Basic Mechanisms of Action in Immuno-Oncology

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Immune System Function and Immune Response

Identify and destroy foreign or abnormal cells in the body

**Innate Immunity**
- Nonspecific
- First line of defense
- WBCs (natural killer cells, neutrophils)
- Activation of adaptive immunity

**Adaptive Immunity**
- Specific target recognition
- Slower to develop
- Antibody or cell mediated
- Memory
  - Faster, stronger subsequent responses

Immuno-Therapy: Does it Work in Cancer? Paul Ehrlich’s Immunosurveillance Concept

100+ years of progress

- Magic Bullet (Paul Ehrlich)
- Immunosurveillance (P Ehrlich 1909)

Paul Ehrlich
Hallmarks of cancer

Resisting cell death

Sustaining proliferative signaling

Evading growth suppressors

Inducing angiogenesis

Activating invasion and metastasis

Enabling replicative immortality

Hanahan2011
Emerging hallmarks

- Avoiding immune destruction
- Deregulating cellular energetics
- Genome instability and mutation
- Tumor-promoting Inflammation

[Hanahan2011]
Proper T-Cell Activation Requires 2 Signals\(^1\)

- To be properly activated, a T cell MUST receive 2 signals\(^1\)
  - Binding of an MHC-antigen complex to TCR
  - Binding of a second costimulatory signal
- This initiates intracellular signaling that activates the T cell, which can then kill infected or cancer cells or help support other immune functions\(^1,2\)
T-Cell Response: First Signal

T-Cell Response: Second Signal to Accelerate or Brake

- **Activating Signals**
  - CD28
  - OX40
  - GITR
  - CD137
  - CD27
  - HVEM

- **Inhibitory Signals**
  - CTLA-4
  - PD-1
  - TIM-3
  - BTLA
  - VISTA
  - LAG-3

T-Cell Stimulation

T-Cell Inhibition

Cancer Immunoediting

The immune system is capable of recognizing and eliminating tumor cells in the tumor microenvironment. Innate and adaptive immunity act as a complementary network of self-defense against foreign threats.

Tumors can use various mechanisms to escape detection and enable growth.
Tumors Evade Immune Detection and Destruction

- The immune response to tumor cells can be evaded by a number of mechanisms:
  - Reduced antigen presentation
  - Resistance to T-cell–mediated killing
  - T-cell inhibition and anergy (eg, by upregulation of coinhibitory molecules, including PD-L1)
  - Treg-mediated immunosuppression
Regulatory T cells
Regulatory T cells

Flow cytometry plot gated on human CD4 T cells
Regulatory T cells

- T-regs cells which have a role in regulating or suppressing other cells in the immune system.
- T-regs control the immune response to self and foreign antigens and help prevent autoimmune disease.
- T-regs produced by a normal thymus are termed ‘natural’
- T-regs formed by differentiation of naïve T cells outside the thymus, i.e. the periphery, or in cell culture are called ‘adaptive’
Regulatory T cells

• Natural T-reg are characterised as expressing both the CD4 T cell co-receptor and CD25, which is a component of the IL-2 receptor
• Tregs are thus CD4+ CD25+
• Expression of the nuclear transcription factor **Forkhead box P3 (FoxP3)** is the defining property which determines natural T-reg development and function
Regulatory T cells

- FoxP3 is crucial for maintaining suppression of the immune system.
- Naturally occurring mutations in the FOXP3 gene can result in self-reactive lymphocytes that cause a rare but severe disease IPEX (Immune Dysregulation, Polyendocrinopathy, Enteropathy, X-Linked) in humans.
MDSC
Myeloid Derived Suppressor Cell
MDSC

- MDSCs expand in pathological situations such as chronic infections and cancer
- Cancer is associated with altered hematopoiesis and development of MDSC
MDSC

• MDSCs infiltrate tumors
• Inhibition of T cells and NK cells immune
• MDSCs also accelerate angiogenesis, tumor progression and metastasis through the expression of cytokines and factors such as TGF-beta and IL10
• MDSC works on L-arginine metabolism & ROS
MDSC

