Concepts of cancer immunotherapy
Cancer *immunoediting*

- The fact that cancers occur in immunocompetent individuals indicates that immune surveillance is **imperfect**
- it follows that the tumors that do grow out must be composed of cells that are either invisible to the host immune system or that release factors that actively suppress host immunity.
- The term cancer immunoediting has been used to describe the ability of the immune system to shape and mold the immunogenic properties of tumor cells in a fashion that ultimately leads to the darwinian selection of subclones that are best able to avoid immune elimination.
Tumor Antigens

- Product of mutated genes
- Consequence of enhanced or aberrant expression
- Product of oncogenic viruses
- Oncofetal antigens
- Altered cell surface glycolipids and glycoproteins
- Differentiation antigens
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Tumor antigens recognized by CD8+ T cells.
Product of mutated genes

- Cancer mutated genes encode variant proteins that have never been seen by the immune system and are thus recognized as non-self.
- These acquired mutations are likely to be “passengers,” mutations that are neutral in terms of cancer cell fitness and thus unrelated to the transformed phenotype. However, by chance, some of these passenger mutations may fall in the coding sequences of genes and give rise to protein variants that serve as tumor antigens.
- The products of altered proto-oncogenes, tumor suppressor genes, and “passenger” genes are translated in the cytoplasm of tumor cells, and like any cytoplasmic protein, they may enter the class I MHC antigen-processing pathway and be recognized by CD8+ T cells.
- In addition, these proteins may enter the class II antigen-processing pathway in antigen-presenting cells that have phagocytosed dead tumor cells, and thus be recognized by CD4+ T cells also.
- In animals, immunization with mutated RAS or p53 proteins induces CTLs and rejection responses against tumors expressing these mutated proteins. However, the tumor-specific neoantigens that are recognized by CTLs in patients with cancer are for the most part currently unknown.
Overexpressed and aberrantly expressed proteins

- Tumor antigens may also be normal cellular proteins that are abnormally expressed in tumor cells.
- Examples: tyrosinase, expressed only in normal melanocytes and melanomas
  - tyrosinase is normally produced in such small amounts and in so few normal cells that it is not recognized by the immune system and fails to induce tolerance.
- Cancer-testis antigens, are encoded by genes that are silent in all adult tissues except germ cells in the testis.
  - sperm do not express MHC class I antigens, so these proteins are not immunogenic normally.
  - Melanoma antigen gene (MAGE) family. Although originally described in melanomas, MAGE antigens are expressed by a variety of tumor types.
Products of oncoviruses

• Oncoviruses produce proteins that are recognized as foreign by the immune system.
• Examples in humans include human papilloma virus (HPV) and Epstein-Barr virus (EBV).
  – Abundant evidence that CTLs recognize antigens of these viruses and that a competent immune system plays a role in surveillance against virus-induced tumors
  – the concept of immune surveillance against tumors is best established for DNA virus-induced tumors.
Oncofetal proteins

- Oncofetal antigens are proteins that are expressed at high levels on cancer cells and in normal developing (fetal) tissues.
- Amounts of these proteins are increased in tissues and in the circulation in various inflammatory conditions, and they are even found in small quantities in normal tissues.
- There is no evidence that oncofetal antigens are important inducers or targets of antitumor immunity.
- Oncofetal proteins are sufficiently specific that they can serve as markers that aid in tumor diagnosis and clinical management.
- The two most thoroughly characterized oncofetal antigens are carcinoembryonic antigen (CEA) and α-fetoprotein (AFP). These are used extensively as tumor markers in clinics.
Cell-type specific differentiation antigens

• Tumors express molecules that are normally present on the cells of origin, called differentiation antigens because they are specific for particular lineages or differentiation stages of various cell types.

• Differentiation antigens are typically normal self-antigens, and therefore they do not induce immune responses in tumor-bearing hosts.
  – Their importance is as potential targets for immunotherapy and for identifying the tissue of origin of tumors.
An example: CD20

- CD20 is a transmembrane protein that is expressed on the surface of all normal mature B cells
- Antibodies against CD20 have broad cytocidal activity against mature B-cell lymphomas and leukemias and are widely used in the treatment of these tumors.
- These antibodies are believed to induce cell killing through several mechanisms, including opsonization and phagocytosis of tumor cells, antibody-dependent cell-mediated cytotoxicity and complement fixation.
- Anti-CD20 antibodies also kill normal B cells, but because hematopoietic stem cells are spared, normal B cells reemerge following treatment.
Mechanism of action of anti-CD20 antibodies
Mechanism of action of anti-CD20 antibodies

Figure 1. Mechanisms of Action of Anti-CD20 Antibodies.

