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Mechanisms and Clinical Implications of Immune Checkpoint Inhibitors PD-1, CTLA-4, and TIM-3 in Cancer

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Abstract

Cancer poses a significant health challenge due to its complex nature and the limitations of current treatments. Over the last decade, emerging research has identified that modulation of immune checkpoint molecules such as CTLA-4, PD-1, and TIM-3 can have potential therapeutic benefits. These molecules are critical regulators of the immune system's ability to target and eliminate cancer cells. This review explores the roles of these immune checkpoints in cancer and hematological malignancies; examining their mechanisms of action and the potential they hold for enhancing therapeutic outcomes. We highlight recent advancements in immunotherapy that leverage these checkpoints within the tumor microenvironment, demonstrating their potential to improve patient outcomes. Additionally, we discuss therapeutic metrics like objective response rates and treatment related adverse events in therapeutic combinations and in monotherapies; providing a comprehensive overview of the potential benefits, risks, and future directions in immunotherapy.

Keywords: Immune checkpoints, Immunotherapy, PD-1, CTLA-4, TIM-3, Cancer

Introduction

Cancer is the second-leading cause of death in the United States, and with an estimated 2 million new cases along with 600 thousand deaths projected in 2024, the urgency to understand its pathology and find effective treatments for patients has never been more apparent [1,2]. Furthermore, hematological malignancies are a leading cause of global tumor burdens, driven not only by the rapidly aging population but also by advances in innovative technologies, such as next-generation sequencing, which can lead to increased reported incidence. Additionally, over time, the criteria for diagnosing and classifying these malignancies has evolved, contributing to the rising incidence rates [2,3]. For decades, the standard of care was limited to surgery, chemotherapy, and radiation therapy, either as single treatments or in combination [4]. Though in recent history, research on cancer pathways and pathology has led to novel approaches to cancer treatment. A finely tuned immune response is crucial in cancer treatment; an excessively robust response risks autoimmunity, while an inadequate one offers minimal therapeutic benefit against cancer [5]. The mechanisms by which the immune system is stimulated or inhibited involve two kinds of immune checkpoint proteins, commonly known as co-stimulatory and inhibitory molecules. Stimulatory checkpoint molecules facilitate the activation and propagation of T cell mediated immune responses, whereas inhibitory molecules promote the inhibition of that immune response. The inhibition of inhibitory immune checkpoint molecules has shown clinical efficacy in many tumors and autoimmune diseases, including melanoma, non-small cell lung cancer, systemic lupus erythematosus, and type 1 diabetes [5]. In this review, we will outline the role of immune checkpoint molecules in cancer, with a specific focus on their mechanisms and clinical implications.

PD-1/PD-L1

Overview

CD279, also known as Programmed Cell Death 1 (PD-1), is a 55-kDa transmembrane inhibitory receptor primarily expressed on activated T cells across conditions including acute and chronic infection, cancer, and autoimmunity [5-7]. The expression of PD-1 is highly conserved in upstream regulatory regions (CR-B/CR-C) of the PD1 gene, *PDCD1*; these regions are involved in regulatory functions that influence PD-1 expression levels [6,7,9,10]. The associated proteins with this receptor include Programmed Cell Death Ligand 1 and Ligand 2 (PD-L1, PD-L2) [6-8]. The spatial discrepancy of the expression of these ligands is that PD-L1 is widely expressed in both hematopoietic and non-hematopoietic cells, whereas PD-L2 expression is exclusive to antigen presenting cells (APCs) **(Table 1)**. Furthermore, the expression of both ligands can be induced by inflammatory signals within the tumor microenvironment [5].

Mechanism

To understand the mechanism by which PD-1 modulates immune response, it is first imperative to identify the mechanisms that regulate the expression of the *PDCD1* gene [8]. The transcription of PD-1 in $CD4^+$ and $CD8^+$ T cells is regulated by various transcription factors, including NFAT (Nuclear Factor of Activated T cells), which is crucial for T cell activation and IRF9 (Interferon Regulatory Factor 9), which plays a role in cancer by regulating immune responses against tumors [6,8,10,11]. The involvement of these, and other transcription factors varies depending on whether the T cell is in a naïve or an exhausted state. In naïve T cells, which are mature but have not yet encountered their specific antigen, the stimulation of their T cell receptor (TCR) upon antigen recognition, combined with intracellular calcium circulation enhances the activity of NFAT, which then translocates to the nucleus to bind to the conserved promoter region of the *PDCD1* gene, ultimately promoting PD-1 transcription **(Figure 1)** [6,8,11,22]. Similarly, in exhausted T cells, which

Table 1. Cell expression of the key immune checkpoints (IC) and their ligands.

