Concepts of cancer immunotherapy
History

• Paul Ehrlich first conceived the idea that tumor cells can be recognized as “foreign” and eliminated by the immune system.
• Subsequently, Lewis Thomas and Macfarlane Burnet formalized this concept by coining the term immune surveillance, which implies that a normal function of the immune system is to constantly “scan” the body for emerging malignant cells and destroy them.
• This idea has been supported by many observations
  – the presence of lymphocytic infiltrates around tumors and reactive changes in lymph nodes draining sites of cancer
  – experimental results, mostly with transplanted tumors;
  – the increased incidence of some cancers in immunodeficient people and mice;
  – the direct demonstration of tumor-specific T cells and antibodies in patients;
  – most recently and most directly, the response of advanced cancers to therapeutic agents that act by stimulating latent host T-cell responses
• 2011: approval of the first checkpoint inhibitors
• 2017: approval of the first CAR T-cell therapies
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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<td><img src="image11.png" alt="Diagram" /> Virus antigen-specific CD8+ CTL</td>
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**Oncogene products:** mutated RAS, BCR/ABL fusion proteins

**Tumor suppressor gene products:** mutated p53 protein

**Various mutant proteins in carcinogen, or radiation, induced animal tumors; various mutated proteins in melanomas**

**Overexpressed:** tyrosinase, gp100, MART in melanomas

**Aberrantly expressed:** cancer-testis antigens (MAGE, BAGE)

**Human papilloma virus E6, E7 proteins in cervical carcinoma; EBNA proteins in EBV-induced lymphoma**

**Tumor antigens recognized by CD8+ T cells.**
Product of mutated genes ("neo-antigens")

• 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.
• Initial trials where patients are injected with cocktails of neoantigens derived from the analysis of the patients’cancer genomes, identification of mutations and selection of best candidate neoantigens
  – Still preliminary, but great promises (especially in combination with other approaches described later)
  – 6 melanoma patients received vaccines with 20 neoantigens each: 4/6 were cancer-free 2 years and 6 months later. The remaining 2 patients were also “cured”, but after the combination with a “checkpoint inhibitor”
    • For now, scientists are focusing on mutation-rich tumors
    • Is this really working? More studies and more patients need to be treated
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 surface glycolipids and glycoproteins

- Most tumors express higher than normal levels and/or abnormal forms of surface glycoproteins and glycolipids
- These altered molecules include:
  - gangliosides
  - blood group antigens
  - mucins.
- Mucins are high-molecular-weight glycoproteins containing numerous carbohydrate side chains on a core polypeptide. Tumors often have de-regulated expression of the enzymes that synthesize these carbohydrate side chains, which leads to the appearance of tumor-specific epitopes on the carbohydrate side chains or on the abnormally exposed polypeptide core.
- Several mucins have been the focus of diagnostic and therapeutic studies, including CA-125 and CA-19-9, expressed on ovarian carcinomas, and MUC-1, expressed on both ovarian and breast carcinomas.
- MUC-1 is an integral membrane protein that is normally expressed only on the apical surface of breast ductal epithelium. In ductal carcinomas of the breast, however, the molecule is expressed in an unpolarized fashion and contains new, tumor-specific carbohydrate and peptide epitopes that induce both antibody and T-cell responses in cancer patients and are therefore considered candidates for tumor vaccines in patients with breast cancer and possibly ovarian cancer as well.
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

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.
Other approaches

- Monoclonal antibodies may also be covalently coupled to drugs, toxins, or radiochemicals
  - the antibody serves as guided missile that delivers a therapeutic warhead to cancers expressing particular surface antigens

- Anti-CD30 antibodies:
  - CD30 is a member of the TNF receptor family of transmembrane proteins that is expressed by particular T cell lymphomas and most Hodgkin lymphomas.
  - Antibodies against CD30 linked to a cytotoxic drug have recently produced remarkable responses in patients with CD30-positive lymphomas that have failed conventional therapies.

