEA-1 [embryonic stem cells])

In adults, most of the proteins that are expressed during embryonal/fetal stage are not expressed in/on cells. In some tumors, these antigens are re-expressed

- Antigens resulting from mutational in protein (beta catenin, RAS, p53, CDK4)
Tumor Antigens

• **Tissue Associated Antigen= TAA**
  Present in normal cells & tumor cells
  (e.g. MART-1, gp100, tyrosinase) expressed in melanomas & normal melanocytes

  Immunity directed against melanomas will also destroy normal melanin-containing cells

***do not confuse with TUMOR-SPECIFIC Ags!***
Tumor Associated Antigens (TAA)

- Over expressed Antigens; not normally expressed at high levels in normal cells

  e.g.

  HER-2 (neu); EGF-R in 30% Breast cancer (present in normal breast & ovary)

  DHFR (dihydrofolate reductase; resistance to methotrexate)

  IGF-I receptor

  PDGF receptor

  integrins

  CD10 and Prostate-Specific Antigen (PSA); expressed in normal B-cells and prostate gland
# Tumor-associated antigens

<table>
<thead>
<tr>
<th>Antigen category</th>
<th>Examples of antigens</th>
<th>Tumor types expressing antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cluster of differentiation (CD) antigens</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD20</td>
<td></td>
<td>non-Hodgkin lymphoma</td>
</tr>
<tr>
<td>CD30</td>
<td></td>
<td>Hodgkin lymphoma</td>
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<tr>
<td>CD33</td>
<td></td>
<td>Acute myelogenous leukemia</td>
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<tr>
<td>CD52</td>
<td></td>
<td>Chronic lymphocytic leukemia</td>
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<td><strong>Glycoproteins</strong></td>
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<td></td>
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<td>EpCAM</td>
<td></td>
<td>Epithelial tumors (breast, colon, lung)</td>
</tr>
<tr>
<td>CEA</td>
<td></td>
<td>Epithelial tumors (breast, colon, lung)</td>
</tr>
<tr>
<td>gpA33</td>
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<td>Colorectal carcinoma</td>
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<tr>
<td>Mucins</td>
<td></td>
<td>Epithelial tumors (breast, colon, lung, ovarian)</td>
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<td>TAG-72</td>
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</tr>
<tr>
<td>Carbonic anhydrase IX</td>
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<td>Renal cell carcinoma</td>
</tr>
<tr>
<td>PSMA</td>
<td></td>
<td>Prostate carcinoma</td>
</tr>
<tr>
<td>Folate binding protein</td>
<td></td>
<td>Ovarian tumors</td>
</tr>
<tr>
<td><strong>Glycolipids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gangliosides (e.g., GD2, GD3, GM2)</td>
<td></td>
<td>Neuroectodermal tumors, some epithelial tumors</td>
</tr>
<tr>
<td><strong>Carbohydrates</strong></td>
<td>Lewis-Y^2</td>
<td>Epithelial tumors (breast, colon, lung, prostate)</td>
</tr>
<tr>
<td><strong>Vascular targets</strong></td>
<td>VEGF</td>
<td>Tumor vasculature</td>
</tr>
<tr>
<td></td>
<td>VEGFR</td>
<td>Epithelium-derived solid tumors</td>
</tr>
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<td>αVβ3</td>
<td>Tumor vasculature</td>
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<td></td>
<td>α5β1</td>
<td>Tumor vasculature</td>
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<td><strong>Growth factors</strong></td>
<td>ErbB1/EGFR</td>
<td>Glioma, lung, breast, colon, head and neck tumors</td>
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<td>ErbB2/HER2</td>
<td>Breast, colon, lung, ovarian, prostate tumors</td>
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<tr>
<td></td>
<td>ErbB3</td>
<td>Breast, colon, lung, ovarian, prostate tumors</td>
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<td>c-MET</td>
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</tr>
<tr>
<td></td>
<td>IGF1R</td>
<td>Lung, breast, head and neck, prostate, thyroid, glioma</td>
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<td></td>
<td>EphA3</td>
<td>Lung, kidney, colon, melanoma, glioma, hematological malignancies</td>
</tr>
<tr>
<td></td>
<td>TRAIL-R1, TRAIL-R2</td>
<td>Solid tumors (colon, lung, pancreas) and hematological malignancies</td>
</tr>
<tr>
<td></td>
<td>RANKL</td>
<td>Prostate cancer and bone metastases</td>
</tr>
<tr>
<td><strong>Stromal and extracellular matrix antigens</strong></td>
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<td></td>
</tr>
<tr>
<td>FAP</td>
<td></td>
<td>Epithelial tumors (colon, breast, lung, head and neck, pancreas)</td>
</tr>
<tr>
<td>Tenascin</td>
<td></td>
<td>Glioma, epithelial tumors (breast, prostate)</td>
</tr>
</tbody>
</table>
TUMOR ANTIGENS