Immune Checkpoints (IC)	IC Ligands	IC Expression	Ligand Expression
PD-1	PD-L1/L2 [5,8,25]	$CD4^+$/$CD8^+$ T cells [5,8,25]	PD-L1: hematopoietic and non-hematopoietic cells PD-L2: Antigen Presenting Cells (APCs) [5,8,25]
CTLA-4	CD80/CD86 [5,24,25]	Tregs/Memory T cells [5,24,25]	APCs CD80: constitutive/ CD86: inducible [5,24,25]
TIM-3	Gal-9/Cecam1/HMGB1/PtdSer [5,34-36]	T cells/NK cells/APCs [5,34-36]	Gal-9: Tregs/APCs HMGB1: secreted in cellular stress Cecam1: $CD4^+$/$CD8^+$ T cells PtdSer: apoptotic cells [5,34-36]

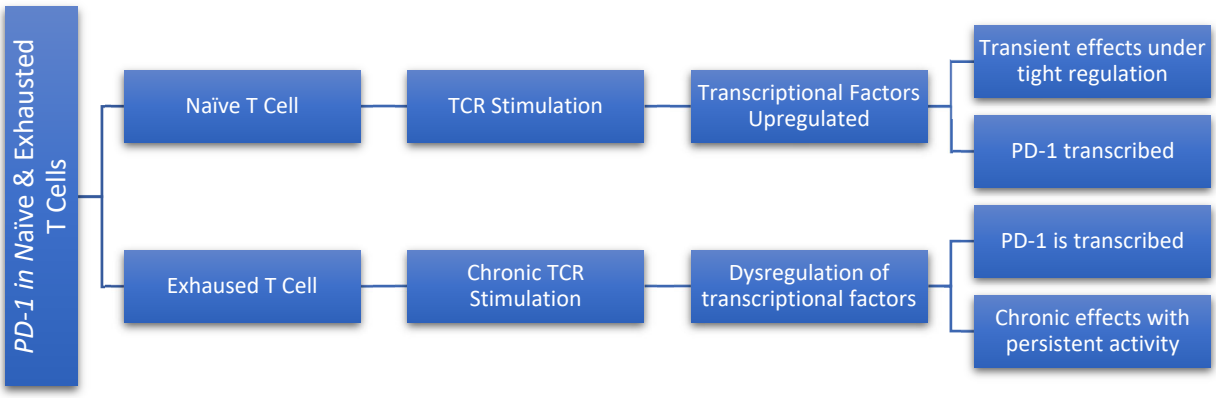

Figure 1. This figure simplifies the differences in PD-1 transcription between naive and exhausted T cells. In naive T cells, TCR stimulation leads to upregulation of transcriptional factors, resulting in transient PD-1 expression under tight regulation. However, in exhausted T cells, chronic TCR stimulation causes dysregulation of transcriptional factors, leading to persistent PD-1 expression and chronic inhibitory effects due to sustained activity.

have undergone chronic stimulation of their TCR due to persistent exposure of their specific antigen, intracellular calcium circulation is necessary, but interferon alpha (IFNα), a cytokine with anti-tumor properties, along with IRF9 binding to the promoter region of the *PDCD1* gene promotes PD-1 transcription **(Figure 1)** [8,11]. Despite the differences in signal transduction molecules associated with *PDCD1* gene expression across various T cell states, the core function of PD-1 remains consistent. The overarching function of the PD-1/PD-L1 pathway is to facilitate a balance of immune homeostasis by inhibiting immune responses and thus facilitating peripheral tolerance. The binding of PD-1 to its associated ligands inhibits TCR signaling, while also downregulating the expression of antiapoptotic molecules and proinflammatory cytokines; ultimately, this binding initiates the negative regulation of activated T cells, subsequently, promoting their inactivation [611,22]. To further understand the mechanism, it is critical that we examine the effects of the absence of PD-1 and PD-L1. Genetic ablation studies have shown that PD-1 receptor deficiencies are linked to various autoimmune phenotypes in murine models, highlighting the regulatory role of PD-1 in suppressing T cell response [23]. Similarly, it was illustrated that PD-L1 deficient mice were prone to autoimmune disease, suggesting that PD-L1 is a critical ligand for T cell immunotolerance.

CTLA-4

Overview

CD152, also known as Cytotoxic T Lymphocyte-associated antigen-4 (CTLA-4), is a transmembrane inhibitory glycoprotein mainly expressed upon activation of regulatory T cells and memory T cells [12,24]. The transcriptional regulation of CTLA-4 remains largely unexplored; however, it is known that CTLA-4 activation relies on NFAT activity. Consequently, modulation of NFAT levels directly impacts CTLA-4 expression [24,25]. CTLA-4 is a co-inhibitory molecule that negatively regulates T cell responses. It is a homolog to CD28, a co-stimulatory molecule that functions to positively regulate T cell responses. Consequently, these two molecules competitively bind the same proteins, CD80 and CD86 [12,25]. The expression patterns of these ligands differ in that CD80 is constitutively expressed on APCs, whereas CD86 exhibits inducible expression on APCs **(Table 1** and **Figure 2)** [13,14,25]. With this in mind, we will examine the mechanism through which CTLA-4 exerts negative regulation on T cells and explore how tumor cells exploit this process to evade immune responses.

