Chemotherapy-induced immunomodulation in non-small-cell lung cancer: a rationale for combination chemoimmunotherapy

Hua Zheng^{1,2}, Masha Zeltsman¹, Marjorie G Zauderer³, Takashi Eguchi¹, Raj G Vaghjiani¹ & Prasad S Adusumilli^{*,1,4,5}

¹Thoracic Service, Department of Surgery, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, NY, USA

²Department of Oncology, Beijing Chest Hospital, Capital Medical University, 97 Machang, Tongzhou District, Beijing, China

³Thoracic Oncology Service, Division of Solid Tumor Oncology, Department of Medicine, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, NY, USA

⁴Center for Cell Engineering, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA

⁵Deputy Chief, Translational & Clinical Research, Thoracic Service, Department of Surgery; Associate Attending, Thoracic Service, Department of Surgery; Director, Mesothelioma Program; Memorial Sloan Kettering Cancer Center, 1275 York Avenue, NY 10065, USA

*Author for correspondence: Tel.: +1 212 639 8093; Fax: +1 646 422 2340; adusumip@mskcc.org

Spurred by the survival benefits seen with the use of checkpoint blockade in non-small-cell lung cancer (NSCLC), there has been a growing interest in the potential applications of immunotherapy. Despite this, the objective response rate for single-agent immunotherapy remains $\leq 20\%$ in patients with advanced NSCLC. A combinatorial approach that utilizes both chemotherapy and immunotherapy is a potential strategy to increase antitumor efficacy. Accumulating evidence has shown that the immunomodulatory effects of chemotherapeutic agents can be exploited in a combinational approach. Herein, we review the influence of specific chemotherapeutic agents on the tumor immune microenvironment in preclinical and clinical studies, and establish the rationale for combination chemoimmunotherapy for the treatment of NSCLC.

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Lung cancer is the leading cause of cancer deaths worldwide [1] with a 5-year survival rate under 20% [2]. Non-small-cell lung cancer (NSCLC) accounts for approximately 80% of all the lung cancer cases [3]. Adjuvant chemotherapy following complete resection is recommended for treatment of stage II–IIIA NSCLC [4]. For advanced NSCLC, platinum-based cytotoxic chemotherapy, tyrosine kinase inhibitor targeted therapy, and checkpoint blockade immunotherapy have all been shown to improve overall survival (OS) [5]. Adjuvant first-line treatment consists of a regimen of doublet chemotherapy that includes paclitaxel, gemcitabine, docetaxel, pemetrexed, vinorelbine or etoposide combined with either cisplatin or carboplatin [6]. In patients with advanced adenocarcinomas that harbor driver gene mutations, targeted therapy enhances survival benefit. Patients who received genotype-directed therapy reached a median OS of 3.5 years, which was significantly longer than patients with oncogenic driver mutations who did not receive genotype-directed therapy (OS: 2.4 years; $p = 0.006$) [7]. To further improve the outcomes, various combination treatments have also been studied. In the BEYOND trial, median progression-free survival (PFS) and median OS were both prolonged in the group that received chemotherapy with bevacizumab compared with the group that received only chemotherapy (PFS: 9.2 vs 6.5 months; $p < 0.001$; OS: 24.3 vs 17.7 months; $p = 0.0154$) [8]. In the FASTACT-2 trial, intercalated chemotherapy with erlotinib significantly improved survival compared with chemotherapy alone (PFS: 7.6 vs 6.0 months; $p < 0.0001$; OS: 18.3 vs 15.2 months; $p = 0.042$) [9]. The JO25567 trial investigated patients with advanced, nonsquamous NSCLC who harbored *EGFR* mutations and found that median PFS was 16.0 months for erlotinib plus bevacizumab compared with 9.7 months for erlotinib alone ($p = 0.0015$) [10]. By disrupting cancer cell proliferation through multiple pathways, these studies have demonstrated how combination therapy has evolved into a promising strategy. However, despite the advances

in lung cancer treatment with third-generation chemotherapies, targeted therapies, and antiangiogenic therapies, prognosis for these patients remains poor.

Recently, immunotherapy has come into the spotlight because of the significant survival benefit seen with immune checkpoint blockade agents in the treatment of bladder cancer, melanoma, lung cancer, Merkel cell carcinoma, and urothelial cancer. Among them is a class of PD-1/PD-L1 antibodies that include pembrolizumab, atezolizumab, nivolumab, avelumab, and durvalumab. Checkpoint blockade agents have been approved by the United States FDA for the treatment of metastatic NSCLC with progression on or after platinum-based chemotherapy. Of those antibodies, pembrolizumab has been approved as a first-line treatment for NSCLC tumors with PD-L1 expression in more than 50% of cancer cells [11].