VEGF, bFGF, MMPs, TGF-β, S100A8/A9

Tumor

Leukocytes
Fibroblasts
Tumor cells

Neoangiogenesis

Invasion

Metastasis

Proliferation

Formation of pre-metastatic niche
TAM
Tumor-associated macrophages
Tumor-associated macrophages

- (TAMs) are a group of cells that originate mainly from the peri-tumoral tissue or bone marrow
- Two main types: M1 and M2
- Infiltrating M1 TAMs present in the early stages of tumorigenesis
- Secrete pro-inflammatory cytokines and in turn inhibit tumor growth
- M2 TAMs are predominant in the late stage of tumor formation
- It remains unclear when M1 TAMs are transformed to M2 TAMs
- Tumor hypoxia is currently thought to be associated with such a shift
Tumor-associated macrophages
Factors inhibiting anti-tumor immune response
HOW CAN TUMORS EVADE THE IMMUNE RESPONSE

APC = antigen-presenting cells; BTLA = B- and T-lymphocyte attenuator; CD = cluster of differentiation; CTLA-4 = cytotoxic T-lymphocyte–associated protein-4; GITR = glucocorticoid induced tumor necrosis factor-related protein; HVEM = herpes virus entry mediator; LAG-3 = lymphocyte-activation gene 3; MDSC = myeloid-derived suppressor cell; MHC = major histocompatibility complex; PD-4 = programmed death receptor-4; TIM-3 = T-cell immunoglobulin domain and mucin domain-3; Tregs = regulatory T cells; VISTA = V-domain immunoglobulin-containing suppressor of T-cell activation.

Immune response and chemotherapy
## Immune response and chemotherapy

<table>
<thead>
<tr>
<th>Drug</th>
<th>Effect on immune system</th>
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| Taxanes       | • Enhances T cell and NK cell function  
• Increases recruitment of TIL  
• Increase efficacy of immuno-stimulatory agents                                                                                      |
| Doxorubicin   | • Induces immunogenic cell death  
• Increases proliferation of CD8 T cells  
• Stimulates antigen presentation by DCs  
• Stimulates MCP1 and M6PR                                                                                                                |
| Cyclophosphamide | • Induces immunogenic cell death  
• Suppresses Treg inhibitory functions and restores the proliferative capacity of effector T cells and NK cell cytotoxicity  |
| Gemcitabine   | • Reduces the number of myeloid suppressor cells  
• Increases the antitumor activity of CD8(+) T cells and activated NK cells                                                                 |
| Oxaliplatin   | • Induces immunogenic cell death  
• Increases MHC I complex  
• Inhibits PD-L2                                                                                                                            |
Chemotherapy: Pleiotropic stimulatory effects on the immune system

T cell function & Th1 polarization

Increase in antigen presentation/crosspresentation via dendritic cells; MHC I upregulation; epitope spreading

Proinflammatory cytokines

Tumor-antigen release/presentation; danger signal release; immunogenic cell death

Chemotherapy

IL-2 IFN-γ

Treg & MSDC depletion
Chemotherapy: Pleiotropic stimulatory effects on the immune system

- T cell function & Th1 polarization
- Increase in antigen presentation/crosspresentation via dendritic cells; MHC I upregulation; epitope spreading
- Tumor-antigen release/presentation; danger signal release; immunogenic cell death
- Proinflammatory cytokines
- IL-2, IFN-γ
- Treg & MSDC depletion
Chemotherapy: Pleiotropic stimulatory effects on the immune system

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Increase in antigen presentation/crosspresentation via dendritic cells; MHC I upregulation; epitope spreading

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IL-2
IFN-γ

Tumor-antigen release/presentation; danger signal release; immunogenic cell death

Treg & MSDC depletion
Chemotherapy: Pleiotropic stimulatory effects on the immune system