Anti-CD20 antibodies bind to the CD20 molecule on the surface of the malignant B cell in non-Hodgkin’s lymphoma (NHL), leading to cell death. Three mechanisms of action of anti-CD20 antibodies have been proposed. In complement-dependent cytotoxicity, the first component of complement (C1) binds to the Fc portion of the anti-CD20 molecule, resulting in the activation of the complement cascade and cell lysis through the formation of membrane attack complexes (MAC). In antibody-dependent cell-mediated cytotoxicity (ADCC), effector cells, such as natural killer cells or macrophages, bind to the Fc portion of the anti-CD20 molecule through Fcγ receptors; the effector cells then release effector molecules such as perforin, which cause cell lysis. In direct cytotoxicity, the anti-CD20 antibody induces internal signaling within the tumor cell, causing antiproliferative effects or cell death, which may involve apoptosis or other cell-death pathways. In the top inset, anti-CD20 antibodies bind to an extracellular portion of the CD20 molecule. Most anti-CD20 antibodies, including rituximab, tositumomab, and obinutuzumab, bind to the larger of two extracellular loops within the CD20 molecule; this loop includes the alanine-N-proline (ANP) residues at positions 170 to 172. Ofatumumab binds to two sites on the CD20 molecule: the smaller extracellular loop and positions 159 to 166 on the larger loop. This unique binding pattern of ofatumumab, which results in increased proximity of the antibody to the cell membrane, may account for the greater potency of the drug in inducing complement-mediated lysis. In the lower inset, the structure of chimeric and human antibodies is shown.
Antitumor Effector Mechanisms

• Humoral immunity: frequently negligible

• Cellular immunity: main mechanism
Cytotoxic T-lymphocytes

• The antitumor effect of cytotoxic T cells reacting against tumor antigens is well established in experimentally induced tumors.
• In humans, CD8+ CTLs have a clear protective role against virus-associated neoplasms (e.g., EBV- and HPV-induced tumors)
• Several studies have shown that the number of tumor-infiltrating CD8+ T cells and the presence of a “gene signature” associated with CD8+ CTLs correlates with a better prognosis in a variety of cancers, not only those caused by oncogenic viruses.
Natural Killer cells

- NK cells are lymphocytes that are capable of destroying tumor cells without prior sensitization and thus may provide the first line of defense against tumor cells.
- After activation with IL-2 and IL-15, NK cells can lyse a wide range of human tumors, including many that seem to be nonimmunogenic for T cells.
- While the importance of NK cells in host response against spontaneous tumors is still not well established, cytokines that activate NK cells are being used for immunotherapy.
Macrophages

• Activated macrophages exhibit cytotoxicity against tumor cells in vitro.

• T cells, NK cells, and macrophages may collaborate in antitumor reactivity, because interferon-γ, a cytokine secreted by T cells and NK cells, is a potent activator of macrophages.

• Activated macrophages may kill tumors by mechanisms similar to those used to kill microbes (e.g., production of reactive oxygen species)
Immune surveillance against cancer

- Increased frequency of cancers in the setting of immunodeficiency.
  - Persons with congenital immunodeficiencies develop cancers at about 200 times the rate in immunocompetent individuals.
  - Immunosuppressed transplant recipients and persons with AIDS also have an increased incidence of malignancies.
  - Particularly illustrative is the rare X-linked recessive immunodeficiency disorder termed XLP (X-linked lymphoproliferative syndrome), caused by mutations in the gene encoding an adapter protein, SAP, which participates in NK and T-cell signaling pathways. In affected boys, EBV infection does not take the usual self-limited form of infectious mononucleosis but instead evolves into a chronic or sometimes fatal form of infectious mononucleosis or, even worse, a lymphoma comprised of EBV-infected B cells.