Mechanism

As previously mentioned, CTLA-4 competes with CD28 for binding of CD80 and CD86. There are two potential mechanisms by which T cells may be inhibited by CTLA-4: through competitive antagonism and direct negative signaling. It is widely accepted that these mechanisms operate in conjunction rather than exclusively [12,13,25]. Competitive antagonism, as it relates to CTLA-4 and CD28, is characterized by the overwhelming avidity that CTLA-4 has for CD80/CD86; this affinity promotes the sequestration of CD80/CD86 molecules to the receptor of CTLA-4, thereby reducing the available co-stimulatory molecules for CD28 [14,15,25]. Inherently, one could understand how this molecular dynamic acts as an evolutionary safeguard against autoimmunity. However, this mechanism is dependent on a higher ratio of expression of CTLA-4:CD28 on T cell surfaces [25]. In the absence of genetic alterations affecting the CTLA-4 phenotype, the second mechanism involves direct negative

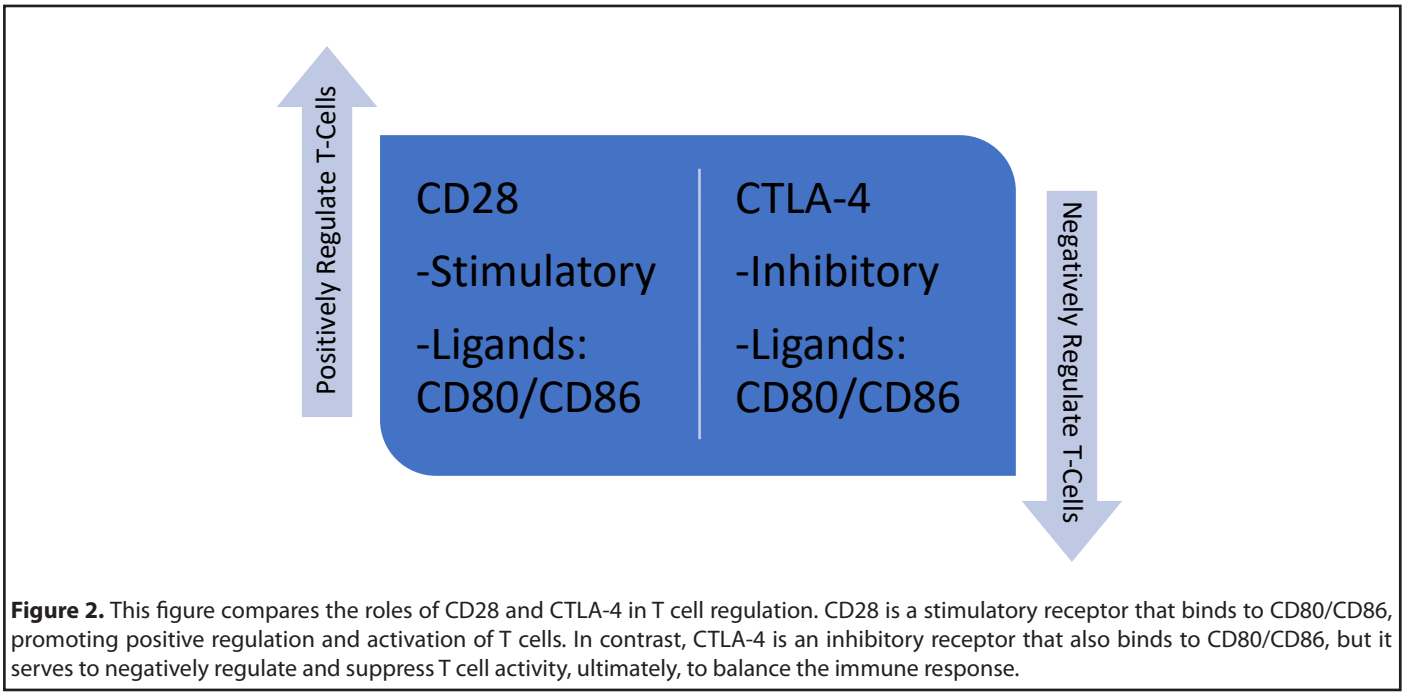

Figure 2. This figure compares the roles of CD28 and CTLA-4 in T cell regulation. CD28 is a stimulatory receptor that binds to CD80/CD86, promoting positive regulation and activation of T cells. In contrast, CTLA-4 is an inhibitory receptor that also binds to CD80/CD86, but it serves to negatively regulate and suppress T cell activity, ultimately, to balance the immune response.

signaling upon ligand binding through CTLA-4's cytoplasmic tail; ultimately facilitating the transmission of inhibitory signals that lead to T cell inactivation [25]. In the tumor microenvironment, tumor cells manipulate these homeostatic mechanisms of regulatory T cells (Tregs) to suppress immune responses. Evidence from an *in vitro* study demonstrated that Tregs mediated apoptosis in self-reactive $CD8^+$ T cells upon recognition of self-antigens; researchers also observed the proliferative preference of self-antigen-specific T cells expressing CTLA-4 over self-antigen-specific T cells lacking CTLA-4 expression [26]. Expectedly, this illustrates how tumor cells directly impact tumor-immune interactions and immune regulation by inducing a tumor microenvironment that is conducive to immunosuppression by inhibiting self-reactive T cells and promoting the proliferation of self-tolerant T regulatory cells.