Immunotherapy aims to treat cancer by either blocking the immunosuppressive tumor microenvironment or enhancing the cytotoxic potential of immune cells; unfortunately, the heterogeneous nature of NSCLC limits the efficacy of single-agent treatment [12–14]. For instance, the objective response rate of anti-PD-1/PD-L1 therapy for nonselected NSCLC patients is 14–20% [15–17] compared with 44.8% for selected patients who are defined as patients with tumors bearing PD-L1 expression on $\geq 50\%$ of cancer cells [11]. To enhance the antitumor efficacy, there are several ongoing clinical trials that utilize multiple combinations of anti-PD-1/PD-L1 therapy with chemotherapy, targeted therapy, or the CTLA-4 antibody.

Conventional combination therapy utilizes combining agents that target nonidentical mechanistic pathways in order to limit resistance to any single therapy. Although chemotherapy can be cytotoxic to immune cells, accumulating evidence shows that chemotherapy can modulate the immune microenvironment through mechanisms such as increasing the immunogenicity of cancer cells, enhancing the cytotoxicity of T cells and NK cells, and fostering the accumulation of IFN- γ and TNF- α [18–20]. These agents have also been found to decrease immunosuppressive immune cells, such as myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs), which results in a subsequent reduction in inhibitory cytokines such as TGF- β [21,22].

A rationale-based combination of chemotherapy and immunotherapy may synergize and enhance antitumor efficacy. In this review, we illustrate how the tumor immune microenvironment changes following chemotherapy and we establish a rationale for combination chemoimmunotherapy for the treatment of NSCLC by exploring supportive preclinical and clinical studies.

Immunomodulatory effects of chemotherapeutic agents

The immunomodulatory effects of conventional chemotherapeutics in NSCLC are summarized in Table 1.

Cisplatin

Cisplatin is a heavy metal complex containing a central atom of platinum surrounded by two chloride molecules and two ammonia molecules in the cis position [23]. Cisplatin exerts its cytotoxic effect by binding genomic DNA within the cell nucleus. This DNA cross-linkage interferes with cell division and replication, ultimately triggering apoptosis. In addition to its cytotoxic effects, cisplatin has multiple immunomodulatory effects that we have reviewed in a previous publication [24].

In preclinical studies, cisplatin has demonstrated immunostimulatory effects. In a study of NSCLC cell lines, cisplatin upregulated the MHC class I chain related molecule A and B expression, and led to enhanced NK cell-mediated cytotoxicity [18]. In addition to higher expression in cancer cells, Jackaman *et al.* illustrated that MHC class I was also increased in APCs, specifically in CD11c⁺ dendritic cells (DCs), in mice treated with cisplatin [25]. Cisplatin, along with paclitaxel and doxorubicin, also increased the permeability to granzyme B released by cytotoxic T lymphocytes. The authors demonstrated that chemotherapy induced permeability to granzyme B increased, not only the killing of tumor cells by antigen-specific cytotoxic T lymphocytes, but also the apoptosis of neighboring nonantigen expressing tumor cells [26]. In murine models of HPV-associated cancer using cell lines TC-1 and C3, Beyranvand Nejad *et al.* demonstrated that cisplatin stimulates tumor infiltration of APCs that express high T-cell costimulatory ligands, CD70, CD80, and CD86. A mouse model that lacked CD80/86 on APCs exhibited a reduction in the treatment effect of cisplatin. The subsequent use of a CTLA-4 blocker led to an enhanced ability of CD80/86 to bind to CD28 and improved cisplatin-mediated tumor eradication [27].

Multiple studies have also shown that cisplatin can downregulate the immunosuppressive microenvironment as evidenced by a reduction in Tregs and MDSCs [28–30]. Chang *et al.* showed that a dose-dense regimen of cisplatin/paclitaxel significantly reduced MDSCs in ovarian tumor-bearing mice compared with a maximum tolerated dose [28].

Table 1. Immunomodulatory properties of conventional chemotherapeutic agents in non-small-cell lung cancer.

