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Analyzing Strategies to Enhance the Efficacy of Immune Checkpoint Inhibitors for Cancer Immunotherapy

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ABSTRACT

The discovery of immune checkpoint inhibitors (ICIs) has been a significant breakthrough in the field of cancer immunotherapy. By blocking T-cell inhibitory signals and allowing the immune system to mount a response against cancer cells, ICIs have been used to treat patients with a variety of cancer types. Currently, the US FDA has approved three categories of checkpoint inhibitors: PD-1 inhibitors (Nivolumab, Pembrolizumab, and Cemiplimab), PD-L1 inhibitors (Atezolizumab, Avelumab and Durvalumab) and one CTLA-4 inhibitor (Ipilimumab). But despite the fact that ICIs have received success in specific cancer types, such as hematological (blood) cancers like leukemia and lymphoma, they have relatively low response rates in patients suffering from epithelial (solid) cancers, limiting their use. This paper discusses possible improvements to checkpoint inhibitor therapy, including current predictive factors for response as well as mechanisms and possible improvements to PD-1/PD-L1 and CTLA-4 inhibitors. Through an exploration of current challenges to ICI therapy, clinical trials, biomarkers like the tumor mutation burden and multivariate model, and combination therapies to improve efficacy, this review aims to provide insight into potential strategies to enhance ICIs to treat a broader spectrum of cancers, leading to a more inclusive and effective treatment. While combination therapies often demonstrate enhanced efficacy, further research must be conducted to optimize treatment specifics for each cancer type. Although this review focuses on the potential of PD-1/PD-L1 and CTLA-4 inhibitors, it overlooks other novel checkpoint targets, which could offer a more broad perspective.

Introduction

In recent years, medical science has witnessed significant advancements in the development of various cancer treatments, including surgery, radiation therapy, chemotherapy, targeted therapy, and immunotherapy. Among these advancements, immunotherapy is emerging as a promising treatment by harnessing a patient's own immune system in order to control, eliminate, or prevent a variety of cancers. One type of immunotherapy, known as immune checkpoint inhibitors (ICIs), has revolutionized cancer treatment, with PD-1/PD-L1 and CTLA-4 inhibitors exhibiting improved outcomes and sometimes curing patients whose disease was previously considered incurable (Rubin & Olszanski, 2020).

Immune checkpoints are crucial regulatory mechanisms in the body that prevent excess activation of the immune system and potential harm to healthy tissues. These checkpoints, such as programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein (CTLA-4), serve as brakes on immune responses and are expressed in many immune cells, especially effector cells such as T-cells. Cancer cells can exploit these checkpoints to evade detection and elimination by the immune system. Blocking these checkpoints with antibodies, or immune checkpoint inhibitors (ICI), releases the brakes on immune response, allowing the effector T-cells to more effectively recognize and target cancer cells. This unleashing of the anti-tumor effector immune function enhances the body's natural ability to mount a targeted immune response.

In spite of the success of CTLA-4 and PD-1/PD-L1 inhibitors, growing evidence suggests that only a small fraction of patients benefit from checkpoint inhibitors and severe adverse effects are common (Jacob and Parajuli, 2021). This literature review is an examination of different strategies aimed to enhance the efficacy and safety of these inhibitors. By exploring a variety of topics, such as the mechanisms of checkpoint inhibitors, the tumor mutation burden (TMB), multivariable model, combination therapies, and activation of receptor ligands, this research aims to synthesize current knowledge and provide a roadmap for the development of more optimized immunotherapy strategies.

Mechanisms of Immune Checkpoint Molecules

Immune checkpoint inhibitors block inhibitory signals of T-cell activation, allowing T-cells to overcome this regulatory mechanism and mount a response against tumor cells (Figure I). Antibodies that block CTLA-4 and PD-1/PD-L1 interactions are the most well studied and are currently used in cancer treatments. CTLA-4 is found on both CD4+ and CD-8+ lymphocytes and binds to CD80 and CD86 receptors on the surface of antigen presenting cells (APC). Binding of CTLA-4 reduces the production of interleukin-2 (IL-2) which stimulates T-cell, natural killer (NK) cell, and B-cell proliferation. PD-1 is a receptor found on a variety of immune cells while the ligand PD-L1 can be found in many cell types, including tumor cells. The interaction of PD-1 and PD-L1 causes the inhibition of previously activated T-cells (Iranzo et al., 2022). Using ICI's to block these pathways overcomes this immune inhibition caused by tumors.