Tumor-associated antigens can also be “neo-antigens”

- mutated proteins (amino acid changes resulting in mutant protein); p53, RAS, PTEN

- Changes in carbohydrate structure
## TUMOR ANTIGENS

- Changes in carbohydrate structure (very common in all tumors)
  - due to altered expression of endo/exoglycosidase, glycosyltransferase genes
  - due to changes in glycolipid structure (genetic, post-translational mods)
  - due to imbalanced uptake of sugars and lipids by cells due to the rapid cell division; “overwhelming” of the glycosylation systems in the ER and the Golgi

<table>
<thead>
<tr>
<th>Structural change</th>
<th>Carriers</th>
<th>Biosynthetic basis of structural change</th>
<th>Potential lectin partners</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased β1,6-branching (N-linked)</td>
<td>N-glycans</td>
<td>Increased GnT5</td>
<td>Galectins, Siglecs</td>
<td>Guo et al. (19), Lagana et al. (20)</td>
</tr>
<tr>
<td>Increased α2,6-sialylation</td>
<td>N-glycans, e.g., β integrin</td>
<td>Increased ST6Gal1 sialyltransferase activity</td>
<td>Selectins, siglecs, galectins</td>
<td>Seales et al. (32)</td>
</tr>
<tr>
<td>General increase in sialylation</td>
<td>Mucins N-glycans</td>
<td>Increased sialyltransferase activity</td>
<td>Selectins, siglecs, galectins</td>
<td>Dall’Olio et al. (30), Gessner et al. (31)</td>
</tr>
<tr>
<td>Increased sialyl-Lewis xkα</td>
<td>Mucins</td>
<td>Increased FUT7, FUT3, FUT6, ST3Gal6</td>
<td>Selectins</td>
<td>Barthel et al. (169), Julien et al. (195), Koike et al. (27), Ogawa et al. (161), Yin et al. (198)</td>
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<tr>
<td>Decreased di-sialyl-Lewis xkα</td>
<td>Mucins, glycolipids</td>
<td>Decreased ST6GalNAc6 GlcNAc6ST1</td>
<td>Selectins, Reduced siglecs binding</td>
<td>Miyazaki et al. (41), Tsuchida et al. (42), Nudelman et al. (43)</td>
</tr>
<tr>
<td>Increased Tn epitopes</td>
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<td>Downregulated T-synthase activity due to Cosmic mutations</td>
<td>Galectins</td>
<td>Ju et al. (40)</td>
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<tr>
<td>Increased sialyl-Tn epitopes</td>
<td>Mucins (e.g., MUC1), CD44, β1 integrin, osteopontin</td>
<td>Increased ST6GalNAc1 expression</td>
<td>Siglecs, Galectins</td>
<td>Julien et al. (67), Ozaki et al. (68)</td>
</tr>
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<td>Increased T antigen (core 1 structure)</td>
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<td>Decreased C2GnT2 Enhanced availability of UDP-galactose</td>
<td>Galectins</td>
<td>Brockhausen et al. (53), Dalziel et al. (55), Kumamoto et al. (73)</td>
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<td>Increased sialyl-T antigens</td>
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<td>Increased levels of α2,3-sialyltransferase (ST3Gal1)</td>
<td>Galectins, Siglecs</td>
<td>Burchell et al. (78), Dalziel et al. (55), Picco et al. (79), Schneider et al. (72)</td>
</tr>
</tbody>
</table>
TUMOR ANTIGENS

In principle, all these changes in tumor cells resulting in expression of tumor antigens should lead to immune cell recognition

USUALLY, NO
How can one make the immune system aware to the tumor antigens, in order to activate an immune reaction that eliminates a tumor?