- Most cancers occur in persons who do not suffer from any overt immunodeficiency. It is evident, then, that tumor cells must develop mechanisms to escape or evade the immune system in immunocompetent hosts.
The 3 “E”s: Elimination

A) Elimination: Immune System Eradicates Cancer Cells

Normal cells/tissue

Immune Protection

Immune Evasion
The 3 “E”s: Equilibrium

B) Equilibrium: Immune System Controls Cancer Cells

Abnormal cells/tissue outgrowth controlled

Immune Protection

Immune Evasion
The 3 “E”s: Escape

C) Escape: Cancer Cells Evade Immune System

Abnormal cells/tissue continue to replicate

Immune Protection

Immune Evasion
Evasion of the immune response

- **Failure to produce tumor antigen**
  - Antigen-loss variant of tumor cell
  - Lack of T cell recognition of tumor

- **Mutations in MHC genes or genes needed for antigen processing**
  - Class I MHC-deficient tumor cell
  - Lack of T cell recognition of tumor

- **Production of immunosuppressive proteins or expression of inhibitory cell surface proteins**
  - Inhibitory ligand
  - Inhibitory receptor
  - Immunosuppressive cytokines
  - Inhibition of T cell activation
Mechanisms of evasion of the immune response

• Selective outgrowth of antigen-negative variants.
  – During tumor progression, strongly immunogenic subclones may be eliminated, an example of immunoediting that has already been discussed.

• Loss or reduced expression of MHC molecules.
  – Tumor cells may fail to express normal levels of HLA class I molecules, thereby escaping attack by cytotoxic T cells. Such cells, however, may trigger NK cells if the tumor cells express ligands for NK cell activating receptors.
Mechanisms of evasion of the immune response

• Secretion of immunosuppressive factors by cancer cells.
  – Tumors may secrete products that inhibit the host immune response.
    • TGF-β is secreted in large quantities by many tumors and is a potent immunosuppressant.
    • Other tumors secrete galectins, sugar-rich lectin-like factors that skew T-cell responses so as to favor immunosuppression.
    • Many other soluble factors produced by tumors are also suspected of inhibiting the host immune response, including interleukin-10, prostaglandin E2, certain metabolites derived from tryptophan, and VEGF, which can inhibit the diapedesis of T cells from the vasculature into the tumor bed.

• Induction of regulatory T cells (Tregs).
  – Some studies suggest that tumors produce factors that favor the development of immunosuppressive regulatory T cells, which could also contribute to “immunoevasion.”
Mechanisms of evasion of the immune response

• Activation of immunoregulatory pathways.
  – tumor cells actively inhibit tumor immunity by engaging normal pathways of immune regulation that serve as “checkpoints” in immune responses.
• Tumor cells may downregulate the expression of costimulatory factors on antigen-presenting cells, such as dendritic cells.
  – as a result, the antigen presenting cells fail to engage the stimulatory receptor CD28 and instead activate the inhibitory receptor CTLA-4 on effector T cells.
• This not only prevents sensitization but also may induce long-lived unresponsiveness in tumor-specific T cells.
• Tumor cells also may upregulate the expression of PD-L1 and PD-L2, cell surface proteins that activate the programmed death-1 (PD-1) receptor on effector T cells.
• PD-1, like CTLA-4, may inhibit T cell activation.
Forms of Cancer Immunotherapy

• Non-Specific: Generalized, Non-Antigen-Specific Immune Activation

• Specific: Antigen-specific Response Induced in the Mouse or Patient or Passively Transferred in from Donor Source
Forms of Cancer Immunotherapy

**Active:** Induced Directly in the Tumor-Bearing Animal or in the Patient
- Can be Specific or Non Specific

**Passive or Adoptive:** Immunologically Active Material Transferred into Mouse or Patient as a Passive Recipient
- Can be Specific (Antibodies, T-Cells, Antigen-presenting cells – Dendritic Cell Vaccines)
- Or Non-Specific (Non-specifically-activated T-Cells; Cytokines)
Active Non-Specific Immunotherapy

Induced in the Patient or Mouse: Non-Antigen-specific

Bacterial Extracts: Non-Specific Immune Adjuvants
- **BCG**: Bacillus Calmette-Guerin (Attenuated Bovine Tuberculosis Bacterium)
- **Membrane Extracts of BCG**
- **C Parvum**: Corynebacterium parvum (related to diphtheria bacillus)

Bacterial Endotoxins: Muramyl Dipeptide

Chemical Adjuvants:
- **Levamisole**
- **Poly IC** (Poly-inosinic-Poly-cytidyllic acid)

