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Immunopharmacology of Immune Checkpoint Inhibitors: A Comprehensive Review on Mechanisms, Clinical Advances and Emerging Perspectives

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A B S T R A C T

Immunology-based therapies are emerging as an effective cancer treatment, using the body's immune system to target tumors. Immune checkpoints, which regulate immune responses to prevent tissue damage and autoimmunity, are often exploited by cancer cells to avoid destruction. The discovery of checkpoint proteins like PD-1/PD-L1 and CTLA-4 was pivotal in developing cancer immunotherapy. Immune checkpoint inhibitors (ICIs) have shown great success, with FDAapproved drugs like PD-1 inhibitors (Nivolumab, Pembrolizumab, Cemiplimab), PD-L1 inhibitors (Atezolizumab, Durvalumab, Avelumab), and CTLA-4 inhibitors (Ipilimumab, Tremelimumab), alongside LAG-3 inhibitor Relatlimab. Research continues on new checkpoints like TIM-3, VISTA and TIGIT. Despite their effectiveness, not all patients experience the same level of benefit, and organ-specific immune-related adverse events (irAEs) such as diarrhea, alopecia, nausea and anemia may occur. Given the rapid advancements in this field and the variability in patient outcomes, there is an urgent need for a comprehensive review that consolidates the latest findings on immune checkpoint inhibitors, covering their clinical status, biomarkers, resistance mechanisms, strategies to overcome resistance, and associated adverse effects. This review presents a comprehensive overview of recent findings on ICIs, providing insights into their clinical applications, mechanisms of action, resistance strategies, future directions and current challenges with immune checkpoint inhibitors.

Keywords: Immune checkpoint inhibitors; PD-1; CTLA-4; cancer immunotherapy; pharmacology; resistance; biomarkers; adverse events.

Introduction:

A rapidly expanding field of study, cancer immunology examines the interactions between the immune system and cancer cells in an effort to find biomarkers for cancer immunodiagnosis and create novel cancer immunotherapeutic approaches. The immune response, particularly the discovery of antigens specific to cancer, is of particular interest in the field of cancer immunology since it can help advance the development of innovative vaccines and antibody therapies. The immune system's ability to identify antigenic alterations in cancer cells and produce antibodies against these cellular antigens generally referred to as tumor-associated antigens, or TAAs has also been extensively shown. Current developments in cancer immunotherapy scientific and clinical research, as well as the detection and assessment of TAA and anti-TAA biomarkers in cancer immunodiagnosis [1]. Immunocheckpoint inhibitors (ICIs) are a class of cancer treatment that targets tumor cells by utilizing immune system components. Immunotherapy has been a very successful standard treatment for many malignancies, either by itself or in conjunction with other traditional treatments like chemotherapy and radiation. Nevertheless, tumor cells use these inhibitory substances to induce T cell depletion and tumor tolerance. T cell-surface co-inhibitory receptors, cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed cell death 1 (PD-1), adversely control T cell-mediated immune responses. The immune response against tumor cells can therefore be reactivated by ICIs such anti-CTLA-4, anti-PD-1, and antiPD-L1 attaching to these co-inhibitory receptors. The US Food and Drug Administration (FDA) has authorized three distinct classes of ICIs for the treatment of different cancer types: PD-1 inhibitors (Nivolumab, Pembrolizumab, and Cemiplimab), PDL-1 inhibitors (Atezolimumab, Durvalumab, and Avelumab), and CTLA-4 inhibitors (Ipilimumab) [2].

IMMUNE CHECKPOINTS: PHYSIOLOGICAL ROLES AND TUMOR IMMUNE EVASION:

One of the main barriers to the induction of antitumor immune responses has been found to be immunological checkpoints [3]. The majority of immunological checkpoint molecules that have been identified thus far are expressed on innate immune system cells and adaptive immune system cells, especially T cells [4].

The Role of Immune Checkpoints in Immunity and Cancer:

Survival of the host depends on immunological balance. Autoimmune disorders and inflammatory tissue damage can result from overt or uncontrollable immune reactions to infections or mutated/overexpressed self-antigens. Co-stimulatory and inhibitory signals are balanced to control the size and scope of the immune response in order to avoid this. Together, these signals are known as immunological checkpoints, and they are essential for preserving self-tolerance and shielding the host's tissue from harm.

CTLA-4 :

The mechanisms through which CTLA-4 suppresses T cell function are the most well-understood of the immunological checkpoint proteins discovered thus far. A homolog of the immunological co-stimulatory protein CD28, CTLA-4 is a transmembrane glycoprotein.

By inhibiting CD28's function, CTLA-4 plays a crucial part in the development of peripheral tolerance to self proteins. The second signal for T cell activation is sent by CD28 attaching to the CD80 (B7-1) and CD86 (B7-2) proteins on antigen-presenting cells (APCs) following interaction between the T cell receptor (TCR) and the appropriate antigen. Due to its about 20-fold higher affinity for binding these B7 proteins, CTLA-4 can outcompete CD28 for binding.