- Increase in antigen presentation/crosspresentation via dendritic cells; MHC I upregulation; epitope spreading
- T cell function & Th1 polarization
- Proinflammatory cytokines: IL-2, IFN-γ
- Treg & MSDC depletion
- Tumor-antigen release/presentation; danger signal release; immunogenic cell death
Immunogenic Cell Death
ICD

- Agents inducing ICD are targeting endoplasmic reticulum (ER), leading to ER stress and production of reactive oxygen species (ROS)
- Both ER stress and ROS production are key players of intracellular signaling pathways that govern ICD
- ICD is characterized by secretion of damage-associated molecular patterns (DAMPS)
ICD – DAMPs

Calreticulin & Heat-Shock Proteins

- Calreticulin (CRT) "eat me" signal
- Normally in the lumen of endoplasmic reticulum (ER)

- Heat-shock proteins (HSPs), HSP70 and HSP90
- On the cell surface HSPs have an immunostimulatory effect
- HSP Interact with antigen-presenting cell (APC) surface receptors like CD91 and CD40
- Facilitate cross-presentation of antigens derived from tumour cells on MHC class I molecule, which than leads to the CD8+ T cell response
ICD – DAMPS

High Motility Group B1 (HMGB1)

- Late apoptotic marker and its release to the extracellular space seems to be required for the optimal release and presentation of tumor antigens to dendritic cells

- It binds to several pattern recognition receptors (PRRs) such as Toll-like receptor (TLR) 2 and 4, which are expressed on APCs
ICD – DAMPS
ATP

• ATP released during ICD
• Functions as a "find-me" signal for monocytes at the site of apoptosis
Crosspresentation of tumor derived antigens

Cell death induced by immunogenic radio/chemotherapy

Dying tumor cells

Calreticulin

HMGB1

Dendritic cells

TLR4

MHC I

MHC II

Antitumor immune response

CD8+ T cells

CD4+ T cells
Immunogenic vs. Non-immunogenic Tumors
Immunogenic vs. Non-immunogenic Tumors

Inflamed

Respond favorably to checkpoint inhibition

Non-inflamed

How do you convert these tumors to inflamed tumor?
Immunogenic vs. Non-immunogenic Tumors

**Inflamed**
- TILs
- PD-L1 expression
- CD8+ T cells
- Genomic instability
- Pre-existing immunity
- Respond favorably to checkpoint inhibition

**Non-inflamed**
- How do you convert these tumors to inflamed tumor?
Biomarker Analysis:
Tumor-Infiltrating Lymphocytes

Response based on TIL Levels

- TIL Levels
  - > 10% (n = 53)
  - ≤ 10% (n = 55)

ORR + SD Rate

RECIST v1.1

7% 13%
7% 19%

Schmid P, et al. AACR 2017 Phase Ia Atezolizumab in TNBC
Biomarker Analysis: Tumor-Infiltrating Lymphocytes

OS Based on TIL Levels

- Higher ORR and longer OS were seen with higher baseline TIL (CD8) infiltration

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<th>TIL Levels(^a)</th>
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<td>mOS (95% CI)</td>
<td>6.6 mo (4.9, 10.2)</td>
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\(^a\) TIL levels refer to tumor-infiltrating lymphocytes.

Schmid P, et al. AACR 2017 Phase Ia Atezolizumab in TNBC
Immunogenic vs. Non-immunogenic Tumors

Inflamed

- TILs
- PD-L1 expression
- CD8+ T cells
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Respond favorably to checkpoint inhibition

Non-inflamed

How do you convert these tumors to inflamed tumor?
Immunogenic vs. Non-immunogenic Tumors

Alexandrov et al., 2013
JAX mice were transferred to TAC mice, tumor burden was reduced while tumor-specific CD8+ T cell responses were augmented in TAC mice. In contrast, TAC feces had little effect on JAX recipients. Furthermore, the efficacy of anti-PD-L1 therapy in TAC mice was significantly enhanced when mice received feces from JAX donors. The authors...
Conclusion

• Major advances in the understanding of the immune system in cancer

• Major advances in immunotherapy in cancer treatment

• Additional work is needed

• Long term remissions & possible cures
Thank You
Immune Escape in Cancer

Many tumours escape the immune response by creating an immunosuppressive microenvironment that prevents an effective antitumour response.\textsuperscript{1,2}

The mechanisms tumours use to escape the immune system provide a range of potential therapeutic targets for cancer.