Cytokines: (Can be actively induced or passively transferred)
- **Interferons**
- **Interleukin 2 (IL2)**
- **Tumor Necrosis Factor (TNF)**
Adoptive Immunotherapy of Cancers
(Passive: Donor to Recipient)

Non-Specific:
• Lymphokine-activated Killer Cells (LAK Cells)
• Cytokines (TNF alpha; IL2; Interferon)

Specific: Molecular Transfer
• Monoclonal Antibodies (antibodies are specific)

Specific: Cellular Transfer (antigen-specific)
• Tumor-Infiltrating Lymphocytes (TIL Cells)
• Engineered Antigen-Presenting Cells (Dendritic Cells)
LAK Cells in Mice & Humans

1. Isolate lymphocytes from the spleen of a healthy mouse.
2. Culture the lymphocytes with IL-2 for 3 days.
3. Infuse the cultured lymphocytes (LAK cells) into a mouse with a tumor.
4. The mouse returns the lymphocytes to the blood.
5. The machine isolates LAK cells from the blood.
6. LAK cells are filtered and returned to the blood.
Adoptive Immunotherapy using TILs

• Technique involves isolating tumor-infiltrating lymphocytes (TIL’s)
  – Primarily activated cytotoxic T-lymphocytes
  – Lymphocytes with antitumor reactivity found within the tumor

• Expanding their number artificially in cell culture by means of human recombinant interleukin-2.

• The TILs are then put back into the bloodstream, along with IL-2, where they can bind to and destroy the tumor cells.
This figure shows adoptive immunotherapy isolation techniques.
Adoptive T cell therapy: CAR-T cells

**CAR-T cells (Chimeric antigen receptor-T cells)**
- T cells transduced with tumor-specific CAR
- CAR: Single fusion molecule with antigen specificity plus signaling domain
- Three types of CAR: First/second/generations
  - Based on co-stimulatory receptors
- Cancer: Solid tumor & hematological malignancies

**Advantages of CAR T cells**
- “Live drug”
- Tumor recognition independent of HLA (no HLA typing needed)
- Multiple anti-tumor immunomodulators can be engineered
- Target variety of antigens (protein, carbohydrate, glycolipid)

CAR-T therapies are generating huge hope and expectations. This can be translated by the number of clinical trials registered: more than 250 since 2004 and 116 for 2016 alone.

Novartis is currently leading the race for the commercialisation of CAR-T therapies. **Kymriah™** was unanimously approved by the FDA for the treatment of children with ALL*. This CAR-T is directed against cancer cells expressing CD19 on their surface.

*ALL or Acute Lymphoblastic Leukemia is the most common cancer among children. Current treatments are limited to chemotherapy and stem cell transplant.
FDA News Release

FDA approval brings first gene therapy to the United States

CAR T-cell therapy approved to treat certain children and young adults with B-cell acute lymphoblastic leukemia

Aug. 30, 2017

Kymriah™ (Tisagenlecleucel) phase II results
Pediatric patients with relapsed and refractory B-cell ALL*

In Phase II (ELIANA), Kymriah™ has shown an impressive 83% remission rate after six months in clinical trials with patients who failed to respond to standard therapies or relapsed multiple times.

However, half of the patients experienced severe side effects. Unfortunately, several companies had to stop or hold their clinical trials due to patient deaths, highlighting the need of caution in the use of CAR-T cells.
CAR-T production process: Kymriah

1. Patient identified as CTL019 candidate
2. Patient’s T cells harvested and cryopreserved at apheresis center ("leukapheresis")
3. Patient’s T cells transferred to Novartis manufacturing site
   - CTL019 quality controlled before release
   - CTL019 packaged and cryopreserved (reprogrammed T cells)
3a. Modified T cells expanded and harvested
3b. CTL019 cells transferred to infusion center
3c. T cells activated and transduced with lentiviral vector
4. CTL019 infused into patient and CRS\(^2\) monitoring
5. Patient disease state evaluated +28 days after infusion
6. Patient relapse or refractory to prior therapy

Source: Novartis JULIET presentation June 2017
Possible side effects

Cytokine Release Syndrome (CRS)
As CAR-T cells activate the immune system against cancer, a large quantity of cytokines are released. This elevated amount of cytokines can cause high fevers, low blood pressure or poor lung oxygenation. The symptoms are reversible.