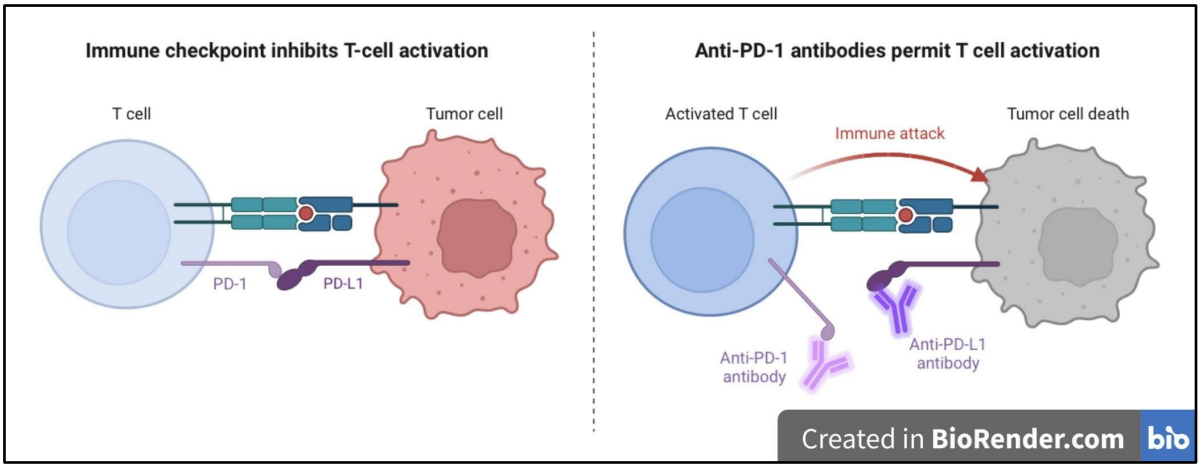

Figure 1. Mechanisms of PD-1/PD-L1 Checkpoint Inhibitors

PD-1/PD-L1 checkpoint inhibitors block the PD-1/PD-L1 interaction, preventing tumor cells from deactivating an immune response (created in Biorender.com by Ananya Devkirti).

FDA Approved Checkpoint Inhibitors

Ipilimumab, the first FDA approved checkpoint inhibitor, was discovered by Dr. James Allison and was used for treating patients with advanced melanoma, targeting cytotoxic T-lymphocyte antigen-4 (CTLA-4). Since its discovery, numerous studies have been conducted regarding its potential implications, especially in regards to its combination with other treatments. When administered in combination with the glycoprotein 100 (gp100) peptide vaccine in patients with metastatic melanoma, patients demonstrated higher survival rates than those administered with just gp100 (Hodi et al., 2010). The use of ipilimumab in combination with dacarbazine

(DTIC), a medication used to treat various types of skin cancers, also demonstrated an increased benefit when compared to DTIC alone (Robert et al., 2011). Ipilimumab has also been administered in combination with nivolumab, another ICI. The objective response rate of ipilimumab (30-40%) improves to 60% when combined with other treatments, known as combination therapy (Postow et al., 2015).

Nivolumab and pembrolizumab, other ICIs that target programmed cell death (PD-1), have exhibited positive results in treating patients with melanoma and non-small lung carcinoma (NSCLC). Nivolumb was found to be more effective than docetaxel, a chemotherapy drug used in multiple tumor types, in treating advanced NSCLC (Borghaei et al., 2015). Pembrolizumab as a monotherapy in treatment of NSCLC was found to be moderately effective, displaying an objective response rate of 19.4% (Garon et al., 2018). Another study found that combined administration of ipilimumab and nivolumab was most effective, followed by nivolumab ipilimumab monotherapies (Larkin et al., 2015). In patients with triple-negative breast cancer (TNBC), the effectiveness of nivolumab was demonstrated to be moderate, with an objective response rate (ORR) of 19% (Polk et al, 2018). However, in relapsed or refractory Hodgin's lymphoma, nivolumab was found to be more effective, with an ORR documented in 87% of patients, and a 17% complete response (Ansell et al., 2015). Currently, hundreds of clinical trials are being conducted across the globe that examine the efficacy of new immune checkpoint treatments as well as aim to optimize the safety and efficacy of current treatments (Table 1).