PD-1/programmed death ligand 1 or 2 (PD-L1 or PD-L2):

PD-1 primarily controls effector T cell activity in tissues and malignancies, in contrast to CTLA-4, which primarily controls T cell activation in lymphoid organs. While naïve T cells are primarily impacted by CTLA-4, mature T cells in peripheral tissues and the tumor microenvironment express PD-1. It is also expressed by B cells, professional APCs, and natural killer (NK) cells, among other non-T cell subsets.

LAG-3:

Over 25 years ago, LAG-3, often referred to as CD223, was cloned as a homolog of CD4. The only known ligand for LAG-3 is the major histocompatibility complex (MHC) class II molecule. LAG-3 plays an important role in suppressing overt T cell immunological responses, just like CTLA-4 and PD-1 do. LAG-3 particularly suppresses the functions of CD8+ effector T cells and can increase Tregs' suppressive activity. When inflammation is present, activated CD4+, CD8+, and NK cells express LAG-3 in vivo on their surface. Additionally, anergic T cells exhibit increased expression of LAG-3, which can be partially reversed by antibody inhibition of LAG-3. Dual inhibition of these receptors caused reversed anergy in a chronic infection situation because PD-1 and LAG-3 are frequently co-expressed on anergic T cells.

T cell immunoglobulin mucin 3 (TIM-3):

In 2002, TIM-3, a marker for CD8+ T cells that produce interferongamma (IFN-γ), was identified. It is a glycoprotein with domains for mucin and extracellular immunoglobulin. Many organs and cells, including the liver, small intestine, thymus, kidney, spleen, lung, muscle, and brain, express TIM-3, including activated T cells. Galectin is the most well-known TIM-3 ligand. Nevertheless, additional ligands have been discovered, including high mobility group box 1 and phosphatidyl serine [5].

APPROVED IMMUNE CHECKPOINT INHIBITORS:

ICIs are cancer immunotherapies that boost anti-cancer immune responses by targeting immunologic receptors on T-lymphocyte surfaces (Table 1).

CTLA-4 inhibitor:

There have been several effective trials on antibodies that block the cytotoxic T cell antigen 4 (CTLA-4), hence enhancing the immune response against the tumor cells. The FDA approved ipilimumab (anti-CTLA-4) in 2011 for use in individuals with clinically advanced melanoma based on data from clinical trials. The first ICI to show encouraging activity in cancer patients was anti-CTLA-4. Actually, the first antibody that the FDA authorized and that was used in cancer patients' regular clinical care was ipilimumab. Research has demonstrated that individuals with advanced cutaneous melanoma benefit from a long-term survival advantage when using this fully human anti-CTLA-4 (IgG1) monoclonal antibody [6]. It is anticipated that ipilimumab has an average half-life of 14.7 days, reaching a steady state after 9 weeks [7].

PD-1 inhibitors:

Nivolumab, an ICI, is used to produce humanized anti-programmed death-1 (PD-1) monoclonal antibodies, which are utilized as a second-line treatment for a number of malignancies, including melanoma, non-small cell lung cancer, renal cancer, Hodgkin's lymphoma, urothelial cancer, and head and neck cancers [8]. A retrospective analysis of immune-related adverse cutaneous responses (irAEs) was conducted in 40 patients with metastatic non-small cell lung cancer (NSCLC) who were taking the anti-PD-1 antibody nivolumab [9].

PD-L1 inhibitors:

There are currently three PD-L1 inhibitors approved by the FDA for a variety of cancers, from Merkel cell carcinoma to non-small cell lung cancer [10]. The Fc-engineered humanized IgG1 monoclonal antibody is called atezolizumab. It binds to PD-ligand 1 (PD-L1) and prevents it from interacting with B7.1 and PD-1 receptors [11]. Avelumab is a completely human IgG1 monoclonal antibody (mAb) that is injected intravenously (i.v.) and prevents T cells' PD-L1 receptors from interacting with antigen-presenting cells, PD-1, and B7.1 [12]. A human IgG1 monoclonal antibody called durvalumab has

a high affinity and selectivity. It prevents programmed death ligand 1 (PD-L1) from attaching to CD80 and programmed death 1 (PD-1), enabling T lymphocytes to identify and eliminate tumor cells. For patients with locally advanced or metastatic urothelial carcinoma who underwent platinum-based chemotherapy, durvalumab was recently licensed in the US. This is due to the drug's fascinating anticancer activity in an early-phase clinical study that included stage IIIB or IV NSCLC and several other advanced solid tumors [13].

Table 1: The list of ICIs with the cancer type indication.