B cell aplasia
CAR-T cells can destroy cancerous B and normal cells expressing the target antigen. This results in a low number of normal B cells (aplasia). B cell aplasia limits the production of antibodies and thus the normal immune response.

Tumor Lysis Syndrome (TLS)
TLS results in complications during the treatment as dead cancer cells release their toxic contents in the bloodstream. It is a life threatening complication common to all cancer treatments and requires close monitoring of the patient.
Challenges to overcome

Production Challenges
The production of CAR-T cells is difficult.
- Time: autologous approach takes 14 to 21 days.
- Scaling up: allogenic approach could be difficult.

Handling Challenges
The handling of CAR-T cells is difficult.
- Risk of cross-contamination between patients.
- T cells are extremely sensitive cells.

Challenges with the cancer
- CAR-T cells are currently mainly for liquid tumours.
- Patients need to have T cells for engineering.
- CAR-T cells are an acute tool for difficult patients.

Cost Challenges
Taking in account the other challenges makes this technology very expensive. Kymriah costs $475,000 (not charged if the treatment fails)
IMMUNE SYSTEM has a safety mechanism that prevents a mature T cell from mounting an immune attack against its host. Before a T cell can attack, it must receive two signals. The first is the binding of an antigen to the T cell’s receptor. The second is typically the secretion and binding of a protein, B7, for example (left). If a T cell is exposed to a self-protein that is presented on a nonstimulatory cell, the T cell will die or become inactive (right).

Role of CD28 Antigen: Costimulatory Signals
CANCER CELLS can elude attack by lymphocytes even if they bear distinctive antigens. That absence of immune response may occur because cancerous cells lack the proper costimulatory molecules (left). Researchers are attempting to induce the body to fight tumors by inserting the molecule B7 into cancer cells (center). When B7 engages CD28, a complementary molecule on the surface of T cells, it generates a signal that instigates an assault on the cancer cells (right).

Co-Stimulatory Signals in T-Cell Mediated Tumor Cell Cytotoxicity
Chekpoints and checkpoint inhibition

**A** Suppression of T-Cell Activation in Lymph Node

- T-cell activation in the lymph node requires both immunologic signal 1 and immunologic signal 2
- Binding of CTLA-4 by dendritic cell ligands blocks immunologic signal 2 and therefore T-cell activation

**B** Activation of T Cell by Antibody Blockade of CTLA-4

- Antibody blockade of CTLA-4 (e.g., by ipilimumab or tremelimumab) permits T-cell activation

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*Figure 1: T-cell activation in the immune system.*
Two immunologic signals are required for T-cell activation in the lymph node: stimulation of the T-cell receptor (TCR) by the MHC (immunologic signal 1), and stimulation of CD28 by the B7 costimulatory molecules (immunologic signal 2). However, binding of the B7 costimulatory molecules to CTLA-4 blocks immunologic signal 2, and therefore blocks T-cell activation. Antibody blockade of CTLA-4, for example, by ipilimumab, derepresses signaling by CD28, permitting T-cell activation.
Chekpoint inhibition in the tumor environment

A. Suppression of T-Cell Activation by Tumor
   - SUPPRESSED T CELL
   - PD-1
   - TCR
   - MHC
   - PD-1 ligand
   - Signal 1
   - Binding of PD-1 by one of its ligands blocks TCR signaling and therefore blocks T-cell activation.

B. Activation of T Cell by Antibody Blockade of PD-1 Signaling
   - ACTIVATED T CELL
   - TCR
   - PD-1
   - Signal 1
   - Antibody blockade of PD-1 (e.g., by pembrolizumab or nivolumab) or one of its ligands permits T-cell activation
Chekpoint inhibition in the tumor environment

Figure 2. T-cell Activation in Tumor Milieu.

During long-term antigen exposure, such as occurs in the tumor milieu, the programmed death 1 (PD-1) inhibitor receptor is expressed by T cells (Panel A); it suppresses the effect of the TCR on T-cell activation. Blockade of PD-1 or its ligand (Panel B) (e.g., by pembrolizumab or nivolumab) derepresses TCR signaling, thereby permitting T-cell activation.
Immunomodulatory monoclonal antibodies and armoured chimeric antigen receptor (CAR) T cells overcome immune suppression