Apart from ipilimumab, nivolumab, and pembrolizumab, several other inhibitors have gained attention in cancer therapy (Table 1). For instance, atezolizumab and avelumab target the programmed death ligand (PD-L1), disrupting its interaction with PD-1 to unleash an antitumor immune response. Tremelimumab is a monoclonal antibody that targets CTLA-4 and is being investigated for its uses in various cancers (Shiravand, 2022).

Table 1. ICIs in Stage III and IV Clinical Trials

Drug	**Cancer Type**	**Clinical Trial ID**
Ipilimumab (Anti-CTLA-4)	Melanoma	NCT03445533, NCT01515189, NCT03873402, NCT02545075, NCT02599402, NCT02905266, NCT01866319, NCT00636168, NCT01844505
	Renal Cell Carcinoma	NCT03138512, NCT02982954, NCT03873402, NCT02231749, NCT03141177, NCT03937219, NCT03793166
	Non-Small Cell Lung Cancer (NSCLC)	NCT03469960, NCT03302234, NCT03351361, NCT04026412, NCT02279732, NCT02864251, NCT03215706, NCT02477826, NCT02998528
	Prostate Cancer	NCT00861614, NCT01057810
Pembrolizumab (Anti-PD-1)	Melanoma	NCT01866319, NCT05986331, NCT05665595, NCT05727904
	Merkel Cell Carcinoma	NCT03783078, NCT03712605
	Non-Small Cell Lung Cancer (NSCLC)	NCT04629027, NCT06052852, NCT04738487, NCT04547504, NCT02220894, NCT02142738, NCT03134456, NCT04613596, NCT03774732

	Multiple Myeloma	NCT02579863, NCT02576977, NCT02579863
Nivolumab (Anti-PD-1)	Melanoma	NCT05297565, NCT04309409, NCT06116461, NCT04949113, NCT06112314, NCT05002569, NCT04695977, NCT04410445, NCT02599402
	Non-small cell lung cancer (NSCLC)	NCT03444766, NCT03195491, NCT02066636, NCT03906071, NCT03417037, NCT03351361, NCT04026412
	Hepatocellular Carcinoma	NCT02576509, NCT04044651
	Renal Cell Carcinoma	NCT04810078, NCT02596035, NCT03383458, NCT04987203, NCT02231749
Avelumab (Anti-PD-L1)	Merkel Cell Carcinoma	NCT03271372
	Non-Small Cell Lung Cancer (NSCLC)	NCT02576574, NCT02395172
	Renal Cell Cancer	NCT02684006, NCT03013946, NCT04510597
	Urothelial Cancer	NCT02603432, NCT04637594, NCT05059522, NCT05092958
Atezolizumab (Anti-PD-L1)	Non-Small Cell Lung Cancer (NSCLC)	NCT03285763, NCT03922997, NCT05047250, NCT03735121, NCT04513925, NCT04471428, NCT04294810, NCT02657434
	Hepatocellular Carcinoma	NCT04487067, NCT05665348, NCT05185505, NCT04732286, NCT04102098, NCT04803994, NCT03434379, NCT05904886
	Renal Cell Carcinoma	NCT04338269, NCT03024996, NCT02420821, NCT04157985, NCT04637594,
Tremelimumab (Anti-CTLA-4)	Non-Small Cell Lung Cancer (NSCLC)	NCT06008093
	Squamous Cell Carcinoma	NCT02551159, NCT02369874

Stage III and IV clinical trials on checkpoint inhibitors. Data obtained from clinicaltrials.gov.

Challenges to CPI Therapy

However the success of anti-PD-1/PD-L1 and CTLA-4 therapies is limited and challenges such as resistance to inhibitors still persist. Accumulating research also suggests that many patients experience severe immune-related adverse effects (irAEs) when undergoing checkpoint inhibitor therapy (Yan et al., 2013). irAEs are caused by the inhibition of immune checkpoints, the body's normal barriers against autoimmunity, resulting in various undesirable immune responses. These adverse effects tend to be organ specific, with skin related irAEs being the most common, followed by gastrointestinal toxicity and endocrine irAEs (Yin et al., 2023).