\textbf{Recruitment of immunosuppressive cells}:
- Tregs
- MDSCs

\textbf{Ineffective presentation of tumour antigens to the immune system}:

\textbf{Downregulation of MHC Expression} vs \textbf{Suppression of APC}.

\textbf{Tumour Cell} vs \textbf{APC}.

\textbf{Tumour Microenvironment}.

\textbf{Release of immunosuppressive factors}:
- Factors/enzymes directly or indirectly suppress immune response.

\textbf{T-cell checkpoint}:
- Dysregulation
- Co-inhibitory receptors
- Co-stimulatory receptors

\textbf{APC}=antigen-presenting cell; \textbf{MDSC}=myeloid-derived suppressor cell; \textbf{MHC}=major histocompatibility complex; \textbf{Treg}=regulatory T cell.

Combining immune pathways to refine response

- Immune balance is maintained through the combination of activating and inhibitory signaling pathways.\(^8,9\) Signaling pathways can work in combination to directly or indirectly modulate the activity of effector cells such as cytotoxic T cells and NK cells.

Immune pathways that involve molecules found on the surface of effector cells can \textbf{directly} inhibit or activate their antitumor activity.\(^{77-79}\)

Inhibitory signals from other immune-related pathways can \textbf{indirectly} augment immune suppression.\(^{25}\)

Modulation of two immune pathways can more effectively activate immune activity compared with either pathway alone, as suggested by preclinical data.\(^{80-83}\)
KEYNOTE-010: OS for PD-L1 TPS ≥ 1% Stratum
KEYNOTE-010: OS for PD-L1 TPS

≥ 50% Stratum

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<td>Docetaxel</td>
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2 vs 10 mg/kg:
HR: 1.12 (95% CI: 0.77-1.62)

*Comparison of pembrolizumab vs docetaxel.

Two Paradigms for Advancing the Therapy of Metastatic Melanoma

Immunotherapy

Target host

Targeted Therapy

Target tumor
CTLA-4 and PD-1/PD-L1 Checkpoint Blockade for Cancer Treatment

CTLA-4 mAbs: Ipilimumab Tremelimumab

PD-1 mAbs: Nivolumab Pembrolizumab

PD-L1 mAbs: Atezolizumab Avelumab Durvalumab

HOW CAN TUMORS EVADE THE IMMUNE RESPONSE
Empowering the immune system: innate and adaptive immunity

**Innate immune response**
The first line of defense, it identifies and attacks tumor cells without antigen specificity.\(^1\,4-6\) Natural killer (NK) cells are the main effector cells of innate immunity.

**Adaptive immune response**
A durable response that attacks tumor antigens.\(^1,6\) Once activated, it can be sustained through a memory response.\(^7\) Cytotoxic T cells are the main effector cells of adaptive immunity.

The antitumor activity of NK cells and cytotoxic T cells is regulated through a network of **activating** and **inhibitory** signaling pathways:\(^8-10\)

**ACTIVATING**
Stimulating pathways trigger immune responses

**INHIBITORY**
Pathways that counterbalance immune activation such as checkpoints
Antitumor activity of the innate and adaptive immune responses

The antitumor activity of NK cells and cytotoxic T cells is regulated through a network of activating and inhibitory signaling pathways. The balance between activating and inhibitory pathways normally enables the immune system to attack tumor cells, while sparing healthy cells.