Reminders:

- Tumors can evade immune surveillance (nurture immunosuppressive environment (TH2 cytokines); convert innate immune cells into suppressors (macrophages, neutrophils)

- Immune editing (strongly-immunogenic cells are deleted, leaving behind highly-mutated, “stealthy” cells to expand)

Induce inflammation, could be a solution; But is it a good thing?

Question: Pros and Cons of Inflammation to combat malignancy
END OF PART I
Tumor Immunology II:

-Can the immune system be harnessed to control/eradicate tumors?
Question: Pros and Cons of Inflammation to combat malignancy

Pros:
- Triggering the innate immune cells would “force” tumor cells to react by increasing expression, levels, and activity of STRESS FACTORS (e.g. Heat Shock Proteins; Highly-immunogenic) and APOPTOSIS

- Cytokines produced would recruit APC and condition them to phagocytose apoptotic tumor cells; processing of tumor Ags and loading onto class I MHC to trigger ADAPTIVE ARM of immune system (CD4+ CD8+ T-cells)

Cons:
- Reactive Oxygen Species produced by activated innate immune cells (macrophages, neutrophils) will damage DNA in tumor cells, further exacerbating genomic instability and potentially making the tumor cell even more stealthy through new mutations
- Exhaustion of innate immune cell activity
- Conversion of pro-inflammatory phenotype of innate immune cells into immunosuppressive character and activity
Tumor immunotherapy

Objectives:

- ACTIVE Immunity to the tumor
  - Cellular
    - Cytotoxic T-lymphocytes
    - NK Cells/Large Granular Lymphocytes (LGLs)
    - Dendritic Cells
  - Non-Cellular
    - Immunokines (cytokines)
    - Growth Factors
  - "Personalized Therapy"
    - Theranostics (See below, can be passive too)

COMBINATIONS OF ABOVE

- PASSIVE Immunity to the tumor
  - ADOPTIONTIVE CELLULAR THERAPY (LAKs/TILs)
  - Monoclonal antibodies targeting tumor antigens
  - Immunoonjugates
  - Theranostics (can also be active)
Tumor immunotherapy

Objectives:

- ACTIVE Immunity to the tumor
  - Cellular
    - Cytotoxic T-lymphocytes
    - NK Cells/Large Granular Lymphocytes (LGLs)
    - Dendritic Cells
  - Non-Cellular
    - Immunokines (cytokines)
    - Growth Factors
- "Personalized Therapy"
  - Theranostics (See below, can be passive too)

COMBINATIONS OF ABOVE

PLUS

TUMOR ANTIGENS

= "Antigen-Specific Immunotherapy"
Immunotherapy for tumors:

More specific and fewer side effects than current therapies (radiation, chemotherapy).
Stimulation of active host immune responses

- Vaccination with tumor antigens
- Use of costimulators and cytokines
- Blocking inhibitory pathways
- Nonspecific stimulation
# Types of Tumor Vaccines

