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Immune checkpoints inhibitors in cancer therapy-current status and future prospects

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***Abstract*---**The field of oncology is revolutionized by immunotherapy. immunotherapy is a fundamental breakthrough in cancer treatment that focuses on boosting the natural defense for malignant cell elimination. Cancer immunotherapy is of different forms including, virus therapies, adoptive cell transfer, cytokine therapies, immune checkpoint inhibitors, and cancer vaccines, all of which have promise future developments and clinical applications. To maintain homeostasis several pathways of immune signaling are employed that inhibit or stimulate immune responses. These immune signaling pathways help to keep immune responses maintained by avoiding autoimmunity and chronic inflammation, these immune signals that

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regulate immune responses in the body are also known as immune checkpoints. The responses of numerous self-regulating checkpoints of the immune system are exploited by the cancerous cells. Immune checkpoint inhibitors and monoclonal antibodies (mAbs) are becoming the most important immunotherapies, despite the progress of Acceptance and Commitment Therapy (ACT). ICIs are the antibodies that inhibit the receptors of the immune checkpoint, PD-1/PD-L1, and CTLA-4. It has potential benefits in cancer treatment for long-term survival and antitumor effect production in malignancies of a broad spectrum. Numerous patients with a wide range of solid and hematological malignancies now have better prognoses because of immunotherapy, which has established itself as a key component of cancer treatment. The two main reasons that have contributed to this success are chimeric antigen receptor (CAR) T cells, and checkpoint inhibitors (CPIs) . This review article is aiming to summarize immune therapeutic methods used in cancer therapy, the role of different immune checkpoints in immune system cells, and different types of immune checkpoint inhibitors and their applications.

***Keywords*---**checkpoint receptor, immune checkpoint inhibitors, checkpoint ligands, CTLA-4, PD-1, immunotherapy, cancer treatment.

Introduction

A biological therapy called immunotherapy targets the body's immune system to combat cancer. [1]. Usually, the immune system does not recognize cancer in the body, hence it survives unchecked. The immune system is supported by immunotherapy to detect and treat cancer. Cancer is characterized by genomic instability as it is a genomic disease [2]. Numerous structural alterations and point mutation accumulation occur in the tumor progression process. Tumor antigens are risen by genomic variations, these tumor antigens are recognized as elicit, and non-self cellular immune responses by the immune system [3]. Malignant cells are eradicated or impaired based on their functions and phenotypes by the effective immune responses. Multiple evolving mechanisms like negative regulatory pathways upregulation, immunosuppressive cell populations recruitment, and antigen presentation machinery defects, for immune surveillance escaping, as a result, it shows antitumor immune responses abrogation and immune cells functioning as impeded effector [4].

The field of oncology is revolutionized by immunotherapy. immunotherapy is a fundamental breakthrough in cancer treatment that focuses on boosting the natural defense for malignant cell elimination [5]. In recent years noticeable advances and developments have been achieved in clinical investigations. Sustainable clinical responses to immunotherapy have been shown in multiple cancer types [6]. Cancer immunotherapy is of different forms including, virus therapies, Immune checkpoint inhibitors, adoptive cell transfer, cancer vaccines, and cytokine treatments all exhibit promise development and clinical uses [7]. Each strategy’s basic principle and the molecular and cellular underpinning are depicted in each step concern to (NK) natural killer, (TCR) T-cell receptor, (CAT-T)

chimeric antigen receptor T-cell, and (DC) dendritic cells. Including ACT, immune Checkpoints inhibitors (ICI), and cancer vaccines, cancer immunotherapy have great advantages and applications [5].

To date, there are several treatments available for cancer. The treatment to be given depending on some factors like the type of cancer, stage of cancer, patients' general health, and treatment preferences [8]. The treatment is being finalized mutually by the doctor and patients. The several methods of cancer therapy include Chemotherapy, Bone marrow transplant, Surgery, Hormone therapy, Radiation therapy, Immunotherapy, Targeted drug therapy, Radiofrequency ablation, Cryoablation, and Clinical trials [9].

