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REVIEW ARTICLE

Clinical Combinatorial Treatments Based on Cancer Vaccines: Combination with Checkpoint Inhibitors and Beyond

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Abstract: The efficacy of the cancer vaccine is influenced by several factors, but one of the most important is the immunosuppressive tumor microenvironment, which can attenuate treatment effects. The combination of therapeutic cancer vaccines with other immunotherapies or conventional therapeutic approaches can promote vaccine efficacy by increasing immune surveillance and tumor immunogenicity and modulating immune escape in the tumor microenvironment. Inhibitory checkpoints have a significant role in the modulation of anticancer immune responses, and according to preclinical and clinical trials, administration of immune checkpoint inhibitors (ICIs) in combination with cancer vaccines can markedly improve their therapeutic effects, considering their low clinical efficacy. In addition, these combinatorial therapies have acceptable safety and minimal additional toxicity compared to single-agent cancer vaccines or ICIs. In this review, based on the results of previous studies, we introduce and discuss treatments that can be combined with therapeutic cancer vaccines to improve their potency. Our major focus is on checkpoint blockade therapies, which are the most well-known and applicable immunotherapies.

Keywords: Cancer vaccine, immune checkpoint inhibitors, combinatorial therapies, therapeutic cancer, tumors, carcinogenesis.

1. INTRODUCTION

Therapeutic cancer vaccines, which have been the subject of researchers' attention for almost one century, are considered promising and reasonably safe approaches for cancer treatment, especially for tumors that do not elicit a strong immune response [1-3]. In addition, the greatest benefit provided by a vaccine is that effective treatment can be achieved with a few injections, and the immunological memory developed by the immune system can also protect patients during relapse events. In contrast to classical preventative vaccination against infectious disease, cancer vaccines are mainly administered after the onset of the disease and rely on tumor-specific or tumor-associated antigens based on different platforms, such as peptide- or protein-based, nucleic acid-based, microbial-based, and cell-based constructs [4-6]. Despite mostly favorable preclinical outcomes of this therapeutic strategy, cancer vaccination is still not considered a definitive treatment for patients. Even after antigen selection and vaccine design, which are the most difficult steps in developing these therapeutics, cancer vaccines may not be efficient or adequately immunogenic [7, 8]. Several aspects, such as the immunosuppressive tumor microenvironment (TME), can influence vaccine efficacy. Therefore, overcoming inhibitory signals in immune cells by blockade of immune checkpoints or their ligands is a promising strategy to improve the clinical benefits of cancer vaccines [9-11]. Immune Checkpoint Inhibitors (ICIs), such as blockers of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed death protein-1 (PD-1), and programmed death ligand-1 (PD-L1), improve anticancer immune responses by blocking negative regulatory signaling. The treatment of some types of cancers, such as lung, renal cell and bladder cancers and melanoma, was revolutionized after the discovery of ICIs; however, other malignancies, such as prostate and pancreatic cancers, have not demonstrated sufficient clinical improvement in response to single-agent ICIs [12-14].

Thus far, three therapeutic cancer vaccines have been approved by the Food and Drug Administration (FDA): (1) talimogene laherparepvec (T-VEC), which is a viral-based vaccine for advanced melanoma, (2) Bacillus Calmette-Guerin (TheraCys®), which is a live attenuated vaccine for

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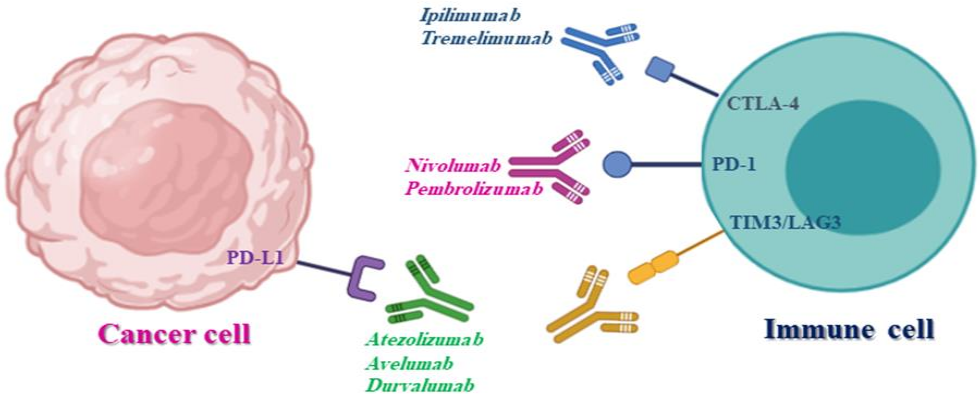

Fig. (1). Some inhibitory checkpoints/ligands and their FDA-approved blocker antibodies. CTLA4 (cytotoxic T-lymphocyte associated protein 4), PD-1/PD-L1 (programmed-death 1/ligand), TIM-3 (T- cell immunoglobulin and mucin domain 3), and LAG-3 (lymphocyte activation gene-3) are some inhibitory checkpoints or ligands along with their specific blocker antibodies that are used in therapeutic approaches. These FDA-approved monoclonal antibodies are Ipilimumab and Tremelimumab (CTLA-4 blocker), Nivolumab and Pembrolizumab (PD-1 blocker), and Atezolizumab, Avelumab, Durvalumab (PD-L1 blocker). The figure was produced with the assistance of Biorender (https://biorender.com/). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

non-muscle invasive bladder carcinoma, and (3) Sipuleucel-T (Provenge®), which is a dendritic cell (DC)-based vaccine for metastatic castration-resistant prostate cancer (mCRPC) [15-18]. PROSTVAC was another promising cancer vaccine, but it failed in a phase III clinical trial [19]. Furthermore, the therapeutic DNA vaccine encoding the E6/E7 fusion protein of human papillomavirus (HPV) subtypes 16 and 18 (HPVE6/E7GX-188E vaccine) is a widely used vaccine for the prevention of cervical carcinogenesis [20]. There are also several cancer-treating FDA-approved ICI monoclonal antibodies, but most of them are based on the blockade of CTLA-4, PD-1, and PD-L1. The most prominent ones are Ipilimumab, Nivolumab, Pembrolizumab and Atezolizumab [21].