<table>
<thead>
<tr>
<th>Type of vaccine</th>
<th>Vaccine preparation</th>
<th>Animal models</th>
<th>Clinical trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Killed tumor vaccine</td>
<td>Killed tumor cells + adjuvants</td>
<td>Melanoma, colon cancer, others</td>
<td>Melanoma, colon cancer</td>
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<tr>
<td></td>
<td>Tumor cell lysates + adjuvants</td>
<td>Sarcoma</td>
<td>Melanoma</td>
</tr>
<tr>
<td>Purified tumor antigens</td>
<td>Melanoma antigens</td>
<td>Melanoma</td>
<td>Melanoma</td>
</tr>
<tr>
<td></td>
<td>Heat shock proteins</td>
<td>Various</td>
<td>Melanoma, renal cancer, sarcoma</td>
</tr>
<tr>
<td>Professional APC-based</td>
<td>Dendritic cells pulsed with tumor antigens</td>
<td>Melanoma, B cell lymphoma, sarcoma</td>
<td>Melanoma, non-Hodgkin's lymphoma, prostate cancer, others</td>
</tr>
<tr>
<td>vaccines</td>
<td>Dendritic cells transfected with genes encoding tumor antigens</td>
<td>Melanoma, colon cancer</td>
<td>Various carcinomas</td>
</tr>
<tr>
<td>Cytokine- and costimulator-</td>
<td>Tumor cells transfected with cytokine or B7 genes</td>
<td>Renal cancer, sarcoma, B cell leukemia, lung cancer</td>
<td>Melanoma, sarcoma, others</td>
</tr>
<tr>
<td>enhanced vaccines</td>
<td>APCs transfected with cytokine genes and pulsed with tumor antigens</td>
<td></td>
<td>Melanoma, renal cancer, others</td>
</tr>
<tr>
<td>DNA vaccines</td>
<td>Immunization with plasmids encoding tumor antigens</td>
<td>Melanoma</td>
<td>Melanoma</td>
</tr>
<tr>
<td>Viral vectors</td>
<td>Adenovirus, vaccinia virus encoding tumor antigen + cytokines</td>
<td>Melanoma, sarcoma</td>
<td>Melanoma</td>
</tr>
</tbody>
</table>

Abbreviations: APC, antigen-presenting cell.
Dendritic cell-based tumor vaccines

**A**
- Vaccinate with tumor-antigen pulsed dendritic cell
- Dendritic cells pulsed with tumor antigens
- CD8⁺ T cell
- Activation of tumor-specific T cells

**B**
- Plasmid expressing cDNA encoding tumor antigen
- Vaccinate with DNA or transfected dendritic cell
- Dendritic cells transfected with plasmid expressing tumor antigen
- APC producing tumor antigen
- CD8⁺ T cell
- Activation of tumor-specific T cells

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Autologous Dendritic Cell Immunotherapy for Cancers

General Approach
Enhancement of tumor immunogenicity by transfection of costimulator and cytokine genes
### Immunotherapy with cytokine gene-transfected tumor cells

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Tumor rejection in animals</th>
<th>Inflammatory infiltrate</th>
<th>Immunity against parental tumor (animal models)</th>
<th>Clinical trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin-2</td>
<td>Yes; mediated by T cells</td>
<td>Lymphocytes, neutrophils</td>
<td>In some cases of renal cancer, melanoma</td>
<td>Renal cancer, melanoma</td>
</tr>
<tr>
<td>Interleukin-4</td>
<td>Yes</td>
<td>Eosinophils, macrophages</td>
<td>No long-lasting immunity in human trials</td>
<td>Melanoma, renal cancer</td>
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<tr>
<td>Interferon-γ</td>
<td>Variable</td>
<td>Macrophages, other cells</td>
<td>Sometimes</td>
<td></td>
</tr>
<tr>
<td>TNF</td>
<td>Variable</td>
<td>Neutrophils and lymphocytes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Yes</td>
<td>Macrophages, other cells</td>
<td>Yes (long-lived T cell immunity)</td>
<td>Renal cancer</td>
</tr>
<tr>
<td>Interleukin-3</td>
<td>Sometimes</td>
<td>Macrophages, other cells</td>
<td>Sometimes</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** GM-CSF, granulocyte-macrophage colony-stimulating factor; TNF, tumor necrosis factor.
Autologous Cytotoxic T-Lymphocytes
Potential for tumor-activated inhibition of CTLs (direct and indirect mechanisms)
Autologous NK Cell Therapy (with or without antibody to tumor Ag)
Neutralizing inhibition of T-cells
Nivolumab: Anti PD1 receptor
Ipilimumab: Anti CTLA-4

(PD1 and CTLA-4 dampen T cell activity.)
Non-specific stimulation by systemic cytokine therapy (a limited success).