TIM-3

Overview

CD366, commonly known as T cell immunoglobulin domain and mucin domain 3 (TIM-3), is a type 1 transmembrane inhibitory glycoprotein that functions to inhibit both adaptive and innate immune responses; TIM-3 expression is detectable across a variety of immune cell types, including T cells, macrophages, natural killer (NK) cells, dendritic cells (DCs), and, adaptively, on tumor cells [34-36]. Furthermore, substantial evidence suggests that TIM-3 primarily functions as a negative regulator of $CD4^+$ and $CD8^+$ T cells that secrete interferon-gamma (IFN-γ), a pro-inflammatory cytokine critical for immune responses [34-37]. The TIM-3 protein, encoded by the *HAVCR2* gene, is transcriptionally upregulated in the presence of pro-proliferative interleukins, particularly IL-12 and IL-27. This induced upregulation in $CD4^+$ and $CD8^+$ T cells is mediated through the STAT1/T-bet and STAT3/NFIL3 pathways, respectively [16,35]. Furthermore, TIM-3 interacts with four ligands: Galectin-9 (Gal-9), Carcinoembryonic antigen cell adhesion molecule 1 (Cecam1), High-mobility group box 1 (HMGB1), and Phosphatidylserine (PtdSer), each utilizing distinct biochemical pathways to exert differential modalities of immunosuppression **(Table 1)** [34-38]. As we continue to elaborate on TIM-3, we will critically assess the proposed mechanisms by which each of these ligands induces immunosuppression via TIM-3 stimulation, while also elucidating the clinical implications of these pathways.

Mechanism

To understand the receptor-ligand interaction of these distinct ligands, it is imperative to delineate the structural arrangement of the TIM-3 protein and its influence on the signaling pathway. The TIM-3 transmembrane inhibitory protein consists of four signaling domains: an extracellular variable immunoglobulin domain (IgV), a mucin-like domain, a transmembrane domain, and an intracellular cytoplasmic tail [34-37]. The IgV domain serves as the principal site for receptor-ligand interactions. This domain is composed of beta strands that form loops, which are stabilized by disulfide bonds, thereby resulting in a pocket-like structure consisting of the functional CC' and FG loops [34,36,37]. When TIM-3 is engaged by ligands, the tyrosine residues within its cytoplasmic tail become phosphorylated via an interleukin-inducible kinase. This state facilitates the release of HLA-B-associated transcript 3 (BAT3) from the cytoplasmic tail of TIM-3; which initiates FYN, another tyrosine kinase, ultimately, negatively regulating T cell activation [34,38]. As aforementioned, the associated ligands of TIM-3 induce an immunosuppressive affect in T cells upon TIM-3 stimulation; the mechanisms by which this occurs differ in each ligand. Galectin-9, or Gal-9, is an approximate 36 kDa soluble protein that binds to the IgV domain of TIM-3 resulting in cell death of T cells, specifically Th1 cells; thereby, suppressing pro-inflammatory cytokine secretion and tissue inflammation **(Figure 3)** [17,36,37]. Moreover, it has been illustrated that Gal-9 induces programmed cell death in $TIM\text{-}3^+$ $CD8^+$ tumor-infiltrating-lymphocytes (TILs) in colon cancer; conversely, Gal-9 exhibits contrasting effects in NK cells, where it has been observed to enhance IFN-γ secretion; thereby promoting a cell-mediated immune response [18,37]. These opposing immune responses at the Gal-9-TIM-3 immunological synapse suggests that the effects of Gal-9 binding to TIM-3 are dependent on the cell of which TIM-3 is expressed. The second TIM-3 ligand, Cecam1, has unique properties and expression that allow for *cis* and *trans* type binding at the TIM-3-Cecam1 immunological synapse **(Figure 3)** [34,36,37]. Interestingly, TIM-3's inhibitory function is contingent upon co-expression with Ceacam1. This dependence on Ceacam1 expression suggests that Ceacam1 acts as a requisite ligand facilitating the intrinsic *cis*-binding mechanism necessary for TIM-3 to exert its inhibitory effects. Moreover, *trans*-binding of Ceacam1 facilitates similar downstream effects to that of *cis*-binding Ceacam1, ultimately leading to the suppression of effector T cell responses [37]. Despite these unique binding properties, $CD8^+$ T cells that co-express Cecam1 and TIM-3 are subjected to T cell exhaustion and thus have dampened IFN-γ secretion, resulting in suppressed antitumor immunity [34]. The third associated ligand of TIM-3, high-mobility group box 1 (HMGB1), is a damage-associated molecular pattern (DAMP) protein that is secreted into the extracellular space by various cells in response to cellular stress [34,36,37,39]. While HMGB1 is recognized by TIM-3, it is also recognized by toll-like receptors (TLRs) and pattern-recognition receptors (PRRs), where it exerts effects counteractive to those mediated by TIM-3. Specifically, HMGB1 interaction with TLRs, such as TLR4, induces immune cell recruitment and pro-inflammatory cytokine secretion, thereby promoting anti-tumor immunity **(Figure 3)** [39-42]. Furthermore, TIM-3 competes with nucleic acids for HMGB1 binding; successful binding of HMGB1 by TIM-3 effectively inhibits nucleic acid interaction with HMGB1, thereby attenuating immune cell recruitment and activation. In contrast, formation of an HMGB1-nucleic acid-PRR complex triggers PRR-mediated immune responses, which have effects opposite to those of TIM-3 [39-42]. Lastly, the phospholipid,