Considering that only a limited number of patients successfully respond to a single treatment of immune checkpoint blockers (ICBs) or cancer vaccines alone, and according to preclinical and clinical research, combinatorial approaches, including concurrent administration of ICIs and cancer vaccines, have been explored for their synergistic effects because the potential drawbacks of each approach alone can be rectified by the other [21, 22]. For instance, robust T-cell activation induced by a vaccine can cause the expression of regulatory checkpoints, so administering ICIs simultaneously with or after cancer vaccination is a logical strategy. Indeed, these active immunotherapies lead to a robust and long-lasting immune response in cancer patients [11]. Synergistic effects of these combinational therapies have previously been shown in some *in vitro* studies. These studies proved that combining CTLA-4 or PD-1/PD-L1 blockade with therapeutic cancer vaccines can reinforce tumor antigen-specific immune responses to cancerous cell lines or tumor-bearing mice. The immunologic mechanism of this amplified immune response is not completely known, although based on these *in vitro* investigations, it may be through an increase in tumor-infiltrating lymphocytes (TILs) numbers and activities, such as $CD8^+$ T cells, as well as decreases in suppressing immune cells, such as T_{reg} cells [23-25]. This review reports the most recent and remarkable clinical trial outcomes of combinatorial therapies that use cancer vaccination alongside ICIs and other classical therapies.

2. COMBINATORIAL CANCER IMMUNOTHERAPY

2.1. Checkpoint Inhibitors Combined with Cancer Vaccines

Fig. (**1**) illustrates some inhibitory checkpoints/ligands and their FDA-approved blocker antibodies that are discussed below.

2.1.1. Anti-PD-1 (Nivolumab and Pembrolizumab)

2.1.1.1. Pembrolizumab

Pembrolizumab is an anti-PD-1 antibody that first received FDA approval for use in patients with non-squamous NSCLC or ipilimumab-refractory advanced melanoma [21, 26, 27]. Various preclinical and clinical trials of immunotherapies have investigated its efficacy when used alone and in combination with other cancer therapies for different malignancies, and most of them have reported promising outcomes. Tested treatments include coadministration of pembrolizumab with radio/chemotherapy, metabolic regulators, costimulatory receptor agonists, other checkpoint blockers, nanoparticles and cancer vaccines. Its combination with therapeutic cancer vaccines appears to be the most common [28-33] (Tables **1** and **2**).

A phase Ib study investigated the effects of pembrolizumab combined with T-VEC in advanced head and neck squamous cell carcinoma patients. According to initial data from this study (n = 36), the disease control rate (DCR) and overall response rate (ORR) were 38.9% and 16.7%, respectively. In this trial, 200 mg of pembrolizumab was administered by *i.v.* every 3 weeks after intratumoral T-VEC injection [34]. A similar study evaluated this combinatorial approach for unresectable IIIB-IV stage melanoma and reported

Table 1. Selected cancer vaccines in combination with one or more ICIs and other therapeutic compounds.

Vaccine	ICI	Other Treatments	Malignancy	Phase	NCT	Number of Patients
DPX-Survivac	Pembrolizumab	Cyclophosphamide	Peritoneal, ovarian, or fallopian tube cancer	II	NCT03029403	42
GVAX	Pembrolizumab	Cyclophosphamide, SBRT	Pancreatic cancer	II	NCT02648282	58
GVAX, CRS-207	Pembrolizumab	Epacadostat	Pancreatic cancer	II	NCT03006302	44
NeoVax	Pembrolizumab	Temozolomide, Radiotherapy	Glioblastoma	I	NCT02287428	56
GVAX	Nivolumab	Ipilimumab/CRS-207, Cyclophosphamide	Pancreatic cancer	II	NCT03190265	63
pTVG-HP	Nivolumab	GM-CSF	Non-metastatic, PSA-recurrent prostate cancer	II	NCT03600350	41
Dendritic cell-based p53 vaccine	Nivolumab Ipilimumab	-	Small-cell lung cancer	I/II	NCT03406715	14

Abbreviations: NCT, number of clinical trials; ICI, immune checkpoint inhibitor; NCT, number of clinical trials; GM-CSF, granulocyte-macrophage colony-stimulating factor, PSA, prostate-specific antigen.

Table 2. Selected trials administering immune checkpoint blockers in combination with cancer vaccines.

