Advances in Cancer Immunotherapy

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MacMillan Group Meeting

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The Impact of Cancer on Society

class of diseases arising from abnormal, rapid and uncontrolled growth of cells
malignant tumors are defined as those which can expand and invade other parts of the body

the challenge in developing new treatments is in how to differentiate cancer cells from healthy ones

- Second leading cause of death globally (1 in 6 of total deaths)
- 14 million newly reported cases annually, along with 8 million deaths
- Total global economic cost of approx 1.6 trillion USD annually

http://www.who.int/en/news-room/fact-sheets/detail/cancer
Traditional Cancer Therapy

surgery  radiotherapy  chemotherapy

focus on factors such as anatomical location or unique features of tumor cells for targeting

many cancers remain difficult to treat, particularly in advanced stages following significant metastasis

negative side effects and toxicity to healthy cells remain a challenge in development of new therapeutics

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Introduction to Immunotherapy

- Immunotherapy harnesses the ability of the immune system to identify and destroy tumors, as opposed to targeting them directly.

- Potential advantages of this strategy include a high degree of selectivity for tumor cells and decreased toxicity to the patient.

- However, tumors can develop a range of means to suppress the immune response, often exploiting the body’s own regulation system.

*tumor cells present distinct antigens to healthy cells due to a range of genetic and epigenetic changes*

- Oncogenic virus
  - eg HPV, HBV

- Mutated DNA
  - eg through UV exposure

Farkona, S.; Diamandis, E. P.; Blasutig, I.M. *BMC Medicine*, 2016, 14, 73.
Introduction to Immunotherapy

1893- William Coley utilized live bacteria to stimulate immune response

1909- Paul Ehrlich proposes surveillance of cancer by immune system

1967- T-cells first identified and characterized

1970- Discovery of dendritic cells

1973- CTLA-4 discovered

1990- Elucidation of CTLA-4’s role as an immune checkpoint

1995- Evidence for T-cell mediated tumor immune surveillance

1998/2001- FDA approves ipilimumab

2001- FDA approves sipuleucel-T

2011- FDA approves tisagenlecleucel

Farkona, S.; Diamandis, E. P.; Blasutig, I. M. BMC Medicine, 2016, 14, 73.
**The Immune System**

**innate immune system**
- Component of the immune system that responds generically to all pathogens
- Does not confer immunological memory and generally provides immediate response

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**adaptive immune system**
- Component of the immune system mediated by antigen-specific lymphocytes and antibodies
- Components include B and T cells, includes the development of immunological memory

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- Antigen-presenting cells (APCs) display foreign antigens in the form of complexes with MHCs to T-cells
- MHC= Major Histocompatibility Complex, a distinct protein which displays an antigen on a cell surface for recognition
- An important subcategory is dendritic cells (DCs), which are located throughout tissues and in lymph nodes
- Binding to foreign antigens causes DCs to mature and express costimulatory ligands on their surface

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**T-cell activation requires both antigen and co-stimulant**

Farkona, S.; Diamandis, E. P.; Blasutig, I. M. *BMC Medicine*, 2016, 14, 73.
A (Very) Brief Overview of the Immune System

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A (Very) Brief Overview of the Immune System

**CD4+ (helper) T cells**
- Responsible for the activation and inhibition of other immune cells

**CD8+ (killer) T cells**
- Responsible for destroying virally infected and tumor cells

**Regulatory T cells (T\(_{\text{Reg}}\))**
- Suppress the action of other activated cytotoxic T-cells

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How Tumor Cells Evade the Immune Response

The immune response imposes a selection pressure on tumors, allowing resistant variants to proliferate.

Antigen deficient → evades T-cell detection

MHC deficient

Suppression of immune response

How can we stimulate an immune response to overcome these defence mechanisms?

Mature DC

Active T-cells

Suppression of immune response

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Immunotherapy Strategies

- Therapeutic cancer vaccines
- Immune checkpoint blockade therapy
- Adoptive cell transfer therapy
Cancer Vaccines via Antigen Stimulated Dendritic Cells

cancer vaccines attempt to stimulate T cell activation pathways by APCs

early attempts to use peptides based on tumor antigens were largely unsuccessful

- Only one approved drug to date (Spileucel T) for castration resistant prostate cancer patients
- Shows 4 month improvement in median survival, but no meaningful decrease in tumor volume
Immune Stimulation via Checkpoint Blockade

The amplitude of T-cell response is mediated by a balance of stimulatory and inhibitory signals.

- Inhibitory ligands and receptors which regulate T-cell functions in tissues are often overexpressed by tumors, leading to immune suppression in the tumor environment.
**Immune Stimulation via Checkpoint Blockade**

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- **CTLA-4** is an inhibitory receptor which is expressed exclusively on T-cells and is responsible for down-regulating the initial stages of naive T-cell activation.