Drug	**Target**	**Approval**	**FDA-Approved Indications**
Nivolumab	PD-1	March 2015	Stage III-B or IV Squamous NSCLC
Pembrolizumab	PD-1	October 2016	Stage IV nonsquamous and squamous NSCLC
Atezolizumab	PD-L1	October 2016	Stage III-B or IV nonsquamous and squamous NSCLC
Cemiplimab	PD-1	September 2018	metastatic cutaneous squamous cell carcinoma
Ipilimumab	CTLA-4	August 2010	stage 3 or 4 malignant melanoma
Avelumab	PD-L1	March 2017	histologically confirmed metastatic Merkel cell carcinoma
Durvalumab	PD-L1	February 2016	Stage III non-small-cell lung cancer (NSCLC)

MECHANISMS OF ACTION:

In the host immune system, tumor cells generate a large number of foreign antigens. The recognition of these tumor antigens is carried out by antigen presentation cells (APCs), just like with viral antigens. Once the foreign antigen has been identified, the APCs move to lymphoid organs and present it to T-cells. The major histocompatibility complex, the T-cell receptor, and additional costimulatory pathways must all be activated for this process to occur. One of the most significant costimulatory pathways is the interaction between the CD80 and CD86 receptors, which are produced on mature APC and, when bound to CD28, activate cytotoxic T-cells to eradicate foreign antigens [14]. In addition to preventing tumor growth, the immune system alters the immunogenicity of tumors. Some tumor cells use various strategies involving antigens, cytokines, and immunological checkpoint proteins to evade the antitumor immune response during this phase. To understand tumor immunology, one must combine the changes in the peripheral immune system with the local immune response in the tumor microenvironment. Since distinct cell types in various tissues govern immunity in cancer, cancer immunotherapies that activate or inhibit this immunity may cause immunological responses that may affect several organs. Monoclonal antibodies that block the regulatory immunological targets CTLA-4, PD-1, and programmed death-1 ligand (PD-L1) are the most studied and well-supported cancer immunotherapies. CTLA4, which is present on the surface of CD4-positive and CD8-positive lymphocytes, binds to T-cell-costimulatory factors on the surface of APC. CTLA-4 binding inhibits the synthesis of interleukin 2 (IL-2) and T-cell proliferation. Immune cell types that express the PD-1 receptor on their surface include T cells, B cells, and NK cells. Tumor cells and other cell types have PD-L1, one of its ligands. It helps to suppress T cells that have already been activated. The authorized ICIs include anti-PD1 antibodies (pembrolizumab, nivolumab, cemiplimab), anti-PD-L1 antibodies (atezolizumab, avelumab, and durvalumab), and anti-CTLA4 antibodies (ipilimumab, tremelimumab) [15].

CLINICAL APPLICATIONS AND EFFICACY:

In solid tumors, ICIs have been created quickly, but they are successful in hematological malignancies, which have advanced quickly in recent years. However, because of the infancy of the technology and other factors like resistance and side effects, there are still a lot of undesirable clinical issues with the use of ICIs. More attention has been paid in recent years to studying immunological checkpoints, such as biomarkers, ICI combination treatments, resistance, and toxicities [16]. In conclusion, immunotherapy with chemotherapy or immunotherapy plus chemotherapy produced a notable improvement in OS. Front-line metastatic non-small cell lung cancer (NSCLC) without molecular changes is now treated with pembrolizumab, atezolizumab, cemiplimab, nivolumab plus ipilimumab for PD-L1 ≥50%, pembrolizumab plus chemotherapy, ABCP regimen, and nivolumab plus ipilimumab for PD-L1 ≥1%–49%.Additionally chemoimmunotherapy is a viable choice for individuals with high volume tumors and PD-L1 ≥1%– 49% [17].

IMMUNE-RELATED ADVERSE EVENTS (irAEs):

Common organ systems affected:

ICIs can have toxic effects on any organ because they cause autoreactive T cells to activate and damage host tissues [18]. Numerous organ systems, including the neurological, gastrointestinal, dermatological, cardiovascular, rheumatologic, and endocrine systems, have been observed to be impacted by these chronic adverse events [19]. All 543 (100%) of the patients in the pembrolizumab group and 547 out of 549 (100%) of the participants in the placebo group suffered adverse events. 386 (71%) and 348 (63%) individuals in the pembrolizumab and placebo groups, respectively, experienced grade

3 adverse events (Table 2). Anemia [pembrolizumab group, 284 patients (52%); placebo group, 283 patients (52%)], nausea [pembrolizumab group, 239 patients (44%); placebo group, 264 patients (48%)], diarrhea [pembrolizumab group, 219 patients (40%); placebo group, 207 patients (38%)], and alopecia [pembrolizumab group, 341 patients (63%); placebo group, 355 patients (65%)] were the most common adverse events (40%) in both treatment groups (Table 2). AEs in the safety population. Six (1%) patients in the placebo group and 14 (3%) patients in the pembrolizumab group died as a result of adverse events (AEs); the investigators did not believe that any of the deaths were connected to treatment. AEs caused 129 (24%) patients in the pembrolizumab group and 90 (16%) patients in the placebo group to stop taking any study medication, and 63 (12%) and 14 (3%) patients, respectively, to stop taking pembrolizumab and placebo [20].