Checkpoint Blockers	Cancer Vaccines	Type of Malignancies	Study Phases	References or NCT
Pembrolizumab	T-VEC	Melanoma/ Advanced head and neck squamous cell carcinoma	Phase III	[31-33]
	DNA vaccine encoding PAP	mCRPC	Ongoing trials	NCT02499835
	HPVE6/E7GX-188E	Cervical cancer	Phase I/II trial	NCT03444376
	PVX-410	HLA-A2+ metastatic triple negative breast cancer	Phase I	NCT03362060
Nivolumab	NEO-PV-01	Melanoma/lung cancer/bladder cancer	Phase I	NCT02897765
	Multi-peptide vaccines	Unresectable naive or Ipilimumab-refractory stages III to IV melanoma	Phase I	[42-45]
	Viagenpumatucel-L	NSCLC	Phase II	[46, 47]
Atezolizumab	A personalized RNA mutanome vaccine (RO7198457)	Melanoma, NSCLC, TNBC, HNSCC, colorectal cancer and RCC	Phase I	NCT03289962
	Sipuleucel-T and RO7198457 (an mRNA vaccine)	mCRPC/locally advanced or metastatic tumors	Phase I	NCT03024216 NCT03289962
Durvalumab	DC/AML vaccine (fusion of autologous AML	AML	Phase II	NCT03059485
	Neoantigen DNA vaccine	Triple-negative breast cancer	Phase I	NCT03199040
	BCG (Bacterial cancer vaccine)	Bladder cancer	Phase I/II	NCT03317158
Avelumab	TG4001	Oropharyngeal cancer	Phase I/II	NCT03260023
Ipilimumab	PROSTVAC and Sipuleucel-T	mCRPC	Phase I	[59, 60]
	GVAX	chemotherapy-naive mCRPC	Ongoing trials	[61]
	Pexa-vec	Metastatic/advanced solid tumors	Phase I	NCT0297715
	NeoVax	Clear cell RCC	Phase I	NCT02950766
Anti-Lag3 Ab (IMP321)	Peptide vaccines	Metastatic breast carcinoma, metastatic melanoma, and advanced pancreatic adenocarcinoma	Ongoing trials	[69, 71]

Abbreviations: NCT, number of clinical trials; mCRPC, acute myeloid leukemia (AML); metastatic castration-resistant prostate cancer; NSCLC, non-small cell lung cancer; TNBC, triple-negative breast cancer; RCC, renal cell carcinoma; AML, acute myeloid leukemia; HNSCC, head and neck squamous cell carcinoma.

an ORR of 62%, almost twice that of pembrolizumab (34%) and T-VEC (26%) administered alone (n = 713) [35, 36]. Similarly, combining a DNA vaccine encoding prostatic acid phosphatase (PAP) with pembrolizumab has shown promising results in patients with mCRPC (n = 66) *(NCT 02499835)* [37]. In recent trials, pembrolizumab was coadministered with three vaccines, namely, DPX-Survivac, NeoVax, and a peptide vaccine, for the treatment of advanced ovarian cancer, pancreatic ductal adenocarcinoma/colorectal adenocarcinoma, and glioblastoma, respectively. However, some conventional therapies were also included in these clinical trials *(NCT03029403, NCT02600949, NCT02287528)*. HPVE6/E7GX-188E (a DNA vaccine) was also combined with pembrolizumab for cervical cancer patients in a phase I/II trial (n = 60) *(NCT03444376)*. Finally, a phase I study is ongoing in patients with HLA-A2+ metastatic triple-negative breast cancer with a mixture of PVX-410 vaccine plus pembrolizumab *(NCT03362060)*.

Overall, this combinatorial approach did not increase the toxicity of each monotherapy, and it seemed to have a manageable safety profile. The most common adverse effects (AEs) were fever, chills, fatigue, rash and arthralgia, all of which are manageable. However, more critical AEs, such as autoimmune hepatitis, grade 3 aseptic meningitis, and grade 4 pneumonitis, were also reported in a few patients, which led to the exclusion of these patients from the respective studies based on previously defined protocols [34, 36].

2.1.1.2. Nivolumab

Nivolumab is another anti-PD-1 monoclonal antibody that has received FDA approval for several malignancies, such as NSCLC, unresectable/metastatic melanoma, refractory Hodgkin's lymphoma, and advanced renal cell carcinoma (RCC) [21, 38, 39]. It is also being investigated in several preclinical and clinical studies alongside other therapies, such as radio/chemotherapy, costimulatory receptor agonists, other checkpoint blockers, and cancer vaccination [38, 40-43]. Nivolumab and the NEO-PV-01 vaccine were administered in a phase I study *(NCT02897765)* in melanoma, lung cancer, and bladder cancer patients.

Most of the completed or ongoing trials have shown promising results after coadministration of nivolumab and different cancer vaccines, although the addition of nivolumab to the gp100 peptide vaccine did not enhance its clinical benefit in advanced melanoma patients [44-46]. Two similar phase I trials evaluated the treatment efficiency of multi-peptide vaccines plus Nivolumab (MART-1/NY-ESO-1/gp100 with Montanide ISA 51 VG) for patients with unresectable naïve or Ipilimumab-refractory melanoma (stages III-IV). These studies illustrated that this combination could stimulate immunological activity with promising survival outcomes in advanced melanoma patients, and in one of them, a median RFS of 47.1 months was reported, which was significantly longer than the historical median RFS (12-21 months) [47-50]. Viagenpumatucel-L, which is an allogenic cell-based vaccine derived from a gp96-Ig-secreting tumor cell line, was combined with nivolumab in a phase II study for the assessment of remedial effects in NSCLC [51, 52]. Finally, in a preclinical study, a DC tumor lysate-based vaccine combined with PD-1 mAbs demonstrated long-term survival in mice bearing large established glioma tumors [53].

Interestingly, the beneficial effects of anti-PD-1 and vaccine combinations are also dependent on their administration time, so differently timed combination strategies of vaccines and checkpoint blockers will have varying effects on the final results. For example, successful responses to a prostate-specific antigen (PSA)-targeted DNA vaccine against prostate cancer were only observed with concurrent use of PD-1 checkpoint blockade but not with their sequential administration; however, for TG4010 (Muc-1-targeted MVA vaccine), PD-1 blockade should be administered several days after the vaccine [54, 55]. These time-dependent effects were also observed in some other checkpoint blockers. Similarly, it has been observed that CTLA-4 blockade restricted tumor growth more efficiently when administered 1 day after vaccination, whereas same-day administration did not produce antitumor responses [56]. Nevertheless, according to other evidence, CTLA-4 and PD-1 blockade administration before cancer vaccination diminished tumor progression and improved long-term survival.