Checkpoints are the proteins made up of several cells of immune systems, like T cells. Immune responses are prevented from being strong by these checkpoints and it also prevents cancer cell killing from T cells [10]. T cells can kill cancer only when the checkpoints are blocked. The checkpoint proteins found on the cancer cell or T cells include CTLA-4 (B7-1/B7-2 checkpoint ligands), and PD-1 (PD-L1/PD-L2 checkpoint ligands). Most recently immune checkpoint inhibitors are in great demand for the treatment of cancer [11].

The checkpoint inhibitors are drugs of immunotherapy that prevent the binding of immune checkpoints to their partner proteins [12]. This blocking allows T cells to kill cancer cells by preventing the signals from being sent for immunization. Different types of cancers are approved to get treated by immune checkpoint inhibitors, such as bladder cancer, colon cancer, Hodgkin lymphoma, lung cancer, skin cancer, including melanoma, rectal cancer, breast cancer, head and neck cancer, cervical cancer, liver cancer, stomach cancer, renal cell cancer, [13][14]. This review article is aiming to summarize immune therapeutic methods used in cancer therapy, the role of different immune checkpoints in immune system cells, and different types of immune checkpoint inhibitors and their applications.

Basics of cancer therapy

Curing cancer is the aim of cancer treatments. If complete curing is not possible the treatments may apply to shrink the cancer cells, slow down the cancer cell growth, or reduce the adverse effects of cancer cells [15]. Cancer may be treated by using primary treatment which aiming completely remove cancer from the patient's body or to kill all the cancerous cells [16]. Surgery is the most frequent treatment for the most general type of cancer. Chemotherapy and radiation therapy can also be used as primary treatment if the cancer is particularly sensitive to it [17]. Adjuvant treatment is another type that aims to killing the cancer cells that remain un-killed after the primary treatment to reduce the chances of cancer recurring [18]. Hormone therapy, chemotherapy, and radiation therapy can be used as adjuvant therapy. Neoadjuvant therapy is also a similar treatment but it would be used before primary treatment use to increase the effectiveness of primary treatment and to make it easier [19].

Palliative treatment therapy is applicable for relieving treatment signs, symptoms, and side effects caused by cancer itself [20]. For relieving symptoms radiation

therapy, surgery, hormone therapy, and chemotherapy can be used. Shortness of breathing and pain can be reduced by using other types of medications. Palliative treatment can be given simultaneously with other treatments for curing cancer [21].

Surgery is the treatment for cancer that aims to remove cancer as much as possible. Chemotherapy is the process of eliminating cancer cells with chemicals or medications [22]. energy beams of high-powered, such as protons or X-rays, are used in cancer cell killing radiation therapy. In radiation therapy, external beam radiation is used which came from a machine outside the patient's body [15]. Bone marrow is present within the bones responsible for making stem cells of the blood. Stem cell transplant is an alternative term for bone marrow transplant. Both the bone marrow of the patient and the bone marrow of the donor may be used in a bone marrow transplant [23]. In the event of a bone marrow transplant, doctors may provide high dosages of chemotherapy. Immunotherapy is a treatment method that uses patients' immune systems to cure cancer. It is also called biological therapy. Usually, the immune system does not recognize cancer in the body, hence it survives unchecked. The immune system is supported by immunotherapy to identify and treat cancer [24]. prostate cancer and breast cancer are types of cancer that can be fueled by several hormones of the body. To block or remove those hormones from a body is found to be effective for reducing or stopping cancer cell growth. Within the cancerous cells, specific abnormalities can be focused on by the target drug delivery method for cancer therapy. Clinical trials are an investigative method for cancer treatments [25].

Immune checkpoints inhibitors

Different Immune checkpoints and their function

To maintain homeostasis several pathways of immune signaling are employed that inhibit or stimulate immune responses [26]. These immune signaling pathways help to keep immune responses maintained by avoiding autoimmunity and chronic inflammation. These pathways of immune signaling prevent self-harm and show optimal immune responses against foreign antigens as they are designed for that. These immune signals that regulate immune responses in the body are termed immune checkpoints [27]. Some immune checkpoint receptors with their ligands and types of regulations are given in table 1 [28].