Key stages of the antitumor immune response

In both the innate and adaptive immune responses, immune cells have the potential to recognize and eliminate tumor cells. There are three principal stages in this process:

- **Presentation**: The innate immune system rapidly identifies and attacks tumor cells. Tumor cell death releases tumor antigens, which can activate the cytotoxic T cells of the adaptive immune system.

- **Infiltration**: Tumor antigens and other factors attract immune cells to the tumor site, where they invade and attack.

- **Elimination**: Activated cytotoxic T cells recognize tumor cells as the source of the antigen and target them for elimination.
Immuno-surveillance\textsuperscript{1-3}

Dendritic cells activate naïve T cells in lymph nodes

Activated T cells migrate back to the tumor

Receptor is expressed on the T cell and the ligand on APCs or peripheral tissues.\textsuperscript{1}

Dendritic cell

Activated T cell

Checkpoints

Immune checkpoints such as CTLA-4 and PD-1, LAG-3, and TIM-3 function at different phases in the immune response to regulate the duration and level of the T-cell response.

Tumor-specific antigens

CTLA-4 = cytotoxic T-lymphocyte antigen 4
PD-1 = programmed cell death protein 1
LAG-3 = lymphocyte activation gene 3
TIM-3 = T-cell immunoglobulin and mucin protein 3

Pathways that modulate the innate immune response (1/2)

Current research is investigating the following pathways to understand how they can be modulated to restore the innate immune response’s ability to fight cancer:

**SLAMF7** is an activating receptor on the surface of NK cells and other immune cells.\(^\text{11}\) When engaged, SLAMF7 activates NK cells, the rapid responders of the immune system and the body’s first line of defense against cancer.\(^\text{5,12}\)

Continuous activation of NK cells through pathways like SLAMF7 may initiate the development of long-term immunity.\(^\text{4,13}\)

**CD137** is an activating receptor on the surface of NK cells and T cells that can stimulate them to reproduce and generate antitumor activity.\(^\text{14,15}\) In animal models, CD137 also plays a critical role on T cells in the development of immune memory and the creation of a durable immune response.\(^\text{16}\)

**Preclinical data** suggests that activation of CD137 can stimulate NK-cell and cytotoxic T-cell activity and generate a lasting memory response.\(^\text{17,18}\)
Pathways that modulate the innate immune response (2/2)

Current research is investigating the following pathways to understand how they can be modulated to restore the innate immune response’s ability to fight cancer:

**KIR** is an immune checkpoint receptor on the surface of NK cells that acts to stop NK cells from killing normal cells. Tumor cells can use the KIR pathway to disguise themselves as normal cells and escape detection by NK cells.

*Preclinical data* suggests that blockade of inhibitory KIRs can help restore NK cell-mediated immune activity.

**CSF1R** is a receptor on the surface of macrophages and other cells of the myeloid lineage. In the tumor microenvironment, some macrophages evolve from antitumor to protumor in their activity. Protumor, or tumor-associated macrophages (TAMs) can drive immunosuppression and support tumor growth. Mouse models have shown that tumor cells use CSF1 to target CSF1R on macrophages, stimulating the development and survival of TAMs.

*Preclinical data* suggests that blockade of CSF1R can result in depletion of TAMs and improved T-cell responses.
Current research is investigating the following pathways to understand how they can be modulated to restore the *adaptive immune response*’s ability to fight cancer:

**CTLA-4** is an immune checkpoint receptor on T cells that plays a key role in preventing T-cell overactivation. Tumor cells use the CTLA-4 pathway to suppress initiation of an immune response, resulting in decreased T-cell activation and ability to proliferate into memory T cells. CTLA-4 signaling diminishes the ability of memory T cells to sustain a response, damaging a key element of durable immunity.

**Preclinical data** suggests that treatment with antibodies specific for CTLA-4 can restore an immune response through increased survival of memory T cells and depletion of regulatory T cells.

**PD-1** is an immune checkpoint receptor on cytotoxic T cells that plays a key role in T-cell exhaustion and prevention of autoimmunity. Tumor-infiltrating T cells across solid tumors and hematologic malignancies display evidence of exhaustion, including upregulation of PD-1.