Chemotherapeutic agent	Cytotoxic mechanism	Immune system	Immunomodulatory properties of chemotherapeutic agents		Ref.
			Function enhanced/numbers increased	Function inhibited/numbers decreased	
Cisplatin	Formation of DNA cross-links	Immune cell	NK; CTL; APCs; Mφ	MDSC; Treg	[18,19,27–30,33]
		Cytokine	IL-1α; IL-2; TNF-α	IL-1; IL-6; IL-8; IL-10; IL-12; TNF-α; TNF-β	
		CD, CAM and MHC	MICA/B; CD70; CD80; CD86		
		Other			
Carboplatin	Formation of DNA cross-links	Immune cell	Mφ; CTL		[19,28,36,38]
		Cytokine	IL-1α; IL-2; TNF-α; IFN-γ	IL-10; VEGF; TGF-β; CCL2	
		CD, CAM and MHC			
		Others	H ₂ O ₂ ; O ₂ ^{•-} ; lysozyme; β-N-hexoseaminidase; Gal-1	Arginase	
Paclitaxel	Inhibition of tubulin depolymerization	Immune cell	Mφ; DC; Teff	Treg; MDSC	[33,40,41,43–46,48,50,51]
		Cytokine	IL-2; IL-12	IL-1; IL-6; IL-8; IL-10; IL-12; TNF-α; TNF-β; TGF-β; VEGF; CCL2; CCL22	
		CD, CAM and MHC			
		Other		NF-κB; COX-2; STAT3	
Gemcitabine	Inhibition of DNA polymerase	Immune cell	NK; CTL; Tm	MDSC; Treg; Mφ	[21,22,53,54,56,58]
		Cytokine		TGF-β1	
		CD, CAM and MHC	CD80; CD86; CD40; ICAM-1; VCAM-1; P-selection		
		Other			
Docetaxel	Inhibition of tubulin depolymerization	Immune cell	CTL; Th; NK; LAK;	MDSC; Treg	[20,61,63–65]
		Cytokine	IFN-α; IL-6; GM-CSF; IFN-γ; TNF-α	TNF; TGF-β; IL-1	
		CD, CAM and MHC	CD13; CD15; CD44s; CD44v6; LFA-3; ICAM-1; VLA-4; VLA-5; VLA-6		
		Other		PGE2	

CAM: Cellular adhesion molecule; CCL: Chemokine ligand; CD: Cluster of differentiation; CTL: Cytotoxic T lymphocyte; DC: Dendritic cell; H₂O₂: Hydrogen peroxide; LAK: lymphokine-activated killer cell; LFA: lymphocyte function associated antigen; MDSC: Myeloid-derived suppressor cell; MICA/B: MHC class I chain related molecule A and B; Mφ: Macrophage; O₂^{•-}: Superoxide anion; PGE2: Prostaglandin-E2; Teff: Effector T cell; Tm: Memory T cell; Tregs: Regulatory T cell; VLA: Very late antigen.

In clinical studies, Roselli *et al.* showed that using cisplatin plus vinorelbine treatment for NSCLC patients significantly increased the ratio of effector T cells (Teff) to Tregs [31]. In patients with muscle invasive urothelial carcinoma of the bladder who received cisplatin-based neoadjuvant chemotherapy, response to treatment correlated with the ratio of CD8⁺ tumor-infiltrating lymphocytes to Tregs in pretreatment biopsies ($p = 0.0003$) [32]. High MHC class I chain related molecule A and B expression in resected tissue was also associated with a better prognosis for patients with NSCLC [18]. Koufos *et al.* found that patients with stage III/IV NSCLC who responded to paclitaxel–isofosphamide–cisplatin chemotherapy had a significant increase in IL-2 levels, a decrease in IL-1, TNF-α, TNF-β, IL-6, IL-8, IL-10, and IL-12 levels, and showed a significantly longer OS than nonresponders (26.0 vs 7.5 months; $p < 0.001$) [33].

Carboplatin

As an analog of cisplatin, carboplatin also contains a central atom of platinum but is surrounded by cyclobutane-decarboxylate [34]. Carboplatin has different pharmacokinetic properties from cisplatin and is associated with significant myelotoxicity but less nephrotoxicity and fewer neurological sequelae. The mechanism of action is similar to cisplatin in that it forms DNA cross-links that interrupt cellular DNA functioning, thereby inducing apoptosis [35].

Preclinical studies indicate that carboplatin has an immunostimulatory effect. One study investigated the supernatants of carboplatin-treated (50 µg/ml) or cisplatin-treated (10 µg/ml) macrophages (Mφ) and demonstrated enhancement of IL-1α and TNF-α activity [19]. Upon exposure to tumor cells, carboplatin-activated Mφ demonstrated enhanced tumoricidal activity by increasing the transfer of lysosomes, which leads to tumor lysis. *In vitro* treatment of Mφ with either carboplatin (50 µg/ml) or cisplatin (10 µg/ml) showed a significant increase in the release of cytolytic factors, such as hydrogen peroxide, superoxide anion, IL-1α, lysozyme, and β-N-hexoseaminidase, and led to increased tumor cell destruction [36].