Table 1. Immune checkpoint receptors with their ligands and types of regulations [28]

Checkpoint Receptor	Checkpoint Ligand	Regulation
CTLA-4 (CD152)	B7-1 (CD80) and B7-2 (CD86)	Negative
PD-1 (CD279)	PDL-1 (CD274) and PDL-2 (CD273)	Negative
LAG-3 (CD223) and KIR (CD158)	MHC class II	Negative
4-1BB (CD137)	4-1BBL (CD137L)	Positive
GITR (CD357)	GITRL	Positive
TIGIT	Poliovirus receptor (CD155)	Negative

TIM-3	Galectin	Negative

*****CTLA-4 - Cytotoxic T Lymphocyte Antigen, PDL-1 - Programmed Cell Death Ligand 1, PD-1 - Programmed Cell Death Protein, MHC - Major Histocompatibility Complex, LAG-3 - Lymphocyte-Activation Gene 3; GITR - Glucocorticoid-Induced Tumor Necrosis Factor Receptor, TIM-3, T cell immunoglobulin mucin 3, TIGIT - T cell immunoreceptor with Ig and ITIM domains.

The responses of numerous self-regulating checkpoints of the immune system are exploited by the cancerous cells [29]. Immune responses are regulated negatively by manipulating these immune checkpoints. For example, myeloid-derived T cells, stimulation of T regs (regulatory T cells), and some inhibitory molecules like PDL-1 and Fas ligand [30]. Decrease in antibody production, increase in immunosuppressive cytokines production e.g., (TGF) tumor growth factor beta (β), and interleukin (IL)-10 [31]. It permits tumor cell growth by avoiding immune responses. For enhancement of immune responses more efficiently against tumor cells, the manipulation of checkpoints can be done like cancer cells that manipulate various immune checkpoints for their ends. The drawback of increased chances of tissue damage due to the use of checkpoint blockages as a therapeutic agent in immunotherapy [32].

CTLA-4 (CD152) checkpoint receptor is mostly reoresented on T cells (T regs, CD4+, and CD8+). The binding of CTLA-4 (CD152) receptors to the cell's checkpoint ligands, B7-1 and B7-2, causes the cell to be negatively regulated [33]. Consequently, it is the perfect receptor to target for the tumour. During an immunological response, CD80 and CD86 from APCs (antigen-presenting sites) connect to CD28 on T cells, providing T cell activation co-stimulatory signals by MHC II binding to TCR [34]. When T cells express CTLA-4, it commonly binds to CD80 and CD86 B7 protein as it has a comparatively high affinity than theCD28. The interaction of CD28 with both the B7 proteins is prevented by the binding of CTLA-4 with CD86 and CD80. It prevents activation of T cells which regulate immune response negatively [35].

PD-1 is also termed as CD279, a checkpoint having a function of immune response downregulation by promoting self-tolerance and autoimmunity reduction [36]. The PD-1 receptors are presented mostly on T cells that are of the peripheral tissue. PD-1 binds to 2 ligands during an immune response, one is PDL-1 (B7-H1 and CD274) which are found on most tissues. The second is PDL-2 (B7-DC and CD273) found only on hematopoietic lineage cells [36]. The LAG-3 also termed CD223, are an expression of NK cells and $CD4^+$ and $CD8^+$ cells surface. pro-inflammatory cytokines such as the interferon-gamma (IFN-γ) upregulate LAG-3 during immune responses. The binding of class II MHC molecules to TCR is prevented by binding of class II MHC to LAG-3 to inhibit stimulation of T cells. TNF-α and IL-12 upregulating signaling pathways are a result of the LAG-3 binding to MHC II on dendritic cells. KIRs also termed CD158, are usually reported on NK cells but have also been reported on tumor-specific cytotoxic T cells [37].

On $CD4^+$ and $CD8^+$ T cells TIM-3 is found to be a marker. Signaling pathways of immune response negative regulation are expected to be started by following the binding of TIM-3 to galectin. For example, T cell function reduction, increasing death of $CD4^+$ T cell, promoting suppressor cell development that is derived from myeloid. Immunotherapy of Anti-TIM-3 is under development [38].