Similar to the above-mentioned trials on pembrolizumab, these combinatorial approaches with nivolumab were relatively safe and well-tolerated. Mild to moderate fatigue and injection-site reaction were the most common AEs; although grade 3 irAEs (immune-related adverse effects), such as optic neuritis, fever, pneumonitis, and rash, may also occur, they are easily managed with systemic steroids [44, 48].

2.1.2. Anti-PD-L1 (Atezolizumab, Avelumab, and Durvalumab)

2.1.2.1. Atezolizumab

Atezolizumab is an anti-PD-L1 monoclonal antibody that has been FDA approved for some malignancies, including locally advanced/metastatic urothelial carcinoma and NSCLC [21]. It is also being tested in combination with various vaccine platforms in order to improve its therapeutic capability (Table **2**). A personalized RNA mutanome vaccine (RO7198457) in combination with atezolizumab is being explored for the treatment of melanoma, non-small cell lung cancer (NSCLC), triple-negative breast cancer (TNBC), head and neck squamous cell carcinoma (HNSCC), colorectal cancer, and renal cell carcinoma (RCC) (*NCT03289962*) in a phase I trial. Furthermore, in a phase II trial, the CDX-1401 vaccine (DEC-205/NY-ESO-1 fusion protein) was coadministered with atezolizumab and guadecitabine in recurrent ovarian cancer patients (*NCT03206047*). Finally, Sipuleucel-T and RO7198457 (an mRNA vaccine) in combination with atezolizumab are separately undergoing phase I trials for mCRPC and locally advanced or metastatic tumors (*NCT03024216*, *NCT03289962*).

2.1.2.2. Avelumab

Avelumab is another anti-PD-L1 antibody with FDA approval for locally advanced/metastatic urothelial carcinoma

and metastatic Merkel cell carcinoma [21]. TG4001, which is a viral vector-based HPV-targeted vaccine, is being combined with avelumab for oropharyngeal cancer in a phase I/II study. This clinical trial is still continuing, and currently available outcomes have shown an increase in overall survival up to 3 years. However, some AEs have been reported as well (*n* = 150) (*NCT03260023*).

2.1.2.3. Durvalumab

Durvalumab is also FDA approved for some malignancies, such as locally advanced/metastatic urothelial carcinoma [21]. There are several ongoing combination trials of vaccines plus durvalumab (Table **2**). A neoantigen DNA vaccine for patients with triple-negative breast cancer is being examined in combination with durvalumab (phase I/*NCT03199040*). This mAb has also been combined with MEDI0457 (DNA vaccine) for p16 or HPV16/18^{+} in HNSCC patients (*NCT03162224*). Additionally, for bladder cancer, there is a phase I/II trial in which an intravesical bacterial cancer vaccine (BCG) in combination with durvalumab plus EBRT (*NCT03317158*) is being analyzed. In a phase II study (*NCT03059485*), the DC/AML vaccine (fusion of autologous acute myeloid leukemia (AML) cells and DCs) administered alone or with durvalumab is also being investigated in AML patients after chemotherapy-induced remission. Finally, in patients with platinum-resistant/ refractory peritoneal malignancies, ONCOS-102 (intraperitoneal viral oncolytic vaccine) has been tested alone or in combination with durvalumab and thus far has demonstrated beneficial and safe outcomes. In fact, this study is still continuing; however, current secondary results have shown objective response rate (ORR), and progression-free survival (PFS) prolonged up to 15 months and overall survival up to 4 years (*n* = 67; *NCT02963831*).

2.1.3. Anti-CTLA-4 (Ipilimumab and Tremelimumab)

2.1.3.1. Ipilimumab

Ipilimumab, which is recognized as a CTLA-4 blocking monoclonal antibody, first acquired FDA approval for metastatic melanoma treatment [57]. It caused a dramatic improvement in the overall survival (OS) of melanoma patients, though it failed in achieving significant clinical results in other solid tumors as a single agent [21]. Therefore, in several trials, it is coadministered with other cancer therapies, such as metabolic regulators, chemotherapy, other checkpoint blockers, and cancer vaccines, in order to improve its efficacy in solid tumor treatment [32, 58-60].