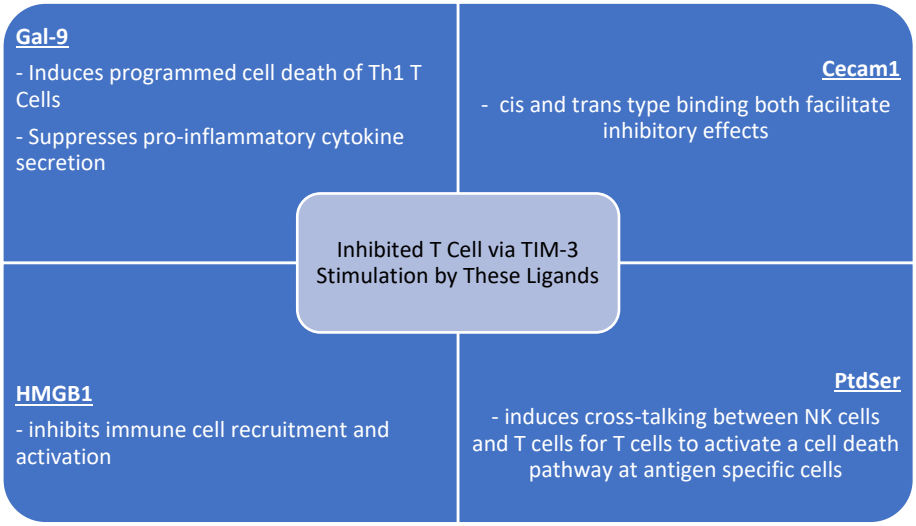

Figure 3. The figure illustrates how TIM-3 stimulation by its respective ligands inhibits T cell activity.

Phosphatidylserine (PtdSer), is one of the four ligands related to TIM-3. PtdSer is expressed on the cell surface of apoptotic cells. Biochemical studies have proposed that a metal cation, specifically calcium, is necessary for PtdSer to bind to the IgV domain of TIM-3 [34-37,43]. Once bound, the TIM-3-PtdSer complex in DCs promotes and initiates phagocytosis of apoptotic cells, while also inducing a cross-talking mechanism to present specific antigens, including PtdSer to T cells **(Figure 3)**, who cannot directly phagocytize apoptotic cells, but instead employ a programmed cell death pathway directed at antigen-specific cells, similar effects to that of the Gal-9-TIM-3 complex [34-37,43,44]. Thus, in the PtdSer-TIM-3 interaction, TIM-3 functions as an immunomodulator that enhances the effects of immune tolerance and facilitates apoptotic cell clearance [36,43,44]. In conclusion, genetic mutations impacting the TIM-3 phenotype or sustained expression or activation by ligands and cytokines, commonly observed in infectious diseases and prominently in cancer, drive the protein into a dysfunctional state, leading to the indication of T cell exhaustion [34,45-47]. Given TIM-3's regulatory role, we will further examine its clinical implications as it relates to immunotherapy, by highlighting preclinical and clinical studies.

Applications

Overview

For well over two decades, cancer researchers have aimed to utilize the inherent mechanisms of the immune system to improve patient outcomes. The traditional standard of care, including surgery, chemotherapy, and radiation therapy, has achieved a measurable amount of success. These modalities, however, have a natural ceiling effect. As a result, scientists began exploring modulation of the immune system through immunotherapy in the 1990s and subsequently subjected it to clinical investigation in the 2010s [27]. These investigative efforts have led to the discovery of immune checkpoints that can be targeted with engineered antibodies (Ab). The rationale behind immune checkpoint inhibitors (ICIs) is based on blocking the molecular interactions between inhibitory molecules, such as PD-1 and CTLA-4, and their respective ligands, PD-L1/PD-L2 and CD80/CD86. Herein, we will evaluate the preclinical and clinical efficacy of ICIs as both monotherapy and in-combination, with a particular emphasis on their mechanism of action and effectiveness.