CD137 is another name for the 4-1BB. It belongs to the family of TNF receptor and is represented on a variety of immune cells, including neutrophils, CD4+ and CD8+ cells, activated NK cells, and dendritic cells (DCs). It binds to its 4-1BB ligand that is found on B cells, macrophages, and DCs. Pathways of pro-inflammatory signaling can be activated by 4-1BB as being a molecule of co-stimulation. The pathway includes NF-kB, p38, and c-jun for the promotion of immune response. 4-1BB becomes an important immunotherapeutic agent as it is a positive immune response stimulator [39].

GITR, also termed CD357, is a CSR found on $CD4^+$, $CD8^+$ T cells, and T regs, along with its ligands (GITRL), which are located on various epithelial cells and APCs. GITR and its ligand binding induce the signaling pathways to increase immune response by effector T cell and T cell enhancement and T reg activity reduction [40]. The TIGIT is found on many lymphocytes like NK cells and T cells. NK cell and T cell activation and TIM-3 expression stimulation are inhibited by the binding of TIGIT to its ligands CD155 (A Poliovirus Receptor). CD155 is located on immune cells like DCs and macrophages. T cells are activated by blocking TIGIT binding resulting in potential therapeutic advantages [41].

Types of Immune checkpoint inhibitors and their function

In the last decades, ICIs development has the greatest advancements in immunotherapeutic methods of cancer treatment. ICIs are the antibodies that inhibit the receptors of the immune checkpoint, PD-1/PD-L1, and CTLA-4. It has potential benefits in cancer treatment for long-term survival and antitumor effect production in malignancies of the broad spectrum [42].

Table 1. Drugs used as immune checkpoint inhibitors, their target checkpoints, and approved uses in cancer treatment [43]

Immune Checkpoints Inhibitor Drugs	Target	Uses
Ipilimumab	CTLA-4	Melanoma
Nivolumab	PD-1	renal cell carcinoma, Melanoma, classical Hodgkin lymphoma, NSCLC, urothelial carcinoma.
Atezolizumab	PD-L1	NSCLC, Urothelial carcinoma
Pembrolizumab	PD-1	head and neck squamous cell carcinoma, Melanoma, classical Hodgkin lymphoma, NSCLC, urothelial carcinoma.
Durvalumab	PD-L1	Urothelial carcinoma

* CTLA-4 - Cytotoxic T Lymphocyte Antigen, PD-1 - Programmed Cell Death Protein, PDL-1 - Programmed Cell Death Ligand 1.

Immune checkpoint inhibitors and monoclonal antibodies (mAbs) are becoming the most important immunotherapies, despite the progress of Acceptance and Commitment Therapy (ACT). The immune checkpoint is a coinhibitory signaling pathway molecule that functions as immune tolerance maintenance. Yet they evade immunosurveillance, they can be utilized. To promote the immune-mediated malignant cells elimination and coinhibitory signaling pathways interruption are generated as an antitumor immune response by the ICIs design and functioning. CTLA-4 , PD-1, and PD-L1 are the most frequently used targets for ICIs [44]. The coinhibitory molecule CTLA-4 is expressed on T cells and it functions for negative regulation of activation of T-cells. The reinforcement of antitumor immune responses by the immune cell breaks by utilizing antibodies, regeneration of tumor, and inducement of effective immune responses are demonstrated by the pioneering study of blocking of CTLA-4. CTLA-4 (mAb), ipilimumab was the very first approved and accepted ICI for the treatment of cancer after the efficacy evaluation and several clinical trials. Ipilimumab has induced durable responses and activation of enhanced T-cells. In parallel with that discovery, PD-1 became expressed on T cells surface and it was originally expected for taking part in (PCD) programmed cell death. After that it was found to be act as an immune response negative regulator. The PD-1s regulatory mechanism remained incomprehensive till its ligand (PD-L1) discovery which represents normal tissue and functioning to immune tolerance regulation by suppressing the proliferation of TCR-mediated lymphocytes and secretion of cytokines while binding to the PD-1. However, the tumor cells express the PD-L1 abnormally for immune surveillance escape. As per the study inducement of tumor regeneration and reinvigoration of T cells' cytotoxic ability is due to the PD-1 or PD-L1 inhibition [45]. It suggests that several therapeutic targets can be served by PD-1 or PD-L1. Extraordinary clinical outcomes are achieved by PD-2 pathways blocked. The multiple cancer treatment is done by PD-1 or PD-L1 are antibody targeting has been approved.