Although initial research results in humans failed to demonstrate that cancer vaccine and ipilimumab combinations had clinically relevant benefits, more recent and advanced vaccine platforms have considerably improved by adding ICIs (Table **2**). [61, 62]. For instance, in a phase II study, the ORR and OS of patients with pretreated advanced melanoma were markedly improved by using a combination of DC vaccine plus ipilimumab compared to either one alone (*n* = 39) [63]. Several investigations have analyzed the potential benefit of combining ipilimumab with mCRPC vaccines. Two-phase I trials assessed PROSTVAC and Sipuleucel-T with escalated-dose ipilimumab in mCRPC patients (1, 3, 5, and 10 mg/kg) [64, 65]. The average OS of patients receiving subcutaneous PROSTVAC alongside 10 mg/kg ipilimumab was 37.2 months, which was significantly longer than historical controls of PROSTVAC or ipilimumab alone (*n*=30) [64]. In another study, fixed-dose GVAX plus escalated-dose ipilimumab were coadministered in chemotherapy-naive mCRPC. In 25% of patients, PSA declined by< 50% from baseline and, interestingly, four patients attained a stable condition (*n* = 28) [66]. Other studies evaluated the curative efficacy of ipilimumab with vaccines for advanced melanoma. A phase II trial analyzed T-VEC combined with intravenous ipilimumab (*n*=198) and indicated that ORR was markedly higher (39% *vs*. 18%) in the combination therapy group compared to subjects who received ipilimumab alone [67]. Another trial indicated that advanced melanoma patients who received ipilimumab with a gp100 peptide vaccine had an OS of 10 months, which was higher than the 6.4-month OS in patients who received the gp100 vaccine alone. Nevertheless, another phase II trial that used peptide vaccines with an extended dose of ipilimumab failed to show significant responses [53]. In other malignancies, vaccine-ipilimumab combinations have shown significant effects. For instance, ipilimumab with intratumoral injections of Pexa-Vec and NeoVax was tested in phase I clinical trials for metastatic/advanced solid tumors and clear cell RCC (*NCT0297715, NCT02950766*), respectively. In another study, 30 previously treated pancreatic adenocarcinoma patients were divided randomly into groups receiving ipilimumab alone or ipilimumab plus GVAX. Overall survival for one year was superior in the combination group (7 *vs*. 27%) [68].

Combining ipilimumab with therapeutic cancer vaccines is not only able to enhance antitumor activity and the ultimate survival of patients but also a relatively safe and well-tolerated approach with no additional toxicity. The most frequently occurring AEs in groups that received combination therapies seemed to be fatigue, chills, diarrhea, pruritus, rash, colitis and endocrine events, which were all manageable and responded to steroids and hormone replacement. However, in trials that used GVAX, injection-site reactions and influenza-like illness were also reported as immune-related AEs [68, 69].

2.1.3.2. Tremelimumab

Tremelimumab, which is a fully human anti-CTLA-4 monoclonal antibody, is being investigated in the treatment of several cancers, such as melanoma, mesothelioma, and non-small cell lung cancer. Not many trials have examined the therapeutic effects of this checkpoint blocker in combination with cancer vaccines. A phase I/II study combining an mRNA vaccine, BI1361849, with tremelimumab and durvalumab on patients with NSCLC has indicated promising results. This clinical trial (initiated in May, 2017) is continuing, although its current outcomes have shown an increase in overall survival up to 5 years. (*n*=59) [70]. It seems that more studies are needed to evaluate the remedial

effects of this checkpoint blocker in association with cancer vaccines.

2.1.4. Anti-LAG-3 and TIM-3

LAG-3 (CD223) and TIM-3 (CD366; also known as HAVCR2) are other inhibitory receptors that are expressed on dysfunctional or exhausted TILs upon persistent antigenic stimulation [71, 72]. In several trials, their specific blocking antibodies are being tested alone or in combination with other conventional therapies for the treatment of various malignancies, including hematologic or solid tumors, and have demonstrated promising outcomes; however, none of them are FDA approved yet [73, 74]. Furthermore, numerous studies investigate the therapeutic effects of LAG-3 and TIM-3 blockade in combination with other ICIs or even with cancer vaccines. They are usually coexpressed with conventional inhibitory checkpoints, particularly PD-1, and their dual blockade has shown more clinical benefits than the blockade of only one of them [75]. Although these checkpoint blockers seem to be capable and beneficial adjuvants for cancer vaccines, to date, not many studies have tested this combinatorial approach. A LAG-3-targeting agent known as *IMP321*, which is a soluble recombinant fusion protein, has been administered as monotherapy or in association with other treatments, such as peptide vaccines, in metastatic breast carcinoma, metastatic melanoma, and advanced pancreatic adenocarcinoma patients; these combinations were able to induce specific CD4 and CD8 T-cell responses [74, 76]. More investigations are needed in this field, especially for combinatorial approaches using cancer vaccines with anti-TIM-3 antibodies.

2.1.5. Checkpoint Blocker Cocktails in Combination with Cancer Vaccines

Some analyses with similar approaches have applied more than two therapeutic components in cancer combinatorial therapy. For instance, several studies are concurrently examining the curative efficacy of the GVAX vaccine in combination with dual checkpoint inhibitors, such as ipilimumab and anti-PD-1 antibodies (Nivolumab and Pembrolizumab), or with neoadjuvant and other conventional cancer therapies, such as cyclophosphamide for pancreatic cancer (*NCT03190265*). They have progressed to phase II, and given that pancreatic cancer is considered a cold tumor that does not elicit a strong immune response, these approaches are anticipated to produce favorable outcomes for this malignancy [77]. Following are some of the most recent clinical trials examples using therapeutic cocktails containing ICIs:

2.1.5.1. Pembrolizumab Cocktails

a) **Pembrolizumab** plus cyclophosphamide was coadministered with DPX-Survivac (peptide vaccine) in patients with peritoneal, ovarian or fallopian tube cancer. The trial is in phase II and has shown promising data (*NCT03029403*).

b) **Pembrolizumab** plus cyclophosphamide, SBRT, and Adjuvant was coadministered with the GVAX vaccine in pancreatic cancer patients in the phase II trial and has shown favorable outcomes (*NCT02648282*).

c) **Pembrolizumab** plus Epaccadostat+CRS-207 was coadministered in combination with GVAX in patients who have been diagnosed with pancreatic cancer, and the trial is in phase II (*NCT03006302*).

d) **Pembrolizumab** plus temozolomide and radiotherapy was coadministered in combination with a personalized neoantigen peptide vaccine (NeoVax) in patients with glioblastoma. The trial is still in phase I (*NCT02287428*).