Monotherapy (Anti-PD-1/Anti-PD-L1)

Preclinical monotherapy: As aforementioned, the binding of PD-L1 to PD-1 promotes the negative regulation of T cell activation; moreover, this molecular interaction also contributes to the downregulation of regulatory T cell proliferation [19,22,28]. Within the tumor microenvironment, this attenuated immune response contributes to the increased PD-1 expression on regulatory T cells, thereby enhancing the immunosuppressive environment [28]. Investigators have identified two potential molecular targets for disrupting these interactions and signaling pathways. The first target, Anti-PD-1 blockade, has demonstrated in *in vitro* studies on non-small cell lung carcinoma (NSCLC) and ovarian tumor cells an increase in antigen-specific $CD8^+$ T cells, leading to elevated cytokine production [28]. In the same study, the second target, Anti-PD-L1 blockade, also demonstrated a significant augmentation in $CD8^+$T cells [28].

Clinical trial monotherapy: Since the latter half of the 2010s, the FDA has sanctioned various PD-1/PD-L1 inhibitors, including atezolizumab. Phase I clinical trials of atezolizumab have demonstrated favorable tolerability and efficacy in

patients with NSCLC, with a minimal incidence of treatment-related adverse events (10%) and an objective response rate of 23% across the cohort. Additionally, the one-year survival rate reached 63% for all participants [29]. Furthermore, in a phase II clinical trial involving a sub-cohort of 27 patients, from a larger cohort, with stage III or IV cervical cancer, 44% demonstrated no progression of their cancer after 6 weeks of treatment with atezolizumab **(Table 2)** [62]. Despite the promising outcomes observed in preclinical and clinical studies, further research is necessary to optimize pharmacodynamics and enhance the objective response rate.

Monotherapy (Anti-CTLA-4)

Preclinical monotherapy (Anti-CTLA-4): As previously noted, CTLA-4 competes with the co-stimulatory molecule CD28 for the binding of CD80/CD86. Consequently, antibodies targeting these ligands would be counterproductive, hence the investigation into Anti-CTLA-4. Inherently, blocking CTLA-4 increases the available ligands, CD80/CD86, for CD28 to bind and disrupt the immunosuppressive environment [30]. In preclinical murine models, researchers illustrated that CD28 blockade suppressed antitumor immunity. In contrast, they observed that CTLA-4 blockade led to increased cytokine production via CD28 stimulation, including IL-2, which in turn upregulated anti-apoptotic genes and disrupted the immunosuppressive tumor microenvironment [31].

Clinical trial monotherapy (Anti-CTLA-4): The FDA approval of the ICI, ipilimumab, demonstrated an improved overall survival (OS) in stage IV melanoma patients in its phase 3 trial [30]. The hypothesized mechanism underlying these patient outcomes suggests that anti-CTLA-4-mediated tumor eradication correlates with an elevated ratio of $CD4^+$ effector cells to Tregs, as well as a similar effect on $CD8^+$ effector cells [30]. Moreover, the preponderance of preclinical and clinical studies, suggests that CTLA-4 blockade induces a diffusive immunity, rather than a tumor-specific immunity; evident by trials in advanced melanoma and renal cell carcinoma, showing minimal objective responses [31]. While this drug proves effective, the consequent immune-related adverse events (irAEs) possibly stem from the elevated infiltration of T cells, most commonly manifesting as a rash [32].

Combination therapy (Anti-PD-1/Anti-PD-L1 + Anti-CTLA-4)

Preclinical combination: Both Anti-PD-1 and Anti-CTLA-4 individually confer therapeutic benefits. As a result, researchers have endeavored to combine the two treatments to investigate potential synergistic or additive effects. Investigators have shown in preclinical models that simultaneous blockade of PD-1 and CTLA-4 enhances OS and reduces tumor growth in both small and large established flank melanoma tumors [33]. Furthermore, they postulated that the simultaneous blockade significantly increased the ratios of both $CD4^+$ and $CD8^+$ effector T cells to Tregs [33]. In a separate preclinical investigation, scientists illustrated that inhibiting both immune checkpoint molecules, PD-1 and CTLA-4, during T cell priming led to increased T cell proliferation and cytokine secretion [28].

Clinical trial combination: Moreover, in a longitudinal clinical trial monitoring patients with metastatic melanoma receiving both nivolumab (anti-PD-1) and ipilimumab (anti-CTLA-4), the overall response rate was 40%, and 65% of the cohort exhibited varying degrees of tumor regression [28]. These studies indicate that, although ICIs act to modulate the immune response, they do so through distinct mechanisms. Future research will focus on elucidating the specific mechanisms of PD-1 and CTLA-4. Furthermore, advancements in combination therapy will address the unmet clinical needs in immunotherapy, which will be pivotal in counteracting the diverse strategies tumor cells employ to manipulate the immune system.