**Preclinical data** suggests that PD-1 blockade reinvigorates exhausted T cells and restores their cytotoxic immune function. Inhibiting both PD-1 ligands (PD-L1 and PD-L2) may be more effective at reversing T-cell exhaustion than inhibiting PD-L1 alone.
Pathways that modulate the adaptive immune response (2/4)

Current research is investigating the following pathways to understand how they can be modulated to restore the **adaptive immune response**’s ability to fight cancer:

**LAG-3** is an immune checkpoint receptor on the surface of both activated cytotoxic and regulatory T cells (Tregs).[^44][^45] When bound to the antigen-MHC complex, LAG-3 can negatively regulate T-cell proliferation and the development of lasting memory T cells.[^46] Repeated exposure to tumor antigen causes an increase in the presence and activity of LAG-3, leading to T-cell exhaustion.[^47][^48]

**Preclinical data** suggests that inactivation of LAG-3 allows T cells to regain cytotoxic function.[^49]

**CD73** is a cell-surface enzyme on Tregs. CD73 is a critical checkpoint in the production of adenosine, which has been demonstrated to be a powerfully immunosuppressive molecule in cellular studies.[^50] Tumor cells exploit this pathway by expressing CD73 and releasing adenosine into the tumor microenvironment.[^51][^52][^53]

**Preclinical data** suggests that inhibition of CD73 activity can stimulate T-cell activity.[^54]
Pathways that modulate the adaptive immune response (3/4)

Current research is investigating the following pathways to understand how they can be modulated to restore the adaptive immune response’s ability to fight cancer:

**IDO** is an intracellular enzyme that initiates the breakdown of tryptophan, an amino acid that is essential for T-cell survival.\(^{55-57}\) Tumor cells can upregulate IDO activity in order to suppress T-cell antitumor function.\(^{58,59}\)

**Preclinical data** suggests that blockade of IDO can restore cytotoxic T-cell function.\(^{60,61}\)

**CD137** is an activating receptor on the surface of NK cells and T cells that can stimulate them to reproduce and generate antitumor activity.\(^{14,15}\) CD137 also plays a critical role on T cells in the development of immune memory and the creation of a durable immune response, in animal models.\(^{16}\)

**Preclinical data** suggests that activation of CD137 can stimulate NK-cell and cytotoxic T-cell activity and generate a lasting memory response.\(^{17,18}\)
Pathways that modulate the adaptive immune response (4/4)

Current research is investigating the following pathways to understand how they can be modulated to restore the **adaptive immune response**’s ability to fight cancer:

**GITR** is an activating receptor on the surface of T cells and other immune cells that helps to enhance cell reproduction and generate antitumor activity.\(^\text{62-64}\) GITR signaling can also block the suppressive abilities of regulatory T cells (Tregs), further enhancing cytotoxic T-cell function.\(^\text{65}\)

**Preclinical data** suggests that activation of GITR signaling can help enhance immunity through the activation of cytotoxic T cells and inhibition of Treg activity.\(^\text{66}\)

**OX40** is an activating receptor on the surface of activated cytotoxic T cells and Tregs.\(^\text{67-69}\) OX40 plays a dual role in the immune response, both activating and amplifying T-cell responses. This dual effect helps create a tumor microenvironment that is more favorable to antitumor response.\(^\text{70-73}\)

**Preclinical data** suggests that OX40 increases the number and activity of cytotoxic T cells and curtails the immunosuppressive impact of Tregs.\(^\text{74-76}\)
Tumors Evade Immune Detection and Destruction

- The immune response to tumor cells can be evaded by a number of mechanisms:
  - Reduced antigen presentation
  - Resistance to T-cell–mediated killing
  - T-cell inhibition and anergy (eg, by upregulation of coinhibitory molecules, including PD-L1)
  - Treg-mediated immunosuppression