2.1.5.2. Nivolumab Cocktails

a) **Nivolumab** plus ipilimumab, CRS-207, and cyclophosphamide was coadministered with GVAX (GM-CSF-secreting tumor cells) in pancreatic cancer patients in a phase II study (*NCT03190265*).

b) **Nivolumab** plus GM-CS was coadministered with a DNA vaccine encoding PAP antigen (pTVG-HP) in nonmetastatic, PSA-recurrent prostate cancer patients in a phase II study (*NCT03600350*).

c) **Nivolumab** plus ipilimumab was coadministered in combination with a dendritic cell-based p53 vaccine in patients with small-cell lung cancer. The trial is in phase I/II and has reported promising data (*NCT03406715*).

2.1.6. Predictive Biomarkers

To date, no effective biomarkers have been identified to predict which patients will respond to immunotherapy, although recent studies have indicated that a high mutational burden in addition to mismatch repair deficiency can produce neoantigens and ultimately increase the immunogenicity of tumors [78, 79]. These features may be biomarkers for the response to ICI treatment in general. The predictive value of these biomarkers changes with combination therapies. Evidence suggests that the numbers of regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) may be biomarkers in these approaches because a marked increase in peripheral Tregs has been detected in non-responder patients in trials that used nivolumab or ipilimumab plus vaccines. This trend of suppressive immune cells was proven to correlate with progressive disease [44, 80]. Treg cell frequency in the tumor microenvironment and peripheral blood is an important predictive factor in ICI-based immunotherapy. Numerous studies have reported positive correlations between intratumoral $FoxP3^+$ Treg infiltration with poor prognosis/clinical outcomes in various tumor types [81].

Furthermore, tumor mutational burden or tumor mutational burden (TMB) is an emerging biomarker of sensitivity to immune checkpoint inhibitors. According to numerous studies, patients with high TMB status treated with immune checkpoint blockade therapy have shown prolonged PFS and OS compared to those with low or intermediate TMB

[82, 83]. Immunohistochemistry (IHC) PD-L1 expression pattern is perhaps the most widely used predictive biomarker for distinguishing patients who effectively respond to ICIs [81].

2.2. Classical Cancer Therapies Combined with Cancer Vaccines

2.2.1. Chemotherapy

Chemotherapy, as a prevalent and conventional therapy for cancer treatment, mainly utilizes cytotoxic agents, which give rise to DNA damage and tumor phenotype alterations that eventually make cancerous cells more susceptible to cytotoxic T lymphocytes (CTL) killing [84, 85]. Furthermore, these agents have some direct or indirect effects on antitumor immune system functions and thus might be effective adjuvants for cancer vaccines [86]. Chemotherapy is proven to not only decrease tumor burden and suppressive immune cells, such as Treg and MDSC but also reinforce the immune response by enhancing dendritic cells (DCs) maturation, increasing natural killer cells (NK)-mediated immunosurveillance, and expanding immune-supportive cells, such as M1 macrophages and CD4+ and CD8+ T cells [87-89]. Several chemotherapeutic agents, including *Docetaxel*, *Gemcitabine*, *Cyclophosphamide (CTX)* and *Irinotecan*, have been coadministered with vaccination in various clinical trials. Metastatic breast cancer patients were evaluated in a phase II trial using docetaxel alone or in combination with PANVAC (which contains MUC-1, CEA, and costimulatory molecules B7.1, ICAM-1, and LFA-3). Median PFS was considerably increased in the combination therapy group (7.9 months *vs*. 3.9 months; *n*=48 $p = 0.09$). According to their results, there was no significant difference in adverse events between the two arms of the study, and most of AEs were mild and manageable [90]. Similarly, in another study, the clinical benefits of *docetaxel* in combination with Ankara vaccine (TroVax; targeted tumor antigen 5T4) were indicated in mCRPC patients because better median PFS was observed in the combination therapy group (*n*=80, 9.67 months *vs*. 5.1 months). Comparative safety and immunological/clinical efficacy were also reported [91].

In a phase II study on resected pancreatic cancer patients, *gemcitabine* in combination with the Algenpantucel-L vaccine and 5-fluorouracil-based standard adjuvant chemoradiotherapy improved the median PFS and OS in 12 months compared to previous data [92, 93]. Similar research was conducted with a p53 synthetic long peptide (SLP) vaccine in ovarian cancer patients, although the results were mixed. According to its result, predominant grade 3/4 toxicities were just nausea/vomiting and dyspnea. Grade 1/2 toxicities consisted of fatigue (78%) and Pegintron-related flu-like symptoms (72%) [94]. Additionally, clinical benefits of the *irinotecan* plus G17DT vaccine were demonstrated in metastatic colorectal cancer patients. Study outcomes demonstrated that treatment with G17DT in combination with irinotecan results in an acceptable anti-G17 immune response, which correlated with promising survival. Survival was significantly longer for anti-G17 responders than for non-responders (9.0 *vs*. 5.6 months; $P < 0.001$). They also showed that the combination approach did not significantly increase toxicity [95].

Despite mostly promising outcomes, there are also some contradictory and unfavorable data on this approach. In a clinical study ($n = 22$), an allogeneic HER2+ GMCSF-secreting whole-cell breast cancer vaccine was administered to metastatic breast cancer patients one day after receiving 300 mg/m^2 *CTX* and 2 mg/kg *trastuzumab* (*NCT00399529*). Based on its outcomes, this combination immunotherapy was safe, with clinical benefit rates at 6 months and 1 year of 55% (95% confidence interval (CI), 32%-77%; $P = 0.013$) and 40% (95% CI, 19%-64%), respectively. Median progression-free survival and overall survival durations were 7 months (95% CI, 4-16) and 42 months (95% CI, 22-70), respectively [96], and in a similar phase II/III study, *CTX* was combined with OPT-822. However, vaccination + *CTX* did not improve PFS or OS in any of these studies (*NCT01516307*) [97]. The Viagenpumatucel-L vaccine plus *CTX* was used in patients with advanced NSCLC who failed to respond to multiple prior therapies (*NCT02117024*). Evidently, more investigations are required in order to improve the efficacy of this treatment cocktail.