Table 2. AEs in the safety population

AE	Pembrolizumab chemotherapy (n 543) D	Placebo D chemotherapy (n 549)
Any AE	543 (100)	547 (100)
Grade 3-5	386 (71)	348 (63)
Led to discontinuation of any treatment	129 (24)	90 (16)
Pembrolizumab or placebo	63 (12)	14 (3)
Carboplatin	35 (6)	21 (4)
Cisplatin	19 (3)	14 (3)
Paclitaxel	74 (14)	63 (11)
Docetaxel	2 (<1)	5 (<1)
Radiotherapy	7 (1)	3 (<1)
Led to death	14 (3)	6 (1)

Management of irAEs:

Corticosteroids and other immunomodulatory drugs are the cornerstone of treatment to reduce the risk of both short-term and long-term effects. Careful management of irAEs is necessary to optimize quality of life and long-term outcomes [21].

Early identification:

Although there is a growing amount of research and use of ICIs, only a small percentage of patients with a limited number of tumor types respond to and benefit from ICI treatment, resulting in needless side effects and resource loss. As a result, there is much debate on whether it is possible to differentiate between intolerance and ICI recipients. If identified early, the majority of irAEs can be reversed. Certain biomarkers can assist identify the target populations and forecast the positive and negative outcomes of ICI therapy, allowing for the early implementation of preventative measures to lessen immune-related harm.

Interventions:

The way irAEs are managed is comparable to that of autoimmune disorders, and more especially, to the way exacerbations of autoimmune diseases are treated. The mainstays of irAE care include immunomodulators, steroids, and immune-oncologic (IO) cessation; a variety of immunesuppressing drugs may also be employed. For certain especially severe irAEs, a number of immunomodulating medications that are frequently used in rheumatism are available. The intensity and subtype of the irAE that is displayed determine the precise strategy and dosage [22].

Personalized treatment plans:

Since each patient may experience a unique range of toxicities with a questioned risk-benefit ratio of continuing immunotherapy, the development of individualized treatment strategies is crucial in the management of iRAEs. Treatment continuance or modification must be evaluated in light of the patient's age, comorbidities, and adverse reaction history. Second, decision-making must take into account the patients' desire to continue therapy as well as the potential for long-term repercussions. This guarantees individualized care based on the patient's values while also controlling side effects.

Multidisciplinary approach:

Given the several organ systems that may be involved, managing irAEs necessitates a multidisciplinary strategy involving oncologists, immunologists, rheumatologists, and nephrologists. The management of adverse events would be comprehensive in this interdisciplinary endeavor, including input from a range of professionals regarding the best course of action for each patient. Since certain toxicities are persistent, preventing problems through holistic

treatment includes communication and coordinated care. All things considered, immunotherapy adverse events require prompt responses to acute toxicities, long-term monitoring in situations of chronic toxicities, and customized therapies based on patient-centered and clinical considerations [23].

ICIS RESISTANCE MECHANISMS:

ICI resistance is multifactorial and can be either primary (no initial reaction) or acquired (progression after initial response). As previously stated, the anticancer effects of ICIs depend on the process of T-cell activation. Although there appears to be some overlap, the T-cell activation process in situations of resistance is inhibited by a variety of factors and can be divided into three primary pathways. Steps 1–6 are inhibited by the first group, "resistance mechanisms related to antigen recognition," whereas steps 4 and 5 are inhibited by the second category, "resistance mechanisms related to T-cell migration and/or infiltration." Step 7 is inhibited by the third category, which is "resistance mechanisms related to effector functions of T cells." We shall discuss these resistance mechanisms collectively because they can cause both initial and developed resistance [24].

Resistance mechanisms intrinsic to tumors:

"Immune checkpoint blockade" for cancer refers to the use of therapeutic antibodies that disrupt negative immune regulatory checkpoints and trigger preexisting anticancer immune responses. The US Food and Drug Administration has licensed antibodies that target the checkpoint molecules cytotoxic T lymphocyte antigen 4 (CTLA4), programmed cell death 1 (PD1), and PD1 ligand 1 (PD-L1) for treatment in various cancer types due to their early clinical success. However, the techniques available to clinicians to determine which patients will react to treatment and which will not are still quite restricted. Interferon signaling and antigen presentation, two biological processes essential to antitumor immunity, have been rediscoverable as a result of the surge in research into the molecular basis underlying tumor-intrinsic resistance to immune checkpoint blockage. Other efforts have clarified the immunological implications of canonical cancer signaling pathways, such as cell cycle regulatory signaling, mitogen-activated protein kinase signaling, WNT–β-catenin signaling, and pathways triggered by loss of the tumor suppressor phosphoinositide phosphatase PTEN [25].