Mechanism of immune checkpoints inhibitors in cancer therapy
A brief explanation of the mechanism of Immune checkpoints inhibitors in cancer therapy

The immune checkpoints are a normal component of the immune system, and their purpose is to prevent or to stop immune responses from intensifying to the point that they threaten to kill healthy cells in the body. When the immune cell (T cell) proteins bind to its partner protein (tumor cells) then the immune checkpoints get engaged. When the partner protein and checkpoint proteins bind together, it sends a signal ‘off” to the immune cell (T cell). Destroying cancer from the immune system is prevented by these proteins. One of the drugs CTLA-4 is act against the checkpoint proteins. PD-1 or PD-L1 (partner protein) are the checkpoint inhibitors that act on checkpoint proteins. Production of PD-L1 can turn down the T cell or immune cell responses. Interaction of inhibitory receptors with ligands inhibition results in intratumor CD8+ cells. anti-PD-1/PD-L1 or anti-CTLA-4 results in immune system activation due to tumor immunity reversal inhibition roles [46].

Inhibitory receptors of CTLA-4 or blocked PD-1S axis clinical activities accounted by different complementary mechanisms. ADCC is mediated by only NK cells,

and macrophage Fc receptors like ipilimumab, which is an isotopic antibody. T cells metabolic reprogramming is allowed by the transmission of reverse inhibitory signals by the anti-PD-1 or the anti-CTLA-4 antibodies. Blocking the interaction of PD-L1 results in PD-L1 reverse signaling on tumor cells.

Tumor rejection is hypothesized to be induced by CTLA4 inhibition via a variety of different pathways. The primary mechanism seems to be the direct inhibition of CTLA4 competition for the costimulatory ligands B7-1 and B7-2, that are allowing the unlimited CD28-mediated positive co-stimulation. The investigation of crystallographic structural of the ipilimumab: the complex of CTLA-4 shows that the binding epitope of ipilimumab overlaps with the interaction region of B7, suggesting that the primary mechanism of action of the drug is steric suppression of B7 interactions. By raising both the quantity and functional activity of CD8 T cells, PD-1 blockade can lead to tumour rejection by revitalising these cells [46]. Because the PD-1 signalling axis is inhibited, proximal TCR signalling is not diminished by PD-1, allowing worn-out CD8 effectors to be activated. Therefore, exhausted T cells can be resurrected and mount an effective immune response even if PD-L1 is still present in the tumour microenvironment. Clinical findings show that inhibiting the PD-1 signalling axis is very effective in cancers when an response of endogenous T-cell has already been activated but is blocked by the engagement of PD-1 with its ligands PD-L1 and PD-L2. However, some PD-L1-negative tumour responses imply that an immune response is already in place [47]. T cell activation progress is initiated by the therapeutic intervention of anti–PD-1 and anti-CTLA4 antibody-mediated negative regulation using the normal mechanism of regulation. anti–PD-1 and anti-CTLA4 efficacy is thought to be increased by several complementary mechanisms in addition to the effector function intrinsic enhancements of a cell [47]. It includes T regs depletion, positive co-stimulation of T cell enhancement in the microenvironment mediated by the antibodies further the PD-L1 and B7-1 interactions blockade, and the microenvironment is blocked by signals of host-derived PD-L1 from nontumor cells.