IL-2 (Melanoma, renal & colon cancer)
IFN-α (Melanoma, carcinoid tumor)
TNF-α (Sarcoma, melanoma)
GM-CSF (to promote bone marrow recovery; to mobilize DC progenitors)
Immunokines and Growth Factors

-This approach uses recombinant human immunokines (cytokines) singly or in combination to AUGMENT AN ENDOGENOUS IMMUNE RESPONSE AGAINST A TUMOR

e.g. IFN-alpha, beta, gamma
   IL-2, IL-12
   GM-CSF, TNF-alpha

IFNs:
   - Most approaches use IFN-alpha
   - Induces tumor regression in hematologic cancers, melanoma, breast cancer
   - All IFNs induce upregulation of class I MHC expression
   - IFN-gamma increase class II MHC on macrophages, and potentiates activity of T-cells, macrophages and NK cells

IL-2:
   - Mobilises activated T-cells

IL-12:
   - induces maturation of DC and activates immunostimulatory activity
Passive immunotherapy

- Adoptive cellular therapy
- Anti-tumor antibodies
Passive immunotherapy

PASSIVE IMMUNOTHERAPY FOR TUMORS WITH T-CELLS AND ANTIBODIES

(1) Adoptive Cellular Therapy

One approach is to generate lymphokine-activated killer cells (LAK) cells. LAK cells are IL-2 activated NK cells. Peripheral blood leukocytes from patients are cultured in high concentrations of IL-2.

Used in advances cases of metastatic tumors, and the efficiency changes from person to person.

Alternatively, tumor-infiltrating lymphocytes (TIL) from the inflammatory infiltrate present in and around solid tumors can be isolated and expanded by culture in IL-2. TILs may be enriched for tumor-specific CTLs and for activated NK cells. Is now being used in metastatic melanoma.

Patient T-cells can also be transduced with genes encoding TCR specific for a tumor antigen.
Adoptive cellular therapy

Lymphokine-Activated Killer Cells (LAK cells)
Tumor Infiltrating Lymphocytes (TILs)

A

Cell processing center (CPC)

- Tumor sample
- Tumor cell cultivation
- Blood sampling
- pBMCs
- pBMCs + IL-2
- T cell activation

B

- PB #1
- PB #2
- PB #3
- PB #4
- CTL #1
- CTL #2
- CTL #3
- CTL #4

Day -21
Day 0
Day 14
Day 28
Day 42
Day 70

Response

CPA

Into patient treated with preconditioning lymphodepletion

- Tumor cell
- Circulating peripheral lymphocyte
- TIL
- Lymphocytes presenting antitumor TCRs
- Viral vector

Autologous

- Tumor surgically resected
- TILs isolated, selected and grown to large numbers
- Cell culture
- TILs infused with IL-2

Genetically engineered

- Lymphocytes isolated from blood
- Retroviral insertion of TCR gene
- Genetically engineered cells grown to large numbers
- Cell culture
- Genetically engineered cells infused with IL-2
Anti-tumor antibodies.

Mouse Ig is immunogenic for humans. Cannot use antibodies raised in mice

«Humanization» of mouse Ig.
-monab  -ximab  -zumab  -mumab
MAbs used in cancer treatment:

Alemtuzumab
  targets CD52
  Chronic lymphatic leukemia
Bevacizumab
  targets VEGF
  Several cancer forms
Cetuximab, Panitumumab
  Targets EGFR
  EGFR+ colorectal cancer
Rituximab, targets CD20 Non-Hodgkin lymphoma, chronic lymphatic leukemia
Ofatumumab targets CD20 Chronic lymphatic leukemia
Trastuzumab
Her2 Her2+ breast cancer
Pertuzumab
  targets Her2R
Her2+ breast cancer

Ipilimumab
  targets CTLA-4
Malignant melanoma
Catumaksomab targets EpCAM + CD3
Malign ascites (ascites is abnormal accumulation of fluid in the abdominal cavity).
Immunoconjugates
Immunotoxins

Bacterial or natural toxins, or toxin subunits are linked to a surface protein-targeting mAb