- Bispecific antibodies engineered to have two different antigen recognition surfaces, one that binds tumor antigens and a second that binds to the CD3 signaling molecule on T cells, have produced some promising results in clinical trials.
Antitumor Effector Mechanisms

- Humoral immunity: 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
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
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

- **a** Mice with tumor
- **b** Lymphocytes isolated from blood
- **c** LAK cells cultured with IL-2 for 3 days
- **d** IL-2 activation

**LAK Cells**

IL-2

**Human Patient**

- **Lung Tumor**

**Healthy Mouse**

- **Spleen**
  - **Isolated Lymphocytes**

**Lymphocytes cultured with IL-2 for 3 days**

**Malignant Cells**

**LAK Cells**

**Healthy Mouse**

**Lymphocytes cultured with IL-2 for 3 days**

**Malignant Cells**

**LAK Cells**

**IL-2**
Adoptive Immunotherapy

• Immunotherapy
  – IL–2, alone, can be used as a cancer treatment by activation of cells which are cytotoxic for the tumor

• Some success has been obtained with renal cell carcinoma and metastatic melanoma.
  – Rosenberg study
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.
Overview: Adoptive T cell therapy

1. Isolation of TILs or tumor specific T-cells from blood

2. Expand and activate T-cells ex vivo

3. Infuse the "boosted" T-cells into the patient.

Target therapy with Tumor specific T cells
- Cancer: Melanoma
- Autologous tumor infiltrating lymphocytes (TILs); “Live drug”

Advantages
- High response rate (>50%),
- Long-term remission,
- Less toxic & gentler to the patient

Limitation:
- Extraction of TILs,
- Cell manufacturing

Possible alternate
- T cell Engineering (CAR-T cells)

Rosenberg SA & Dudley ME 2009 Current Opinion of Immunology
Adoptive T cell therapy: CAR-T cells

**CAR-T cells (Chimeric antigen receptor-T cells)**

- T cells transduced with tumor-specific CAR
  - A single CAR T cell can destroy thousands of cancer cells
- 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)

## Clinical significance of CAR-T cells

<table>
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<th>Target</th>
<th>CAR</th>
<th>Cancer</th>
<th>Objective response</th>
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<tr>
<td>CD19</td>
<td>CAR:CD28-CD3ζ</td>
<td>Lymphoma and CLL</td>
<td>N=7: 1CR, 5 PR &amp; 1SD</td>
</tr>
<tr>
<td></td>
<td>CAR:CD137-CD3ζ</td>
<td>ALL</td>
<td>2CR</td>
</tr>
<tr>
<td></td>
<td>CAR:CD28-CD3ζ</td>
<td>ALL</td>
<td>5CR</td>
</tr>
<tr>
<td>CD20</td>
<td>CAR:CD137-CD28-CD3ζ</td>
<td>NHL</td>
<td>N=3: 1PR, 2NED</td>
</tr>
<tr>
<td>CEA</td>
<td>CAR-CD3ζ (1st gen)</td>
<td>Colorectal &amp; breast</td>
<td>N=7: minor responses in two patients</td>
</tr>
<tr>
<td>GD2</td>
<td>CAR-CD3ζ (1st gen)</td>
<td>Neuroblastoma</td>
<td>N=19: 3CR</td>
</tr>
<tr>
<td>ERBB2</td>
<td>CAR:CD28-CD137-CD3ζ</td>
<td>Colorectal cancer</td>
<td>N=1, patient died</td>
</tr>
</tbody>
</table>

Kershaw et. al. 2013 Nature Reviews cancer
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.

patients suffered strong cytokine release syndrome (CRS)
CAR-T production process: Kymriah

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

Source: Novartis JULIET presentation June 2017
CAR T-Cell Therapy: ZUMA-1 Trial of KTE-C19

- 101 patients with refractory aggressive NHL, including DLBCL, received CAR T-cell therapy with KTE-C19 (axicabtagene ciloleucel)
- ORR was 82%; 54% of patients had CR
- At median follow-up of 8.7 months, 44% of patients remained in response, 39% in CR
- The median duration of response was 8.2 months
- The most common grade ≥ 3 treatment-emergent AE was neutropenia (66%); cytokine release syndrome occurred in 13%

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 into account the other challenges makes this technology **very expensive**. Kymriah costs **$475,000** (not charged if the treatment fails).
Additional challenges for solid tumors