- **PD-1** is a receptor expressed on activated T-cells, limiting T-cell functions in peripheral tissues and is often overexpressed by cancerous cells.

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CTLA-4 has a considerably greater affinity for APC ligand, but is initially not present on cell surface. Binding to CD28 receptor induces transport of CTLA-4 to the surface where it dampens the activation. Concentration of CTLA-4 on cell surface is proportional to strength of original stimulation.

Activated T-cells upregulate PD-1, which is activated by inflammatory signals in tissues. This downregulates the effect of T-cells, preventing collateral damage to healthy tissues.

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The Importance of CTLA-4

Knockout of genes coding for CTLA-4 demonstrate its importance in immune regulation.

Stem cells deficient in CTLA-4 gene

CTLA-4 deficient mice

Mice die within 3-4 weeks

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Wet weight (mg)</th>
<th>Lymphocytes (10^7)</th>
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<tr>
<td></td>
<td>Lymph nodes</td>
<td>Spleen</td>
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<tr>
<td></td>
<td>Lymph nodes</td>
<td>Spleen</td>
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<td>Ctlα-4^+/+</td>
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<td>69</td>
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<tr>
<td></td>
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<tr>
<td>Ctlα-4^+/−</td>
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</tr>
<tr>
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<td>1.7</td>
<td>3.1</td>
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<td>Ctlα-4^−/−</td>
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<td>28.0</td>
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<td>Ctlα-4^−/−</td>
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<td>501</td>
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</table>

Pancreatic tissue samples in unmodified (D) and modified (E / F) mice

**CTLA-4 Blockade as a Therapeutic Strategy**

**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

**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

Monoclonal Antibodies to Target Specific Sites

Monoclonal antibodies are made by immune cells that are clones of an original parent cell. Specific antibodies can be made for almost any substance, bind to substrate with a high level of specificity.

1984 Nobel Prize

“for theories concerning the specificity in development and control of the immune system and the discovery of the principle for production of monoclonal antibodies”

First in-vivo demonstration of anti-CTLA-4 antibody

mice injected with tumor cells (colon carcinoma) subsequently treated with an anti-CTLA-4 antibody

mice either injected with CDLA-4 or CD28 antibody or left untreated (control)

first in-vivo demonstration of the ability of CTLA-4 monoclonal antibodies to induce a therapeutic antitumor immunity

recovered mice reinjected with new tumor after 70 days

Clinical trial results for Ipilimumab

Clinical trials performed for anti-CTLA-4 monoclonal antibodies vs metastatic melanoma

Initial trials with Ipilimumab

- Objective clinical response in approx 10% of patients
- 25-30% of patients display immune related toxicities

Phase III trials

- Tested using gp100 peptide vaccine specific to melanoma as a control
- Ipilimumab demonstrated a mean survival benefit of 3.5 months compared to control
- 18% of patients survived beyond two years compared to 5% in control group

First therapy to demonstrate a survival benefit for metastatic melanoma

Phan, G. Q. et al. PNAS, 2003, 100, 8372.
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The Role of PD-1 in Immune Resistance

expression of PD-1 ligands is a known means by which tumors block T-cell response

PDL-1 enhanced tumors are found to be considerably less susceptible to T-cell attack

CTLA-4 Blockade as a Therapeutic Strategy

A Suppression of T-Cell Activation by Tumor

B Activation of T Cell by Antibody Blockade of PD-1 Signaling

Binding of PD-1 by one of its ligands blocks TCR signaling and therefore blocks T-cell activation.

Antibody blockade of PD-1 (e.g., by pembrolizumab or nivolumab) or one of its ligands permits T-cell activation.

its role in the suppression of immune response makes PD-1 an attractive target for checkpoint blockade

PD-1 blockade has been explored as an alternative to CTLA-4 with fewer autoimmune side effects

The Role of PD-1 in Immune Resistance

Studies on mice injected with PDL-1 enhanced tumors illustrate PDL-1’s role in tumor immune resistance.

Mice injected with PDL-1 enhanced tumors exhibit increased tumor volume and decreased life expectancy.

The Role of PD-1 in Immune Resistance

injection of mice with anti-PDL-1 monoclonal antibody allows for immune response to tumors

mice injected with anti-PDL-1 antibodies exhibit decreased tumor volume and increased life expectancy

Clinical Results with anti-PD-1 Antibodies

Trials of anti-PD-1 antibodies investigated activity against several cancers (melanoma, lung, prostate).

Nivolumab initially tested against late stage melanoma, gaining FDA approval in 2014.