Mechanisms of tumor extrinsic resistance:

The TME is diverse and includes extracellular matrix, stromal cells, immunological cells, malignant cells, and vasculature. The intricate multifaceted interactions among these various elements have been shown to be crucial in shaping the immunosuppressive TME, influencing the anti-tumor immune responses, and influencing the susceptibility to cancer treatment. Apart from the previously identified tumor intrinsic mechanisms, immunological and non-immunological pathways might cause resistance to ICIs outside of tumor cells [26].

BIOMARKERS FOR RESPONSE AND TOXICITY PREDICTION:

PD-L1 expression: In a variety of malignancies, PD-L1 is an essential biomarker for anti-PD1/PD-L1 therapy. The most common technique for evaluating PD-L1 in patients is immunohistochemical (IHC) labeling. Algorithms for PD-L1 detection scoring are determined by the cell type, location, frequency, and intensity of PD-L1 expression. Three scoring methods are utilized in clinical settings: ICs (immune cells), CPS (combination positive score), and TPS (tumor proportion score, often known as TC for tumor cells). TPS is the percentage of viable tumor cells that exhibit partial or complete membrane staining relative to all viable tumor cells, as assessed by positive or negative PD-L1 staining. TC is the proportion of PD-L1-stained tumor cells. The proportion of PD-L1-positive cells (tumor cells, lymphocytes, and macrophages) to all viable tumor cells is known as CPS. IC is the percentage of the tumor region that is made up of immune cells that are PD-L1 positive, regardless of intensity. The significance of PD-L1 staining varies depending on the type of malignancy. 15, 16 Pembrolizumab, for instance, is approved for stage III non-small cell lung cancer (NSCLC) that is limited to the chest, unsuitable for surgery or chemoradiation, and for advanced NSCLC that has spread. Considerable research indicates that survival results in advanced/metastatic non-small cell lung cancer are correlated with tumor PDL1 expression.

MSI-H/dMMR: Microsatellites are tandem-spreading, repeating DNA sequences with one to six nucleotides that are found throughout the genome. Normally, the DNA mismatch repair (MMR) mechanism (Figure 3) corrects base mismatches caused by DNA polymerases during replication or as a result of DNA damage, preserving DNA integrity and microsatellite length. Microsatellite instability (MSI) is caused by differences in microsatellite length that occur when dMMR is present, as opposed to matched normal DNA. A phenotypic indicator of dMMR is the existence of MSI. The ORR with pembrolizumab was 33.3% for a variety of advanced MSI-H/dMMR solid tumors [27].

Tumor Mutational Burden (TMB): TMB's analytical and clinical usefulness as a biomarker was first examined and confirmed in a number of retrospective studies that demonstrated the potential ability of a large number of mutations or neoantigens to predict the effectiveness of ICIs. The initial research that backed up this theory was released by Rizvi et al. and Snyder et al. Better levels of exonic non-synonymous mutations were linked to better response rates in patients treated with anti-CTLA-4 for melanoma and anti-PD-1 for non-small cell lung cancer, according to these studies. In a variety of cancer types and regardless of the ICI, TMB shows promise as a predictive biomarker of ICI efficacy [28].

Circulating biomarkers: Circulating biomarkers are particularly appealing surrogates for these uses, and they offer several practical benefits. Circulating tumor cells (CTCs) were first identified in 1869 as an uncommon side effect of solid cancers called "carcinocythemia." Since then, their presence in solid malignancies has become more common. According to recent research, CTCs have been assessed as a correlate in therapeutic trials where, predictably, their continued use of medicine consistently results in an unfavorable prognosis. Unbound nucleic acids that circulate, such as cell-free DNA (cfDNA) and RNA (cfRNA), offer an alluring substitute for CTCs. In 1948, circulating nucleic acids were identified, and it was later determined that cancer patients had higher levels of these molecules [29].

COMBINATION THERAPIES:

ICI + ICI: In individuals with metastatic melanoma, combination therapy with nivolumab and ipilimumab has consistently demonstrated success. In a phase 3 trial, nivolumab plus ipilimumab gave patients with advanced melanoma a longer progression-free survival and a greater objective response rate than ipilimumab alone [30].

ICI + chemotherapy: Chemotherapy can promote antigen release and inflammatory signaling; examples include pembrolizumab plus chemotherapy in metastatic NSCLC improving survival [31].

ICI + targeted therapy: In patients with advanced clear-cell renal-cell carcinoma who had not received treatment before, the anti-programmed death 1 (PD-1) monoclonal antibody pembrolizumab and the VEGF receptor tyrosine kinase inhibitor axitinib have both demonstrated anticancer efficacy [32].