2.2.2. Antiangiogenic Therapy

The supply of nutrients and oxygen to cancerous cells is a critical factor in their proliferation, immune escape, and metastatic dissemination. Proangiogenic molecules, such as vascular endothelial growth factor (VEGF), play important roles in this process, and hence, numerous antiangiogenic agents targeting the VEGF/VEGF receptor (VEGFR) pathway (*e.g*., *Bevacizumab*, *Sunitinib and Sorafenib*) have been developed in the cancer treatment [98, 99]. Accordingly, several preclinical studies have demonstrated that these agents also have synergistic effects with therapeutic cancer vaccines. For instance, low doses of anti-VEGFR2 antibody (DC101) combined with a mitomycin C-pretreated MCaP0008 cancer cell vaccine significantly improved tumor control in murine models of breast cancer [100].

To date, these combinatorial approaches have not demonstrated significant clinical efficacy in human trials, although numerous clinical trials are ongoing. A DC-based vaccine (AGS003) was combined with *sunitinib* in intermediate- and low-risk mRCC patients ($n = 462$) [101] (*NCT 01582672*). In addition, in phase II studies, *bevacizumab* was coadministered with different peptide vaccines in glioblastoma patients (*NCT02754362*, *NCT01814813*).

2.2.3. Hormone Therapy

The combination of therapeutic cancer vaccines with hormonal therapy, especially in hormonally driven tumors, such as prostate cancer and breast cancer, is an attractive and potentially effective approach, although not many trials have been conducted in this regard. Two ongoing phase II trials are investigating the treatment efficacy of PROST-VAC in combination with an androgen receptor antagonist (*Enzalutamide*) in metastatic prostate cancer patients (*NCT01867333*, *NCT01875250*). The combination of a PSA

pox virus vaccine with sequential androgen ablation therapy has shown promising outcomes [102, 103].

2.2.4. Radiotherapy

Radiotherapy (RT), as a conventional cancer treatment, is considered a part of standard care for different malignancies. This approach can enhance the destruction of tumor cells *via* direct and indirect effects on both cancerous and immune system cells. According to several studies, it can also be an impressive adjuvant for therapeutic cancer vaccines and exert synergistic effects with them. In preclinical research on mouse models, the combination of vaccines and RT was demonstrated to enhance vaccine-mediated destruction of tumor cells *via* upregulation of ICAM-1, MHC, Fas, and tumor-associated antigens (TAAs) [104-106]. Several ongoing trials are evaluating the safety and efficacy of this combinatorial approach. A phase I study is evaluating the efficacy of local radiation in combination with a self-adjuvanted mRNA cancer vaccine (RNActive(R)) for patients with stage IV non-small-cell lung cancer (NSCLC) (*NCT01915524*). This trial demonstrated that RNActive self-adjuvanted mRNA vaccines have the potential to simultaneously induce immune responses to a wide panel of antigens commonly expressed in tumors [107]. Additionally, in a phase II study, the therapeutic potency of *samarium-153 EDTMP* (Sm-153) (a radiopharmaceutical agent) alone or in combination with the PSA-tricom vaccine is being evaluated in metastatic castration-resistant prostate cancer (mCRPC) patients after receiving *docetaxel* [108, 109]. A phase III trial in patients with intermediate/high-risk localized prostate cancer is investigating the combination of RT and aglatimagene besadenovec (ProstAtak®), which is a prostate cancer vaccine (*NCT01436968*).

2.3. Other Immunotherapies Combined with Cancer Vaccines

As mentioned above, cancer vaccination alone can rarely induce an immune response strong enough for complete tumor eradication, and therefore, some combination strategies are needed to improve their potency and efficiency. Indeed, numerous studies have indicated that immunomodulating treatments may be potential candidates for combination with tumor vaccines and may eventually lead to a more powerful anti-cancer immune response. These agents that can enhance the therapeutic effects of vaccines by reinforcing T-cell activation and expansion, along with DC maturation, contain immune-enhancing cytokines and immune agonists.

2.3.1. Immunocytokines

Coadministration of immunostimulatory cytokines, such as interleukin-2 (IL-2), IL-7, and granulocyte-macrophage colony-stimulating factor (GM-CSF), or blockade of immunosuppressive cytokines, such as transforming growth factor-β (TGF-ß) combined with cancer vaccines, can augment their effects [110]. Multiple clinical trials have investigated these combinatorial approaches. IL-2, which has gained FDA approval for metastatic renal cell carcinoma (mRCC) and metastatic melanoma, was coadministered at a high dose with a gp100 peptide vaccine in 185 patients with locally advanced stage III and stage IV cutaneous melanoma [111, 112]. The trial is in phase III and has demonstrated a better overall clinical response (16% *vs.* 6%, $P = 0.03$) in the combination group [112]. However, a phase I/II investigation that used a DC vaccine plus low-dose IL-2 for mRCC or breast cancer patients failed to show significant clinical responses [113]. The combination of Sipuleucel-T plus subcutaneous IL-7 (CTY107) is currently being evaluated in a phase III study (*NCT01881867*). The most frequently applied cytokine in combination with cancer vaccines appears to be GM-CSF; for instance, Sipuleucel-T and T-VEC, which are FDA-approved vaccines, have been engineered to secrete GM-CSF. However, according to some studies, GM-CSF is an inert adjuvant for vaccines. A phase III trial that administered a peptide vaccine alone or with GM-CSF in patients with stage IV or high-risk stage III melanoma failed to identify any survival benefit [114, 115]. The combination of the PROSTVAC vaccine with GM-CSF is under analysis in a phase III trial (*NCT01322490*).