Some ITXs have modified toxins that can only be activated once inside a cell (e.g. cleavage by endosomal proteinases)

Some toxins are protein-specific; others act at multiple levels in a cell
Radionuclide immunoconjugates

A

Immunotoxin (IT) Protein toxin

Radio-immunoconjugate (RIC) Radionuclide

Antibody-drug conjugate (ADC) Small molecule drug

B

ADC (or IT) RIC

Beta or alpha-particle emission

DNA damage

Microtubule disruption

Protein synthesis inhibition
Pattern Recognition Receptor Activators
(alone or as adjuvants with TSA/TAA)

Toll-like Receptor Ligands
PRR adjuvants
e.g. CpG oligonucleotides
CpGpCpGpCpGpCpGp..(n) [but less than 30 nt]
PRR adjuvants
- TLR3
- TLR4
- TLR7
- TLR9
- TLR7/9
<table>
<thead>
<tr>
<th>Defined Ligand</th>
<th>Receptor</th>
<th>Location</th>
<th>Outcome</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td><strong>Nucleic Acids:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>DNA</td>
<td>HI N200, AIM2, DA1</td>
<td>Cytoplasmic</td>
<td>Interferon</td>
<td>Roberts et al., 2009; Takaoka et al., 2007</td>
</tr>
<tr>
<td>Nonmethylated CpG DNA</td>
<td>TLR9</td>
<td>Endosome</td>
<td>Th1, CTLs</td>
<td>Klinman, 2006</td>
</tr>
<tr>
<td>ssRNA</td>
<td>TLR7/8</td>
<td>Endosome, Cytoplasmic</td>
<td>Interferon, Th1 + Interferon</td>
<td>Pehlmair et al., 2011</td>
</tr>
<tr>
<td>dsRNA</td>
<td>Rig-I (5’ triphosphate) MDA5 (long)</td>
<td>Cytoplasmic, Endosome</td>
<td>Interferon, Interferon</td>
<td>Alexopoulou et al., 2001; Kato et al., 2008</td>
</tr>
<tr>
<td>Imidazoquinolines (Imiquimod, R-848)</td>
<td>TLR7</td>
<td>Endosome</td>
<td>Interferon, Th1</td>
<td>Hemmi et al., 2002</td>
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<tr>
<td>Iminosugiothiazoline (3M-002)</td>
<td>TLR8</td>
<td>Endosome</td>
<td>Interferon, Th1</td>
<td>Philip and Levy, 2007</td>
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<td><strong>Pathogen PAMPs:</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peptidoglycans</td>
<td>TLR2, NOD1, and NOD2</td>
<td>Plasma membrane, cytoplasmic</td>
<td>Th1, CTLs</td>
<td>Ozinsky et al., 2000; Uehara et al., 2006</td>
</tr>
<tr>
<td>Des/nanomylkidep (DMP and MDP)</td>
<td>TLR2</td>
<td>Plasma membrane, cytoplasmic</td>
<td>Th1, CTLs</td>
<td>Duthie et al., 2011</td>
</tr>
<tr>
<td>Lipopeptides (PAM-3-Cys, MALP2)</td>
<td>TLR2+6 or TLR2+1</td>
<td>Plasma membrane</td>
<td>Th1, CTLs</td>
<td>Duthie et al., 2011</td>
</tr>
<tr>
<td>Lipo polysaccharides (LPS, MPL)</td>
<td>TLR4</td>
<td>Plasma membrane</td>
<td>Th1, CTLs</td>
<td>Duthie et al., 2011</td>
</tr>
<tr>
<td>Lipoteichoic acid (LTA)</td>
<td>TLR2</td>
<td>Plasma membrane</td>
<td>Th1, CTLs</td>
<td>Duthie et al., 2011</td>
</tr>
<tr>
<td>Flagellin</td>
<td>TLR5, NLRC4</td>
<td>Plasma membrane, cytoplasmic</td>
<td>Th1, CTLs</td>
<td>Franchi et al., 2006; Hayashi et al., 2001</td>
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<tr>