Additionally, immunotherapy often results in limited success and only a small portion of patients experience lasting benefits. While immunotherapy is largely beneficial in hematological cancers like leukemia and lymphoma, epithelial (solid) cancers, which account for over 80-90 percent of all cancers, have not exhibited the same results. There are several barriers in the tumor microenvironment (TME) that explain this lack of efficacy. One such factor is that epithelial tumors typically reside in non-lymphoid tissues, areas that T-cells are unable to effectively infiltrate (Srivastava & Riddell, 2018).

The TME can be divided into three major types based on the infiltration of immune cells: immune-desert, immune-inflamed, and immune-excluded (Chen and Mellman, 2018). Each phenotype employs distinct mechanisms to hinder immune responses against tumor cells. Immune-inflamed tumors, also called “hot tumors,” are characterized by high T-cell infiltration. Immune-desert and immune-excluded tumors are considered to be “cold tumors.” In immune desert tumors, lymphocytes are absent from the tumor and its periphery while in immune-excluded tumors, lymphocytes accumulate but do not effectively infiltrate the tumor. Variable tumors exist in a variable state between hot and cold tumors. Cold and variable tumors are less responsive to ICI therapy because the lack of T-cell infiltration prevents inhibitors from activating an immune response. These tumors often require the use of other therapies to introduce immune cells into the tumor, essentially converting them into hot tumors, before the use of immunotherapy (Chen and Mellman, 2018).

Additionally, features like the blood-brain barrier (BBB) in gliomas and the desmoplasia in pancreatic cancers present further challenges. The BBB, a semipermeable membrane between the blood and interstitium of the brain, protects the brain from pathogens and controls infiltration. Due to the BBB, which restricts the entry of therapies into the brain tumor, treating glioblastoma and other gliomas is much more challenging than other solid tumors. Therefore, before the use of treatment therapies, it is vital to consider methods to alter the permeability of the BBB, especially with the use of ICIs, which require immune effector cell infiltration (Sanders & Debinski, 2020). The desmoplasia or desmoplastic reaction, the growth of dense connective tissue around pancreatic tumors, creates a microenvironment that both promotes tumor growth and creates a barrier against chemotherapy. This chemoresistance caused by the desmoplasia requires unique methods of treatment such as targeting aspects of tumor stroma in order to break down the desmoplastic reaction (Merika et al., 2012).

Predicting Response to Checkpoint Inhibitors

Older Biomarkers

In order to combat the challenge of resistance in inhibitors, researchers have investigated potential biomarkers, methods of determining which patients will benefit from ICIs. Peripheral blood cell biomarkers and the circulating tumor DNA biomarkers are some of the most well researched patient biomarkers.

Peripheral blood cell biomarkers examine the neutrophil-to-lymphocyte ratio (NLR) amongst other factors. In patients with non-small cell lung cancer (NSCLC) treated with nivolumab, a low neutrophil-to-lymphocyte ratio has been associated with poor tumor response (Bagley et al., 2017). Patients with melanoma treated with pembrolizumab have exhibited a similar pattern (Weide et al., 2016). The detection of circulating

tumor DNA (cDNA) can also gain information related to tumor response to ICIs. Studies have demonstrated that a high mutation number of ctDNA is associated with poor prognosis (Heitzer et al., 2015). Melanoma patients with persistently elevated cDNA during PD-1 therapy displayed worse response and shorter progression-free response (PFS) and overall survival (OS) (Lee et al., 2017).

Despite these successes, the discovery of predictive biomarkers for the efficacy of ICIs has been complicated. Therefore, it is crucial to explore new biomarkers such as the tumor mutation burden and multivariable model.

Tumor Mutation Burden (TMB)

Broad genomic sequencing approaches have been applied to ICI clinical trials. The results suggest that patients with a higher number of somatic tumor mutations displayed more benefits from ICI therapy when compared to patients with less mutations (Rizvi et al., 2015). More effectively designed sequencing measures have since been utilized to assess tumor mutation burden (TMB), defined as the number of somatic cell mutations per megabase (1 million bases).