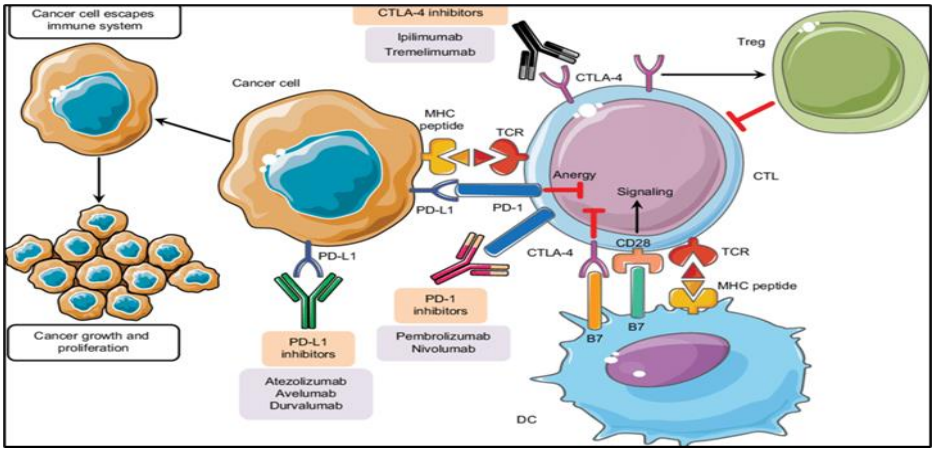

Figure 1. Mechanism of Immune Checkpoints Inhibitors in Cancer Therapy [48]

Immune checkpoint inhibitors include negative effects that can vary from person to person. The adverse effects you may experience and how they affect you will

depend on your pre-treatment health, the type of cancer you have, its stage, the immune checkpoint inhibitor you are using, and the dosage. One of the less frequent negative effects of immune checkpoint inhibitors is widespread inflammation. Symptoms of inflammation can vary depending on the organ of your body that is affected, including changes in skin colour, a rash, and itching in the case of the skin, a cough and chest pains in the case of the lungs, abdominal pain and diarrhoea in the case of the colon, nephritis (inflammation of the kidney) and impaired kidney function, diabetes, which is brought on by pancreatic inflammation, hepatitis (inflammation of the liver), hypophys [48].

Recent advancements in immunotherapy in cancer treatment

Numerous patients with a wide range of solid and hematological malignancies now have better prognoses because of immunotherapy, which has established itself as a key component of cancer treatment. The two main reasons that have contributed to this success are the immune checkpoint inhibitors (CPIs) and the T cell chimeric antigen receptor (CAR). Cancer immunotherapy is a rapidly developing field [49]. The findings of the research on checkpoint blockade that is now being conducted will probably allow CPIs to be used in more patient groups (such as new tumor entities, unique patient populations, and perioperative use,), and they may also help to identify new CPI combo partners. The major challenge for this therapy in the coming years will be the application for solid malignancies of adoptive T-cell therapy. A successful method may include more advanced and effective genetic engineering tools of CAR T cells along with creation of more complex procedures for tumor-reactive (TCR-native) T cells uses. There has been a noticeable shift in clinical trials with respect to regional distribution of immunotherapy [50].

Conclusion

Now there are several treatments available for cancer. The treatment to be given is completely depends on some factors including the stage of cancer, patients' general health, type of cancer, and treatment preferences. The heckpoint inhibitors are immunotherapy drugs that prevent the binding of immune checkpoints to their partner proteins. This blocking allows T cells for killing the cancer cells by preventing the signals from being sent for immunization. To maintain homeostasis several pathways of immune signaling are employed that inhibit or stimulate immune responses. These pathways of immune signaling prevent self-harm and show optimal immune responses against foreign antigens as they are designed for that. These immune signals that regulate immune responses in the body are also known as immune checkpoints. The numerous self-regulating checkpoints responses to the immune system are exploited by the cancerous cells. Immune responses are regulated negatively by manipulating these immune checkpoints. PD-1, and CTLA-4 are the most frequently used targets for ICIs. The coinhibitory molecule CTLA-4 is presented on T cells and it functions for negative regulation of activation of T-cells. CTLA-4 (mAb), ipilimumab became the very first approved ICI for the treatment of cancer after the efficacy evaluation and several clinical trials. Inhibitory receptors of CTLA-4 or blocked PD-1 axis clinical activities accounted by different complementary mechanisms. ICIs are the antibodies that inhibit the receptors of immune

checkpoint, PD-1/PD-L1, and CTLA-4. It has potential benefits in cancer treatment for long-term survival and antitumor effect production in malignancies of a broad spectrum. Numerous patients with a wide range of solid and hematological malignancies now have better prognoses because of immunotherapy, which has established itself as a key component of cancer treatment

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