ICI + radiotherapy: Since radiation and immune checkpoint inhibitor therapy complement each other mechanistically, they may work well together to treat "cold" tumors [33].

NOVEL AND EMERGING IMMUNE CHECKPOINTS:

LAG-3: The structure of the type I transmembrane protein LAG-3 is similar to that of CD4. There is growing evidence that LAG-3 is an inhibitory coreceptor that is essential for anti-infection immunity, tumor immunity, and autoimmune [34]. The combination of nivolumab, a PD-1blocking antibody, and relatlimab, a LAG-3-blocking antibody, has demonstrated antitumor activity and safety in patients with melanoma that has already received treatment; however, further research is required to determine the safety and efficacy in patients with melanoma that has not yet received treatment [35].

TIGIT: Recently, T cell immunoglobulin and ITIM domain (TIGIT), an inhibitory receptor produced by lymphocytes, has drawn interest as a promising novel target for cancer immunotherapy. Through its interaction with CD155, which is expressed on antigen-presenting cells or tumor cells, TIGIT suppresses the activities of T lymphocytes and natural killer (NK) cells. One important inhibitor of antitumor responses that can impede several stages of the cancer immunity cycle is TIGIT. Preclinical studies suggest that TIGIT blocking may provide protection against a variety of hematological and solid cancers [36]. T cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT) is a promising new target for cancer immunotherapy. TIGIT is upregulated by immune cells, including activated T cells, natural killer cells, and regulatory T cells. TIGIT interacts to two ligands expressed by tumor cells and antigen-presenting cells in the tumor microenvironment: CD115 (PVR) and CD112 (PVRL2, nectin-2). The TIGIT pathway is now well established to control tumor detection in vivo and in vitro by T cells and natural killer cells. In vitro, dual PD1/TIGIT inhibition significantly boosts the growth and activity of CD8+ T cells specific to tumor antigens, and in mice tumor models, it encourages tumor rejection [37]. Pre-clinical and clinical research are now being conducted extensively to examine the effectiveness of antibody inhibition of TIGIT in cancer immunotherapy. TIGIT's primary ligand, CD155, is expressed on tumorinfiltrating myeloid cells and increased on cancer cells, which helps to locally reduce immune surveillance. TIGIT is expressed on NK cells, helper T cells, regulatory T cells, and tumor-infiltrating cytotoxic T cells in a range of cancers. Preclinical research shows that co-blockade of the TIGIT and PD-1/PD-L1 pathway results in tumor rejection, especially in anti-PD-1 resistant tumor models, despite the low anti-tumor efficacy of solo TIGIT blockade [38].

TIM-3: In cancer patients, Tim-3 is also present on T cells. About 30% of CD8+ T lymphocytes specific to NY-ESO-1 in patients with metastatic melanoma express Tim-3 [39]. Tim-3 is expressed by approximately one-third of CD8+ tumor-infiltrating lymphocytes (TIL) in non-small cell lung cancer (NSCLC) patients [40]. About one-third of CD4+ and CD8+ T cells in lymph nodes of patients with follicular B-cell non-Hodgkin lymphoma express Tim-3. All of the Tim-3+ T cells in these three malignancies coexpress PD-1 and show abnormalities in effector cytokine production and proliferation. Accordingly, Tim-3 identifies faulty T cells in a variety of cancer types in both humans and experimental settings [41]. Preclinical tumor models and cancer patients' CD8+Tim3+ T cells both exhibit a dysfunctional phenotype that most closely mimics the worn-out or dysfunctional CD8+ T cells that have been linked to persistent viral infections [42]. A distinct immune checkpoint receptor in cancer has been identified as Tim-3. Its preferential expression in tumor tissue and involvement in several immunosuppressive pathways make it a valuable target for cancer immunotherapy. Clarifying the signals that underpin Tim-3-driven immune suppression, the extracellular and intracellular signals that drive Tim-3 upregulation, and the receptor–ligand relationships that are involved in each immune-suppressive mechanism will be crucial next steps in using Tim-3 for anticancer immunotherapy. Together with growing preclinical evidence, Tim3's special characteristics provide compelling evidence for the use of Tim-3-targeted treatments in combination with other checkpoint-based therapies to produce objective responses in a greater number of patients and in cancers that have so far shown resistance to CTLA-4 or PD-1 blockade [43].