TMB was initially measured using whole-exome-sequencing (WES), a technique for sequencing all protein-coding regions of a genome. Studies have demonstrated an association between WES-derived TMB and ICI therapy outcomes in NSCLC (Rizvi et al., 2015). This association has also been demonstrated in desmoplastic melanomas (Eroglu et al., 2018). Although WES-derived TMB improved patient selection, it had limited utility due to its lengthy (6-8 week) sequencing time, and cost. Utilizing next generation sequencing (NGS), which sequences only a subset of the exome, presents a promising alternative method to WES for calculating TMB (Chalmers et al., 2017). Additionally NGS takes a more sophisticated approach than WES, counting both synonymous and nonsynonymous base substitutions as well as short insertion and deletion alterations into TMB calculation. In general, WES only incorporates non synonymous base substitutions. Although synonymous variants don't alter the amino acid sequence of a protein, their presence can be indicative of nonsynonymous substitutions and their inclusion improves TMB detection sensitivity (Chalmers et al., 2017).

There are several factors that link to elevated levels of TMB in patients, including exposure to carcinogens like cigarette smoke and ultraviolet radiation (Rizvi et al., 2015). Changes in DNA mismatch repair (MMR) pathway-associated genes such as MSH2, MSH6, and PMS2 also contribute to high TMB in certain cancer types (Chalmers et al., 2017).

Establishing if any biomarker, including TMB, is able to reliably and accurately separate patients into groups with distinct biological outcomes is essential for determining its clinical use (Hayes, 2014). Although studies have demonstrated a greater benefit in patients treated with ICIs in patients with high TMB when compared to those with low TMB, these analyses utilize a variety of cutoffs in order to define "high" and "low" TMB levels. While analyzing TMB as a response to immunotherapy in diverse cancers, Goodman and team defined high TMB as ≥20 mutations per megabase (Goodman et al., 2017). Other studies include both a quantitative metric using mutations per Mb as well as a qualitative measurement of low, or high (Hellmann et al., 2018). Separate and defined cutoffs, which will vary based on tumor and intervention type, are currently being pursued (Merino et al., s2020). Efforts are currently underway to standardize TMB analysis and interpretation (Miao et al., 2018).

Despite the importance of TMB in predicting patient survival and response to ICI treatment, there are tumors with a high TMB that exhibit no response and tumors with low TMB that benefit from inhibitor therapy. It has become evident that understanding the nuances of TMB will be essential for developing a more robust predictor for ICI response. Anagnostou and team analyzed whole-exome and target sequence data in order in 5,449 tumors and found that, consistent with previous findings, patients with higher observed TMB benefited more from ICI treatment. However, they also recorded a significant correlation between TMB and tumor purity, suggesting that tumors with higher purity are more likely to have inaccurate estimates of TMB. Samples with

low tumor purity tended to have underestimated TMB values, and the team developed correction factors by considering tumor purity, resulting in improved prognostication (Anagnostou, 2020). Based on these findings, it is clear that a multivariate analysis, one that takes multiple variables into consideration, is needed for a more accurate measurement of ICI response.

Multivariate Model

Although many biomarkers have been selected based on biological rationale, they often show limited use in predicting treatment response, indicating that it is likely that one single biomarker isn't sufficient to capture the intricacies of each patient. Previous biomarkers have focused on developing and improving single biomarkers of response to immunotherapy, highlighting the need for more nuanced models that consider multiple factors. The Multivariate model is a more complete approach that combines the improved estimate of TMB that is corrected for tumor purity with human leukocyte antigen (HLA) class I genetic variation, molecular smoking signature, genomic alterations in RTK genes, and genome-wide mutational features in order to capture the multifaceted nature of the tumor immune system (Anagnostou, 2020). In addition to analyzing genome and exome characteristics, TME characteristics could be taken into account for an improved Multivariate model.

Integrating artificial intelligence (AI) into cancer treatment has the potential to revolutionize the field of personalized medicine, particularly by creating a model to analyze and predict response to treatments and determine combination therapies involving ICIs. The multivariate model is a step towards developing a more sophisticated understanding of the tumor microenvironment. As AI evolves, it may have a significant role in improving treatment decision making by considering a variety of factors that are personalized to each patient as well as analyzing vast datasets, similarly to the multivariate model. AI could leverage patient data to identify patterns and trends human physicians may overlook and then determine optimal therapies. For example, AI could analyze a patient's specific profile and then recommend combination therapies. Additionally, AI has the ability to constantly adjust to new data, allowing for more personalized treatments.

Strategies to Enhance Response to CPI

Combination Therapies

It is becoming clear that tumors lacking T-cell infiltration that resist current treatment options can be sensitized to checkpoint inhibitor therapy with various strategies, including immunogenic chemotherapy, radiation therapy, targeted therapy, and cryoablation.