Combination therapy (Anti-TIM-3/Anti-PD-1)

Clinical trial combination: Over years of preclinical and clinical investigations, researchers have identified a correlation between elevated TIM-3 expression on exhausted $CD8^+$ T cells in chronic viral infections [20,21,37]. Subsequent studies have further characterized TIM-3 expression in oncology, recognizing TIM-3 as a checkpoint inhibitor that contributes to the exhausted T cell phenotype within the tumor microenvironment, thereby suppressing antitumor immunity [21,37]. This has led to the immunotherapeutic

Table 2. Clinical information of the key immune checkpoint inhibitors (ICIs).

IC Inhibitors (ICI)	Drug Name	Type of Antibody (Ab)	Common Combination Therapy	Clinical Trial Status
Anti-PD-1/L1	Nivolumab/atezolizumab [29,60]	Monoclonal	Combined with other ICIs; i.e. CTLA-4/TIM-3 [29,60]	Phase III Complete; commercialized (NCT01844505) [29,60]
Anti-CTLA-4	Ipilimumab [60]	Monoclonal	Nivolumab	Phase III Complete; commercialized (NCT01844505) [60]
Anti-TIM-3	Cobolimab (TSR-022) [34,61]	Monoclonal [34,61]	Nivolumab	Phase I - recruiting (NCT02817633) [34,61]

exploration of drug candidates that target TIM-3; despite these investigative endeavors, monotherapy with anti-TIM-3 Ab has shown tolerability, but little clinical efficacy in overall response rates (ORR) [52-54]. In a Novartis-sponsored Phase I/II multicenter, open-label, double-arm clinical trial, investigators enrolled 219 patients with various metastatic solid tumors, predominantly ovarian cancer, to assess the clinical implications of sabatolimab, an anti-TIM-3 Ab, and spartalizumab, an anti-PD-1 Ab, either as monotherapy or in-combination. The study found no objective response rate with sabatolimab alone [49,52]. Moreover, in a phase I clinical trial funded by Tesaro, 173 patients were assigned randomly to three treatment cohorts assessing the ORR of TSR-022 (anti-TIM-3 Ab), TSR-022 + TSR-042 (anti-PD-1 Ab), and TSR-022 + docetaxel (chemotherapy) in the context of advanced solid tumor progression. The study reported an overall response rate (ORR) across all groups, with notable efficacy observed in the TSR-022 + TSR-042 arm. Importantly, grade ≥ 3 TRAEs were limited to 7.5% of participants in this cohort [34]. This study elucidates the specificity of anti-TIM-3 Abs, which contributed to the observed ORR, potentially through the induction of IFN-γ secretion in $CD8^+$ T cells, while mitigating treatment-related adverse events [51].

Preclinical combination: Furthermore, in a preclinical study evaluating checkpoint immunotherapy, investigators inoculated murine glioma into C57BL/6 mice and assessed tumor progression under eight different randomized treatment arms: control, stereotactic radiosurgery (SRS), anti-TIM-3 Ab, anti-PD-1 Ab, anti-TIM-3 Ab + SRS, anti-PD-1 Ab + SRS, anti-TIM-3 Ab + anti-PD-1 Ab, and anti-TIM-3 Ab + anti-PD-1 Ab + SRS. In alignment with the previously referenced clinical study, it was observed that monotherapy with the anti-TIM-3 Ab did not yield a significant therapeutic effect [35]. Additionally, elevated TIM-3 expression, alongside chronic PD-1 expression, characterizes the exhausted T cell phenotype [21,37,52]. Based on this observation, the proposed rationale for employing a dual checkpoint blockade targeting TIM-3 and PD-1 is rooted in the concept of adaptive resistance. Tumor cells that exhibit refractoriness to anti-PD-1 treatment likely employ adaptive resistance mechanisms [37,52]. Given that TIM-3 expression is correlated with PD-1 expression in exhausted T cells, TIM-3 is upregulated, thus contributing to the immunosuppressive milieu [52]. TIM-3 represents a promising secondary target in conjunction with anti-PD-1 therapy; given that TIM-3 is predominantly expressed on IFN-γ-secreting T cells, targeting TIM-3 with specific antibodies can localize the immune response to tumor cells. This specificity has the potential to reduce treatment-related adverse events (TRAEs) [34,37]. In anti-TIM-3 immunotherapy, significant advancements have been achieved through combination approaches, particularly with anti-PD-1, as opposed to anti-TIM-3 monotherapy [36,37,52-54]. Through several preclinical and clinical trials, it is evident that TIM-3 immunotherapy is emerging as a promising immunotherapeutic target capable of reversing the exhausted T cell phenotype in the tumor microenvironment through multiple mechanisms, of particular interest, by inducing IFN-γ secretion through $CD8^+$ T cells. Despite these significant advancements, the limitations of anti-TIM-3 Abs are evident due to the complex interactions with TIM-3 ligands. Future research should focus on augmenting bispecific antibodies that can bind multiple antigens, given the multifaceted ligand interactions of TIM-3. Additionally, considering the observed efficacy of anti-TIM-3/anti-PD-1 combination regimens, further optimization of these therapies could be achieved through in-depth investigations into the molecular interactions of TIM-3, its ligands, and the downstream signaling pathways within the tumor microenvironment.