VISTA: In several malignancies, V-set immunoglobulin domain suppressor of T cell activation (VISTA), a new immunoregulatory receptor widely expressed on myeloid and lymphoid cells, is often linked to poor prognosis. VISTA antibody targeting is significant because it specifically activates both innate and adaptive immunity. Because of this, as well as because VISTA is expressed and exhibits non-redundant functions in comparison to other immune checkpoint regulators, it is a prospective target for advanced cancer immunotherapy [44]**.** On the other hand, V-domain immunoglobulin (Ig) suppressor of T-cell activation (VISTA), a similar immunological checkpoint protein, plays a homeostatic role in controlling the steady state of the system's lymphoid and myeloid lineages. VISTA is also referred to as Dies1, DD1a, Gi24, VSIR, SISP1, B7-H5, PD-1H, and c10orf54. Although VISTA shares a 25% protein sequence with PD-L1 and is comparable to the B7 family of ligands and receptors like the CD28 family, it has distinct structural characteristics, expression patterns, and activities. VISTA functions as a receptor, a ligand, or both, and its expression on immune or tumor cells may have a major impact on antitumor immunity and tumor progression. VISTA was found to be a negative regulatory receptor on T cells in early research

by Chen and associates. Ligands such as PSGL-1 (at low pH), VSIG-3, Galectin-9, LRIG1, and Syndecan-2 have been found to be possible VISTA binding partners [45].

FUTURE DIRECTIONS:

Implementation of next-generation sequencing technologies:

Caner has unstable genomes. New bioinformatics techniques are required because of the characteristics of the cancer sequencing data, which include altered ploidy, heterogeneity, and normal contamination. New information about the molecular mechanisms within cancer cells can be obtained by next-generation sequencing (NGS). throughout addition to assessing the expression of genes and transcripts and detecting alternative splicing, it has enabled the discovery of insertions, deletions, amplifications, interchromosomal rearrangements, and single nucleotide variations (SNV) throughout the whole genome and transcriptome [46].

Biomarker-driven clinical trials:

Tumors will have somatic mutations during the course of cancer growth, and cells that acquire specific mutations have an advantage in survival and will displace cells without these genomic changes to dominate discrete tumor areas. All cancer metastases are dominated by driver mutations, and subclonal mutations are undoubtedly impacted by the heterogeneity. Clonal mutations and tumor heterogeneity, including intra- and inter-tumor heterogeneity, are the main barriers to personalized cancer treatment. Therefore, recurrent biopsies at progression and biomarker-driven personalized therapy are needed to uncover resistance mechanisms and their potential targeted suppression [47–51].

Combinational immunotherapy:

Combining several immune checkpoint inhibitors, such anti-CTLA-4 and anti-PD-1, has demonstrated improved efficacy; the main challenges are figuring out the best combinations and administering the right dosage. Furthermore, it may be possible to combine immunotherapy with other forms of treatment such radiation therapy, chemotherapy, and targeted therapies. Initial data suggested that combining immunotherapy with other forms of therapy would have encouraging synergistic effects [52-54].

ICT'S CURRENT CHALLENGES:

Understanding the primary issues limiting the effectiveness of ICT is crucial for expanding its therapeutic utility. Developing multifactorial and complex irAEs and resistance to immune checkpoint inhibitors are two of the major issues with ICT.

Resistance toward ICT:

The Society for Immunotherapy of Cancer (SITC) Immunotherapy Resistance Taskforce has established three consensus definitions of ICT resistance observed in the clinic based on response kinetics: primary resistance observed in nonresponders, secondary resistance that emerges following a period of response, and third, progression following treatment discontinuation. TME-associated extrinsic factors and intrinsic factors of tumor cells are both biologically responsible for ICT resistance. An overview of the intrinsic and extrinsic factors of tumor cells that may be used to overcome ICT resistance is given in this section.

Intrinsic factors of tumor cells:

The lack of immunogenicity brought on by tumor-specific genetic changes, chromatin remodeling, and oncogenic signaling are among of the intrinsic characteristics of tumor cells that contribute to ICT resistance. While some of these elements may develop over time and result in adaptive resistance to ICT, others may be present at the time of initial presentation and cause de-novo resistance to ICT.

Antigen presentation machinery flaws:

Cancer cells that undergo genetic and epigenetic changes that result in a loss of major histocompatibility complex (MHC)-I, including monoallelic loss, may process and present antigens differently, making it more difficult for T cells to recognize them. Truncating mutations or homozygous loss of beta 2 microglobulin (B2M) in ICT-resistant melanoma and non-small cell lung cancer (NSCLC) restrict T cell-mediated anti-tumor immunity by causing a decrease of MHCI expression on the cell surface. Additionally, deficits in CD8 T cell recognition are caused by transcriptional suppression of the human leukocyte antigen (HLA) class I antigen processing and antigen presentation machinery, which is correlated with the patient's response to ICT.

Genetic changes that converge on the signaling pathway of IFNg:

When IFNg attaches to its receptors, the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway is triggered. This process alters the transcription of genes controlled by IFNg, ultimately leading to T cell activation. Approximately 75% of melanoma patients who did not react to CTLA-4 inhibition displayed copy number changes, including amplification of significant IFNg pathway inhibitors and loss of copy number in the main IFNg pathway genes (compared to 0% in responders). Primary and acquired resistance to ICT in melanoma has also been linked to loss-of-function mutations in the kinase regions of JAK1 and JAK2.