While immunotherapy is less effective in eradicating a large tumor mass, immunogenic chemotherapy can increase efficacy by debulking the tumor mass, decreasing the amount of cells that need to be eradicated by immune cells, reducing the immunosuppressive factors produced by cancer cells, and potentially even directly stimulating antitumor immunity via release of neoantigens and immune stimulatory molecules. Pfirschke and team combined two chemotherapy drugs (oxaliplatin combined with cyclophosphamide) against tumor cells. Instigating T-cell infiltration sensitized tumors for checkpoint blockade therapy to effectively combat the tumor and control cancer durably (Pfirsche, 2016). Common chemotherapeutic drugs may also stimulate anti-tumor immunity by activating T-cells and NK cells as well as targeting the tumor microenvironment. The efficacy of chemotherapeutic drugs is higher in immunocompetent mice when compared to immunodeficient ones (Zitvogel et al., 2016). The type of cell death caused by chemotherapy triggers anti-tumor immunity, releasing neoantigens as well as danger-associated molecular patterns (DAMPs) such as high-mobility group protein B1(HMGPB1, or Alarmin) and cytokines/chemokines into the TME that stimulates the dendritic cells and leads to the activation of immune response against the tumor cells (Kroemer, 2022).

Various studies demonstrate the potential of chemotherapy drugs to improve the efficacy of PD-1/PD-L1 checkpoint blockade, as summarized in Table 2. Preclinical experimentation found that gastric cancers treated with the ICD (immunogenic cell death) inducer were sensitized to PD-1 inhibitors (Liu et al., 2022). Combining trifluridine/tipiracil and oxaliplatin improved efficacy of PD-1 blockade in colorectal cancer (Limagne et al., 2019). In patients with HER-2 negative gastric and gastro-esophageal junction adenocarcinomas, combining oxaliplatin-based chemotherapy with nivolumab significantly improved patient survival (Janjigian et al., 2021). Adding trastuzumab and chemotherapy to the PD-1 blockade significantly improved overall response rate (ORR) in metastatic HER2+ gastric or gastro-esophageal junction adenocarcinoma, bringing it from 51.9 to 74.4% (Janjigian et al., 2021). In squamous NSCLC, pembrolizumab combined with carboplatin and taxane chemotherapies resulted in an improved overall survival when compared to chemotherapy alone (Paz-Ares et al., 2018).

Radiation therapy has also been utilized in combination with ICIs in preclinical trials. A single, strong dose of radiation therapy was found to induce an anti-tumor T-cell response more effectively when combined with immunotherapy (Siva et al., 2015). In mouse models, this combination treatment promoted antitumor immunity, suggesting that PD-1/PD-L1 blockade and RT may prevent tumor immunosuppression, improving the efficacy of RT. This benefit of one single dose of radiation has also been observed in other studies. In a mouse model, combining PD-1 with a single dose of RT led to increased long-term survival in gliomas (Belcaid et al., 2014). Additional studies demonstrate that multiple smaller doses of RT (fractionated RT) are more effective than one stronger dose. Fractionated RT has been demonstrated to cause tumor regression and increase long-term survival in multiple cancers (Dewan et al., 2009). While both single dose and fractionated RT demonstrate positive effects, single use RT is only able to eliminate micrometastases while fractionated RT was more efficient in eliminating both micrometastases and mature tumors (Dewan et al., 2009).

Cryoablation is a technique that utilizes extreme low temperatures to destroy tumors. Multiple preclinical studies have demonstrated the benefits of combining cryoablation with immunotherapy, however, further research is necessary to determine its potential benefits for patients. A preclinical study using mice demonstrated that cryoablation leads to the maturation of DC cells and an anti-tumor immune response, protecting 50% of mice from a new injection of tumor cells. When combined with anti-CTLA-4 checkpoint inhibitors, this rose to 80% of mice exhibiting resistance (Brok et al., 2006). Combining cryoablation with ipilimumab in patients with early-stage breast cancer was found to be a safe option (McArthur et al., 2016). A combination therapy of anti-PD-1, anti-CTLA-4 or placebo with or without cryoablation in prostate cancer demonstrated a delay in tumor growth and decreased mortality in mice (Benzon et al., 2018). Other clinical results were less promising. In a group of patients with hepatitis B-hepatocellular carcinoma, the addition of PD-1/PD-L1 inhibitors after cryoablation led to poor overall survival (Zeng et al., 2011).