<td>Secretion system rod protein</td>
<td>NLRC4</td>
<td>Cytoplasmic</td>
<td>Th1, CTLs</td>
<td>Mao and Warren, 2010; Muller et al., 2009</td>
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<td>Beta Glucan</td>
<td>Dectin 1 (CLEC7a), TLR2</td>
<td>Plasma membrane</td>
<td>Th17 cells</td>
<td>Smekens et al., 2011</td>
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<tr>
<td>Alpha-mannans</td>
<td>Dectin 2</td>
<td>Plasma membrane</td>
<td>Th1, Th17</td>
<td>Drummond et al., 2011; Sajo et al., 2010</td>
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<tr>
<td>Alpha-mannose (Fungi)</td>
<td>Mincle</td>
<td>Plasma membrane</td>
<td>Th1, Th17</td>
<td>Drummond et al., 2011</td>
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<td>Trehalose-6,6-dibehenate (TD3)</td>
<td>Mincle</td>
<td>Plasma membrane</td>
<td>Th1, Th17</td>
<td>Marakalala et al., 2011; Schoenen et al., 2010</td>
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<tr>
<td>Trehalose-6,6-dimycolate (TDM)</td>
<td>Mincle</td>
<td>Plasma membrane</td>
<td>Th1, Th17</td>
<td>Ishikawa et al., 2009; Marakalala et al., 2011</td>
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<td>Lipopramboxosmanann</td>
<td>TLR2</td>
<td>Plasma membrane</td>
<td>Th1, CTLs</td>
<td>Underhill et al., 1999</td>
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<td>Mannose, fucose</td>
<td>MBL, MMR</td>
<td>Plasma membrane</td>
<td>Phagocytosis</td>
<td>Lee et al., 2011; Taylor et al., 2005</td>
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<td><strong>Toxins:</strong></td>
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<td>Anthrax lethal toxin</td>
<td>NLRP1</td>
<td>Cytoplasmic</td>
<td></td>
<td>Newman et al., 2010</td>
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<tr>
<td><strong>Complement:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complement: C3d, C5d</td>
<td>CR2</td>
<td>Extracellular</td>
<td>Phagocytosis, Antibody</td>
<td>Dempsey et al., 1996</td>
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<td><strong>Allergens:</strong></td>
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<td>Allergen glycosals/HDM</td>
<td>Dectin 2</td>
<td>Plasma membrane</td>
<td>Th2</td>
<td>Barrett et al., 2011</td>
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<td><strong>Others:</strong></td>
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<tr>
<td>Advanced glycation products, fibrillar proteins, oxLDL</td>
<td>RAGE</td>
<td>Plasma membrane</td>
<td>Inflammation</td>
<td>Yan et al., 2010</td>
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<td>NALP3</td>
<td>Cytoplasmic</td>
<td>Th2, Antibodies</td>
<td>Hornung et al., 2008</td>
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<tr>
<td>Saponins</td>
<td>NLRP3</td>
<td>Cytoplasmic</td>
<td>Th1, CTLs</td>
<td>Bauernfeind et al., 2011; Duwell et al., 2011</td>
</tr>
</tbody>
</table>