Limitations

While immune checkpoint inhibitors have revolutionized cancer therapy, several limitations must be considered to fully understand their impact and applicability. These limitations encompass variability in patient responses, adverse events, and challenges in biomarker identification, all of which highlight the need for ongoing research and personalized treatment strategies. Inherent to the field of immunotherapy, inducing an immune response is often accompanied with general treatment-related adverse events, which can affect the therapeutic efficacy of the immune check point inhibitor. Moreover, the unpredictability of response rates adds to the inconsistencies seen in objective response rates among patients, and this uncertainty can be amplified in combination therapies. In relation to **Figures 4** and **5**, consolidating data from various clinical trials may introduce biases into the analysis; however, we maintained consistent variables across disease status and treatment type. Though, it is important to note that treatment duration and dosage varied across these trials.

Conclusion

The intricate molecular dynamics of immune checkpoint molecules such as PD-1, CTLA-4, and TIM-3 are pivotal in the regulation of immune responses and have profound implications for cancer therapy. The PD-1 and TIM-3 signaling pathways are crucial for maintaining immune homeostasis and peripheral tolerance; their dysregulation can result in autoimmunity, while in the context of cancer, it often enhances anti-tumor immunity. Likewise, CTLA-4 functions as a pivotal regulator by competing with CD28 for ligand binding, thereby modulating T cell activation. The therapeutic targeting of these checkpoints has ushered in a new modality of cancer treatment, with ICIs demonstrating promising clinical efficacy. Monotherapy with agents such as anti-PD-1 and anti-CTLA-4 has shown promising results, leading to improved patient outcomes and tumor regression. The success of agents like atezolizumab and ipilimumab in clinical trials highlights the potential of these therapies. On the contrary, the associated TRAEs highlight the need for careful management and further optimization of these treatments. Furthermore, combination therapies targeting multiple immune checkpoints offer a compelling strategy, potentially amplifying therapeutic

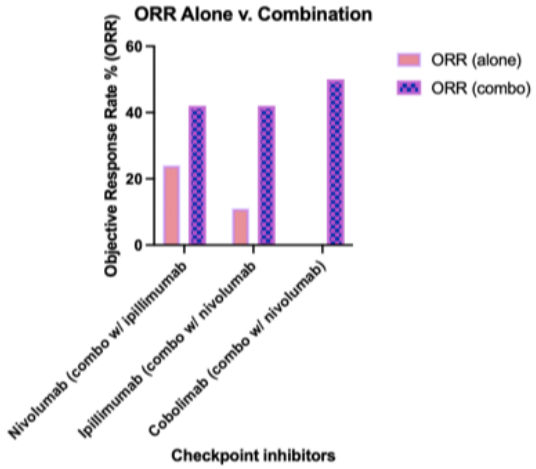

Figure 4. It is important to note that significant limitations exist, as the data has been consolidated from various clinical studies with differing trial designs. Nevertheless, the graph illustrates the relationship between monotherapy and combination therapy on overall response rate (ORR). Despite said limitations, the graph provides a clear comparative illustration of monotherapy versus combination therapy on ORR.

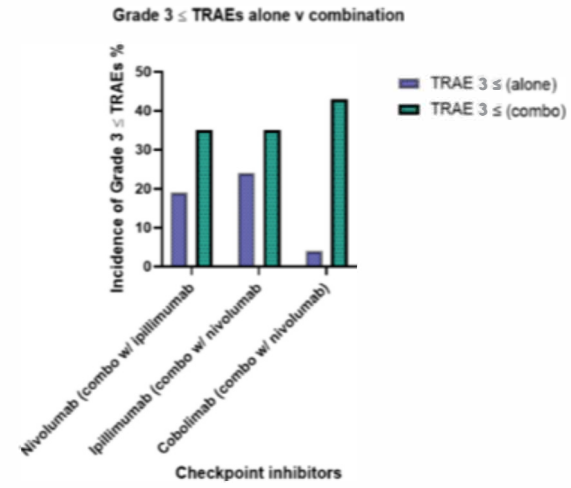

Figure 5. It is important to acknowledge that the data has been derived and consolidated from various clinical studies that differ in trial designs, which introduce significant limitations. Despite this, the graph depicts the comparative impact of monotherapy versus combination therapy on treatment-related adverse events (TRAEs).

benefits through synergistic or additive effects. The enhanced proliferation of effector cells observed in preclinical and clinical studies provides a strong rationale for continued exploration of combination regimens. As we advance, researchers must remain focused on unraveling the precise mechanisms of these immune checkpoints and refining therapeutic approaches to balance efficacy and safety. By harnessing the body's immune system, we edge closer to more effective and personalized cancer treatments, addressing the urgent need to improve outcomes for the millions affected by cancer and hematological malignancies globally.

The Author Disclosure Statement

The authors have nothing to disclose.

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