- Median survival - 16.8 months
- 1 year survival rate - 62%
- 2 year survival rate - 43%
- Tumor regression continued even after treatment was discontinued
- Autoimmune symptoms observed, did not persist after treatment was discontinued


Immune Stimulation via Checkpoint Blockade

Identification of new immune checkpoints provides new therapeutic avenues

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**Immune Stimulation via Checkpoint Blockade**

*identification of new immune checkpoints provides new therapeutic avenues*

- **LAG-3** (Lymphocyte activation gene 3)
  Enhances function of $T_{reg}$ cells and inhibits CD8+ cells

- **TIM3** (T-cell membrane protein 3)
  Inhibits activity of helper T cells

- **A2aR** (Adenosine A2a receptor)
  Drives T cells to become $T_{reg}$ cells

Due to release of adenosine by dying cells and high cell turnover within tumors, this is a potentially potent route for tumor immunity

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To date, the majority of immune checkpoint inhibitors have been focused on monoclonal antibodies.

Small molecule drugs have a number of advantages, such as lower cost as well as no immunogenicity.

The most extensive research thus far has been into blocking the PD-1/PDL-1 interaction.

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How Tumor Cells Evade the Immune Response

The immune response imposes a selection pressure on tumors, allowing resistant variants to proliferate.

- Antigen-deficient tumor cells evade T-cell detection.
- MHC-deficient tumor cells evade T-cell detection.

Suppression of immune response

- Produces cytokines which promote formation of T_{reg} cells around the tumor.

How can we stimulate an immune response to overcome these defense mechanisms?

- Mature DC
- Active T-cells

Suppression of immune response

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Mature DC, active T-cells, Suppression of immune response

Immunotherapy Strategies

- Therapeutic cancer vaccines
- Immune checkpoint blockade therapy
- Adoptive cell transfer therapy
Adoptive T-cell transfer involves extraction of T-cells from tumors, enrichment for an antigen specific variant then re-infusion in theory circumvents the need to break the tolerance of tumor antigens to T-cell response

Adoptive T-cell transfer

Lymphodepletion of target was found to be important to the success of ACT (in mice, 10x more effective)

Thought to be needed in order to deplete T_reg populations, as well as to promote production of growth factors

Adoptive T-cell Transfer - Initial Attempts

Initial human trials of ACT only had significant success against melanoma - likely due to its high mutation rate.

<table>
<thead>
<tr>
<th>CELLS USED FOR ACT</th>
<th>YEAR</th>
<th>CANCER HISTOLOGY</th>
<th>MOLECULAR TARGET</th>
<th>PATIENTS</th>
<th>NUMBER OF ORS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor-infiltrating lymphocytes*</td>
<td>1998</td>
<td>Melanoma (12)</td>
<td></td>
<td>20</td>
<td>55%</td>
</tr>
<tr>
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<td>1994</td>
<td>Melanoma (88)</td>
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<td>86</td>
<td>34%</td>
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<tr>
<td></td>
<td>2002</td>
<td>Melanoma (13)</td>
<td></td>
<td>13</td>
<td>46%</td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>Melanoma (17)</td>
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<td>93</td>
<td>56%</td>
</tr>
<tr>
<td></td>
<td>2012</td>
<td>Melanoma (19)</td>
<td></td>
<td>31</td>
<td>48%</td>
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<tr>
<td></td>
<td>2012</td>
<td>Melanoma (18)</td>
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<td>13</td>
<td>38%</td>
</tr>
</tbody>
</table>

A number of efforts have been made to modify T-cells to allow for expansion to cancers other than melanoma.

Strategies for Genetic Engineering of T-Cells

isolation of high affinity T-cell receptors

- Some patients naturally express T-cells bearing high affinity T-cell receptors for specific tumor types
- Genes encoding the receptor can be extracted and cloned into a virus to make analogous T-cells
- The affinity of such engineered receptors can be increased through directed evolution

Strategies for Genetic Engineering of T-Cells

isolation of high affinity T-cell receptors

- Humanized mice can be used to produce specific human TCR genes upon infection with tumor
- Genes encoding the receptor can be extracted and cloned into a virus to make analogous T-cells
- The affinity of such engineered receptors can be increased through directed evolution

Strategies for Genetic Engineering of T-Cells

*isolation of high affinity T-cell receptors*

- Genetic code from antibodies engineered to encode single chain structure fused to T-cell receptor
- Allows T-cells to recognize structures on cell surface with the specificity of a monoclonal antibody
- First drug using this technology, tisagenlecleucel approved by the FDA in 2017

Cancer immunotherapy has emerged as a distinct approach to longstanding cancer therapies. Manipulation of the immune response shown to be a viable strategy for control of tumor growth. Novel techniques such as immune checkpoint blockade have provided durable remission. Studies are ongoing to determine which patients and which types of tumor will best respond. Severe autoimmunity remains a complication with many successful therapies.