Insufficient neoantigens:

Tumor neoantigens are produced by tumor-specific changes and trigger cytotoxic reactions. Hypermutator phenotypes that are susceptible to ICTs can result from mutations in the mismatch repair machinery and other DNA repair mechanisms. The main factors influencing the ICT impact in these malignancies are the frequency and relative strength of T cell responses to these neoantigens. Low TMB tumors are not immunogenic and do not contain neoantigens. But even in these, the quality of the neoantigen affects patient survival. Prostate cancer patients with low TMB reacted to CTLA-4 inhibition if they had a high intratumoral CD8 density, a strong IFNg gene signature, and antigen-specific T cell responses. There are currently initiatives to integrate individualized neoantigen vaccinations with ICTs.

Pathways for oncogenic signaling:

Immune escape and the maintenance of an immunosuppressive TME are facilitated by tumor intrinsic signaling pathways. Wnt, PTEN/PI3K, and rat sarcoma virus (RAS) are among the pathways that have been identified in many tumor forms as aiding immune exclusion and dysfunction as well as the recruitment and development of immunosuppressive cells. T cell exclusion in bladder, head and neck, gastric, renal, and other malignancies has been linked to Wnt signaling. Interestingly, PD-L1 expression or the expression of class I MHC molecules did not correlate with PTEN status, indicating alternative pathways of immune evasion in the context of PTEN loss, including PI3K signaling. PTEN loss has been linked to a decrease in tumor-infiltrating T cells and a lack of responses to ICT in melanoma patients, PI3K inhibitors are presently undergoing clinical studies after improving responses to ICT in mouse models. Lastly, abnormal RAS signaling promotes immunosuppression by upregulating PD-L1 expression.

Reprogramming epigenetics:

It has also been demonstrated that epigenetic reprogramming, a crucial enabling feature in tumor development and progression, results in ICT resistance. Enhancer of zeste homolog 2 (EZH2)mediated histone modification and DNA methyltransferase1 (DNMT1)-mediated epigenetic silencing of IFNg in tumor cells decreases Th1-type chemokines to inhibit effector T cell homing to TME in in-vivo models and is linked to a decrease in tumor-infiltrating CD8 T cells and patient outcomes in ovarian cancers**.** The impact of methylation status on ICT responses was demonstrated in a study of global methylation patterns of TILs in melanoma. DNA hypermethylation at CpG sites was linked to low PD-L1 expression, which limited ICT efficacy by inhibiting anti-tumor interferon responses, while hypomethylation was demonstrated to promote the expression of PDL1 and other inhibitory cytokines and checkpoints, which contributed to immune evasion.

Factors originating from the extracellular matrix and stroma:

A key role in tumor formation and treatment resistance is played by the coordinated interactions between tumor and immune cells and the stromal cells, vasculature, and extracellular matrix in the microenvironment. Resistance may result from aberrant intratumoral angiogenesis that prevents ICIs from penetrating as well as they should. Furthermore, there is mounting evidence that the very diverse cell population known as cancer-associated fibroblasts (CAFs) contributes to ICT resistance through T cell exclusion, which makes them attractive targets for therapy. Nevertheless, it is still difficult to target CAFs to change the immune milieu, patient benefit from therapeutic treatment has been minimal, and early clinical trials have failed [55].

CONCLUSION:

This comprehensive review consolidates recent findings on ICIs, providing insights into their clinical applications, mechanisms of action, resistance strategies, future directions and current challenges with immune checkpoint inhibitors. There is a great demand for a current resource to direct medical professionals due to the continuous research into novel immune targets and combination medicines. As ICIs develop, they have the potential to revolutionize cancer treatment and open up new therapeutic options for patients everywhere. In a variety of cancers, PD-1, PDL1, and CTLA-4 inhibitors have considerably increased OS and extended PFS. Notwithstanding these achievements, primary and acquired resistance still present a significant therapeutic issue, prompting researchers and medical professionals to investigate other combination tactics in an effort to increase their efficacy. Furthermore, biomarkers like MSI, TMB, and PD-L1 expression are being employed more and more to forecast the effectiveness of ICIs. Additionally, new immune checkpoints including TIGIT, TIM-3, and LAG-3 are being researched to get beyond ICI resistance and increase the uses of immunotherapy. Additionally, ICI is still a mainstay of contemporary and sophisticated oncology; however, improving patient survival and developing the next generation of cancer immunotherapy will require overcoming resistance and limitations through creative combination therapies, such as precision immunotherapy and new immunemodulating techniques. The development of ICI-specific follow-up models for cancer survivors should be prioritized, with a particular emphasis on a multidisciplinary approach to patient care.

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