• Identifying the right targets
  – Solid tumor cells are usually much more heterogeneous than hematological malignant cells
  – More obstacles: reach the tumor, penetration, overcome immunosuppressive stimuli, remain able to kill
    • Immunosuppression of the CAR T cells by the tumor environment seems a strong problem in solid tumors
      – Combination with checkpoint inhibitors?
  – Recent hopes:
    • IL13R in glioblastomas, GD2 in melanomas, mesothelin in ovary
    • (for all cancers including blood) “allogenic” CAR T cells
      – The donor T cells were transduced with lentivirus to express CAR-CD19 and they were engineered to ablate T cell receptor (TCR)-α constant region (TRAC). TRAC was ablated to prevent TCRα–TCRβ expression so as to minimize the risk of graft-versus-host disease (GVHD), which is a concern when using HLA-unmatched cell transplants. These universal CART19 (UCART19) cells were also engineered to express a CD20 epitope so they could be cleared by treatment with rituximab in situations of adverse effects.
Futuristic approaches
STIMULATION by two molecules is needed to activate lymphocytes. The diagrams depict a CD8 T cell and a macrophage. Without the presence of antigens, the T cell is dormant (left). Yet antigen alone cannot induce T cell function (center). In this way, a response to the body’s own antigen does not occur; in fact, this first signal turns off the T cell. If the macrophage is infected, it will produce a molecule called B7, which acts on the T cell’s CD28 surface protein (right). Only when an antigen and the B7 molecule are present on the same cell does the T cell proliferate.

Co-Stimulation by Antigen-presenting Cells of T-Cells

CoStimSA.pox from Scientific American Life, Death, and the Immune System
Chekpoints and chekpoint inhibition

Figure 4: T-cell activation in the lymph node.
Fig. 1. T-cell Activation in the Lymph Node.

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
- Signal 1
- MHC
- PD-1 ligand

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
- Signal 1
- Antibody
- PD-1
- PD-1 ligand

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.
Overview of the immune inhibitory molecules that compromise endogenous T-cell antitumour activity.
Immunomodulatory monoclonal antibodies and armoured chimeric antigen receptor (CAR) T cells overcome immune suppression
Combination immunotherapy

- Combining two different immunotherapic approaches
  - PD-1 and CTLA-4 inhibition: enhanced survival in metastatic melanomas, but also enhanced toxicity
    - Rationale is not very strong: we have those two drugs, let’s combine them together....
  - PD-1 + inhibitors of IDO (indoleamine 2,3 dioxygenase)
    - IDO is an enzyme whose activity correlates with the function of Treg cells

- Combining immunotherapy with other drugs
  - Metastatic lung cancer: chemotherapy (killing cells and inducing inflammation and recruitment of T cells to the tumor) enhances the effect of immunotherapy and extends survival (approved by FDA in 2017)
    - Radiotherapy may work similarly
Minimization of toxicity

• Avoid the “cytokine-release syndrome” after CAR T-cell therapy
  – Identify biomarkers predictive of CRS
  – Antagonizing CRS: tocilizumab (kills the response due to IL6)
    • Can this reduce the efficacy of therapy?

• Unexplained deaths upon treatment with PD1 inhibitors
  – Especially in hematological cancers
  – Maybe due to combination with other used drugs?
Predict efficacy

• As of now, immunotherapy is like playing to the slot machine: a few lucky ones (the oldest survivor is from 2001...), many people do not respond, nobody knows exactly why

• Response can start soon (after weeks from beginning of treatment) or much later (almost a year!)

• Response can be difficult to assess: the tumor may “look” as if it still there (stable disease), but actually all cells are not viable

• Ongoing search for markers
Candidate biomarkers

• Expression of PD-L1 on tumor cells
  – 50% of patients with high PD-L1 respond to immunotherapy, compared to 10% with low PD-L1
    • *Good but not great*
    • *Only partially true in lung cancer, even less predictive for other tumors*

• Tumor types
  – Tumors with high mutation load (lung-smoke; melanoma-UV light) are best responsive
    • High production of neo antigens

• Specific mutations
  – Defects in DNA repair→high mutation→neoantigens
  – 2017: FDA approved pembrolizumab for all tumors showing such defects (Mismatch repair genes)

• Specific gene expression signatures
  – In melanoma, the expression pattern of 26 genes seems to predict resistance with good accuracy (80%)

• Microenvironment of the tumor
  – Presence of a high number of Tcells at the **invasive margin** of the tumor
  – *Search of indicators of T cell response in circulating blood ongoing*

• Microbiota
Considerations on the cost of cancer immunotherapy

• Cost of immunotherapy—new drugs is much higher than conventional therapies
  – Budgetary impact
    • Very high
  – Value
    • Too soon to be known for sure

• Solutions: Performance-based risk sharing agreements
  – The pharmaceutical company get high money only when the drug is working on the patient
    • Still difficult to define efficacy