**Abbreviations:** TLR, Toll like receptor; Th, T helper; NOD, Nod like receptor; NLRP, Nod like receptor protein; IFIT, interferon induced protein with tetratricopeptide repeats; MDA, melanoma differentiation associated; MMR, macrophage mannose receptor; RAGE, receptor for advanced glycation endproducts; oxLDL, oxidized low density lipoprotein; CTL, cytotoxic T cells; CR, complement receptor; HDM, house dust mite; DC, dendritic cell.
Theranostics

Defines a combined THERApeutic + diagNOSTIC package

-Can be tailor-made to the patient, the cancer, the route of administration, the timing of drug release, and the ability to monitor the site of action

-Can control the movement, trafficking, and accumulation of the theranostic By an outside force (magnetic field, ultrasound)

-Can control the timing of drug action/release by external force (heat, light, ultrasound)

REAL PERSONALIZED MEDICINE
When considering everything...

<table>
<thead>
<tr>
<th>Approach</th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ACTIVE IMMUNITY</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytotoxic T-cells</td>
<td>Can be targeted to TSA/TAAs</td>
<td>Cell therapy may need systemic priming with cytokines (side effects)</td>
</tr>
<tr>
<td></td>
<td>T-cells can become memory Tc</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T-cells can frozen (boosters)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Autologous cells (your own cells)</td>
<td></td>
</tr>
<tr>
<td>NK Cells/LGLs</td>
<td>Very effective anti-tumor cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Can drive further adaptive immunity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Autologous cells</td>
<td></td>
</tr>
<tr>
<td>Dendritic Cells</td>
<td>Very powerful and efficient Ag presentation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Can be genetically-engineered to increase efficacy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Can be frozen (boosters)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Autologous cells</td>
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</tr>
<tr>
<td><em><strong>WELL-TOLERATED APPROACH</strong></em></td>
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When considering everything...

<table>
<thead>
<tr>
<th>Approach</th>
<th>Pros</th>
<th>Cons</th>
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<tbody>
<tr>
<td>ACTIVE IMMUNITY</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunokines/Growth Factors</td>
<td>Rapid mobilization of Immune cells</td>
<td>Side effects can be severe and not-well tolerated</td>
</tr>
<tr>
<td></td>
<td>Can be combines with cells, TAAs/TSAs, antibodies</td>
<td>Might activate latent viruses</td>
</tr>
<tr>
<td></td>
<td>Inexpensive to produce and to treat with</td>
<td>Might promote hematologic malignancies that rely on immunokine/GF to cycle (damaged stem cells)</td>
</tr>
<tr>
<td>Approach</td>
<td>Pros</td>
<td>Cons</td>
</tr>
<tr>
<td>------------------------</td>
<td>----------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>PASSIVE IMMUNITY</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adoptive Cell Therapy</td>
<td>Cells are tumor-derived, TAAs are already in the tumor tissue and cells are primed</td>
<td>Cells will not usually induce memory to TAA/TSA</td>
</tr>
<tr>
<td></td>
<td>Cells can be frozen (boosters)</td>
<td>Expensive/laborious</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carryover of suppressive immune cells from tumor biopsy and expansion into therapeutic product</td>
</tr>
<tr>
<td>Monoclonal Abs</td>
<td>Very specific Ags</td>
<td>Body can mount an anti-mAb humoral response</td>
</tr>
<tr>
<td></td>
<td>Mostly easy to manufacture</td>
<td>Body can become refractory immune editing might change expression of tumor Ags</td>
</tr>
<tr>
<td></td>
<td>Can be combined with Immunokine, GF approaches</td>
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</tr>
</tbody>
</table>
When considering everything...

<table>
<thead>
<tr>
<th>Approach</th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>PASSIVE IMMUNITY</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunoconjugates</td>
<td>Very specific Ags</td>
<td>Potential for bystander damage (e.g. radionuclides, toxins)</td>
</tr>
<tr>
<td></td>
<td>Mostly easy to manufacture</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Can be combined with Immunokine, GF approaches</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cytotoxic agent release can be controlled by the cell phenotype</td>
<td></td>
</tr>
<tr>
<td>Theranostics</td>
<td>Very versatile</td>
<td>Pharmacodynamics may not always be predictable in vivo</td>
</tr>
<tr>
<td></td>
<td>Combined diagnostic and therapeutic strategy</td>
<td>Depot effect, spontaneous breakdown; “burst effect”</td>
</tr>
<tr>
<td></td>
<td>Highly-personizable medicines</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Can be tissue-targeted</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Controlled bioactive agent release</td>
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<td>Usually easy to manufacture</td>
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When considering everything...

Never in history has humankind held so many options and combinations of conventional and microsurgery with immunotherapies (active and/or passive) to fight cancer.

Many approaches are in phase II clinical trials at the moment.....

Breakthroughs may not happen as blockbusters, but as incremental advances with outcomes that will increase efficacy, slowly, but solidly.

***Many cancers are now chronic states of tissue instability and are managed, whereas 20 years ago, they were without any hope.
When considering everything...