Targeted therapy has been an essential aspect of cancer treatment for decades that requires a specific drug target. By combining PD-1 blockade with vascular endothelial growth factor receptor 2 (VEGFR2) in a murine cancer model of colon adenocarcinoma, it was found that ICI plus targeted therapy may be an effective treatment method (Yasuda et al., 2013). In a phase III study, patients treated with atezolizumab plus bevacizumab had higher rates of overall survival (OS) and progression-free survival (PFS) when compared to a monotherapy group (Fin et al., 2020). Another study found that the combination of olaparib, a targeted therapy that breaks poly ADP-ribose polymerase, and durvalumab in patients with germline BRCA-mutated metastatic breast cancer displayed promising antitumor activity (Domchek et al., 2020). However, targeted therapy has also shown disappointing results for specific cancer types. A trial combining pembrolizumab with gefitinib in NSCLC patients with EGFR mutations was ineffective due to high levels of liver toxicity in 71.4% of patients (Yang et al., 2019). A phase I trial investigating durvalumab plus trastuzumab in HER2-positive metastatic breast cancer showed no response (Chia et al., 2019). Although a phase III trial combining the PD-1 inhibitor spartalizumab with dabrafenib and trametinib in advanced melanoma patients resulted in modest improvements of PFS, the combined therapy group also had rates of side effects like increased liver enzymes, pneumonitis,

rash, and hyperthyroidism (Drummer et al., 2022). Although a variety of studies have demonstrated the synergistic benefit of combining ICIs with targeted therapy, not all combinations are effective. More studies are needed to improve the clinical outcome of this potentially beneficial treatment strategy.

Table 2. Combination Therapies in Clinical Trials

Drugs	Cancer Type	Clinical Trial ID
Immunogenic Chemotherapy + ICIs	Breast Cancer	NCT03409198, NCT03164993
	Non-Small Cell Lung Cancer (NSCLC)	NCT04043195
	Large B-Cell Lymphoma	NCT03321643
Radiation Therapy + ICIs	Colorectal Cancer	NCT04659382
	Non-Small Cell Lung Cancer (NSCLC	NCT03313804,
	Nasopharyngeal Carcinoma	NCT05290194
Cryoablation + ICIs	Prostate Cancer	NCT02423928, NCT04090775, NCT02489357
	Non-Small Cell Lung Cancer	NCT06127303
	Melanoma	NCT05779423, NCT05302921, NCT03325101,
	Breast Cancer	NCT03546686, NCT01502592, NCT04249167
	Soft Tissue Sarcoma	NCT04118166
	Lung Cancer	NCT04339218, NCT05071014
Targeted Therapy + ICIs		

Checkpoint inhibitors combined with chemotherapy, radiation therapy, and cryoablation. Data obtained from clinicaltrials.gov.

Conclusion

Cancer therapy has shown remarkable progress in the field of immunotherapy especially with the advent of immune checkpoint inhibitors (ICIs). These inhibitors focus on deactivating key regulatory checkpoints such as PD-1/PD-L1 and CTLA-4 and have proven their efficacy in cancer treatment, with results deemed impossible

in the past. Nevertheless, there are limitations in their efficacy such as resistance to ICIs due to suboptimal tumor microenvironments, leading to low response rates. To combat these limitations, the efficacy of recent biomarkers should be enhanced to better discern among patients who will respond to checkpoint blockade therapy. In addition, future biomarkers should incorporate a more multifaceted strategy like the multivariate model, which includes a wide range of factors such as the tumor mutation burden (TMB), human leukocyte antigen (HLA) genetic variation, molecular smoking signature, and others, as opposed to a singular factor to predict treatment response. This serves as a step in the right direction towards a more refined perspective on TME as well as the application of AI in the creation of treatment plans. In addition, the combination of checkpoint inhibitors with other treatments like chemotherapy, radiation therapy, and targeted therapy is promising in resolving cases in which T-cells cannot penetrate into solid tumors. However, despite the challenges that remain, ongoing clinical trials and research studies hold the potential to further refine checkpoint inhibitors and improve outcomes for patients worldwide.

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