2.3.2. Immune Agonists

Stimulation of costimulatory receptors, such as OX40, 4-1BB, CD137, GITR and ICOS, or the use of immunostimulatory adjuvants, such as TLR ligands, can be beneficial when combined with therapeutic cancer vaccines. Concurrent administration of utomilumab (4-1BB agonist) and the ISA101b vaccine in HPV-16-positive incurable oropharyngeal cancer patients is currently in a phase II clinical trial (*NCT0325800*). Furthermore, for resected stage IIb-IV melanoma patients, an allogenic vaccine has been engineered to express the HLA-A2/4-1BB ligand. Coadministration of this vaccine with *cyclophosphamide* has shown some benefits in clinical investigations (*NCT01308294*). In a phase I/II trial (*NCT00534209*), vaccine therapy with allogeneic B7.1/HLA-A1 was administered to patients with stages IIIb/IV NSCLC who completed first-line chemotherapy. Toll-like receptor (TLR) ligands are promising candidates for the development of immune responses induced by cancer vaccines. Some ligands of these receptors are now used as adjuvants in both cancer and infectious disease vaccines. TLR3, 4, 7/8, and 9 agonists are promising cancer immunotherapeutics [116]. According to a study, HPV-16 E7 DNA vaccine in combination with α-GalCer (α-Galactosylceramide) and MPL (monophosphoryl lipid A) that are TLR4 ligand and natural killer T cell, respectively, shows the potential to reinforce immune responses against cervical cancer [117].

CONCLUSION

To date, no effective biomarkers have been confirmed for predicting which patients will respond to immunotherapy, although recent studies have indicated that a high mutational burden, in addition to mismatch repair deficiency, can produce neoantigens and ultimately increase tumor immunogenicity [79]. These features represent nonspecific favorable biomarkers for ICI treatment response. The value of

these predictive biomarkers varies among combination therapies. The numbers of Tregs and myeloid-derived suppressor cells (MDSCs) might represent valid biomarkers because in trials testing nivolumab or ipilimumab in combination with vaccines in non-responder patients, a marked increase in peripheral Tregs was detected. This trend of suppressive immune cells was proven to correlate with progressive disease [80]. According to various preclinical and clinical data, the combination of vaccines and immune checkpoint inhibitors can improve the therapeutic effects of each agent by intensifying immunogenicity and modulating the immunosuppressive tumor microenvironment, and these synergistic effects have been proven in both *in vitro* and clinical investigations [25]. Although the majority of clinical trials support the synergistic effects of combination therapy, some do not obtain such results. Finally, more ongoing investigations evaluating these combinatorial approaches will contribute to improving cancer treatment in the near future.

AUTHOR'S CONTRIBUTIONS

Writing—original draft preparation, M.S.; writing—review and editing, L.V.S., M.G.-H. and A.A.Z.J.; supervision, M.G.-H. and A.A.Z.J.

LIST OF ABBREVIATIONS

AE	=	Adverse Effect
AML	=	Acute Myeloid Leukemia
CI	=	Confidence Interval
CTL	=	Cytotoxic T Lymphocytes
CTLA-4	=	Cytotoxic T-Lymphocyte-Associated Protein 4
DC	=	Dendritic Cell
DCR	=	Disease Control Rate
FDA	=	FOOD and Drug Administration
GM-CSF	=	Granulocyte-Macrophage Colony-Stimulating Factor
HNSCC	=	Head and Neck Squamous Cell Carcinoma
HPV	=	Human Papillomavirus
ICB	=	Immune Checkpoint Blocker
ICI	=	Immune Checkpoint Inhibitor
IHC	=	Immunohistochemistry
IL	=	Interleukin
LAG-3	=	Lymphocyte Activation Gene-3
mCRPC	=	Metastatic Castration-Resistant Prostate Cancer
MDSC	=	Myeloid-Derived Suppressor Cell
MPL	=	Monophosphoryl Lipid A
mRCC	=	Metastatic Renal Cell carcinoma
NCT	=	Number of Clinical Trials
NK	=	Natural Killer Cell
NSCLC	=	Non-Small Cell Lung Cancer
ORR	=	Objective Response Rate
OS	=	Overall Survival
PAP	=	Prostatic Acid Phosphatase
PD-1	=	Programmed Death Protein-1
PD-L1	=	Programmed Death Ligand-1
PFS	=	Progression-Free Survival
PSA	=	Prostate-Specific Antigen
RCC	=	Renal Cell Carcinoma
RFS	=	Relapse-Free-Survival
RT	=	Radiotherapy
TAA	=	Tumor-associated Antigen
TIL	=	Tumor-infiltrating Lymphocyte
TIM-3	=	T- cell Immunoglobulin and Mucin Domain 3
TLR	=	Toll-like Receptor
TMB	=	Tumor Mutational Burden
TME	=	Tumor Microenvironment
TNBC	=	Triple-negative Breast Cancer
Tregs	=	Regulatory T Cells
VEGF	=	Vascular Endothelial Growth Factor
VEGFR	=	Vascular Endothelial Growth Factor Receptor

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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