In a clinical study, Denkert *et al.* detected mRNA expression of immunomodulatory factors in EGFR2 (HER2)-positive and triple-negative breast cancer tissue [37]. They found that chemokine ligand (CCL)5, CD8A, and indoleamine-2,3-dioxygenase 1 can predict response to carboplatin. In another study of relapsed, platinum-resistant ovarian cancer, 14 patients had undergone treatment with dose-dependent chemotherapy that consisted of weekly carboplatin (AUC2) and paclitaxel (60–80 mg/m²) [28]. Of the four patients who achieved stable disease, three were found to have an association between IL-2 and IFN-γ levels and cytotoxic CD8⁺ T-cell activity. Another study with 40 patients who received 3 cycles of paclitaxel–carboplatin or carboplatin monotherapy found significantly lower levels of IL-10 ($p < 0.001$), VEGF ($p = 0.04$), TGF-β ($p < 0.001$) and arginase ($p < 0.001$), and higher levels of gal-1 ($p = 0.016$) after chemotherapy compared with levels at the time of diagnosis [38].

Paclitaxel

Paclitaxel, also known as Taxol, induces cancer cell death by promoting the polymerization of tubulin, which disrupts the normal microtubule dynamics required for cell division [39].

Preclinical studies reveal that paclitaxel has an immunostimulatory effect. A study showed that paclitaxel enhances the tumoricidal activity of M1 Mφ [40] and may partially reverse tumor-induced Mφ suppressor activity by inducing production of IL-12 that enhances T-cell responsiveness [41]. Recently, there have been reports of the immunostimulating effects of noncytotoxic, low-dose paclitaxel. ‘Low-dose’ paclitaxel differs from clinical antineoplastic doses in that it is based on a formula derived from preclinical studies by Reagan-Shaw *et al.* [42]. John *et al.* found that administration of paclitaxel at a concentration of 100 µM did not significantly alter the ability of DCs to induce proliferation compared with the untreated group [43]. By contrast, paclitaxel completely abolished cellular proliferation at a higher concentration of 1 mM, thus indicating that DC toxicity was reached at this level. The stimulatory effect of 1 and 10 µM doses of paclitaxel on DCs were significantly higher ($p < 0.05$) when compared with lipopolysaccharide-matured DCs and untreated controls. Furthermore, it has been shown that, at a low dose of 5 nM, the noncytotoxic effects of paclitaxel enhanced antigen presentation by DCs via an IL-12-dependent mechanism [44].

Multiple studies have shown that paclitaxel can also downregulate the immunosuppressive microenvironment. Zhu *et al.* demonstrated, both *in vivo* and *in vitro*, that paclitaxel can selectively impair Tregs while sparing Teff cells under specific low-dose concentrations of 0.1–1 µM [45]. They also observed a reduction in the amount of IL-10 and TGF-β in the Treg supernatant. Concentrations under 0.1 µM did not affect Treg levels, while concentrations > 1 µM began to impair Teff cell function. Additional investigation has indicated that the selective impairment of Tregs by paclitaxel is independent of toll-like receptor 4, which is a transmembrane protein involved in stimulating inflammatory cytokine production. Sevko *et al.* demonstrated that administration of an ultra-low, noncytotoxic dose of paclitaxel (1 mg/kg) caused a significant reduction in the amount of tumor-associated immunosuppression due to a decrease in the quantity and function of MDSCs that are found in the tumors of melanoma-bearing mice [46]. At this ultra-low dose, paclitaxel can promote MDSC differentiation into functional conventional DCs. Hsu *et al.* found that pretreatment with a single, low-dose of paclitaxel in mice (5–10 mg/kg, equivalent to 15–30 mg/m² in patients) can improve the immunosuppressive tumor microenvironment [48]. This study demonstrated that NF-κB/DNA binding activity and immunosuppressive factors, including TGF-β, CCL2, VEGF, CCL22, COX-2, and IL-10, were decreased in the tumors of mice that were pretreated with a single, low-dose of paclitaxel. Inhibition of NF-κB also enhanced the activation and migration of CD8⁺ T cells. Additionally, paclitaxel has also shown activity

against the immunosuppressive factor signal transducer and activator of transcription 3 (STAT3) by disrupting the interaction of STAT3 with tubulin and by inhibiting cytokine-induced STAT3 activation [49,50].

In clinical studies of NSCLC, paclitaxel treatment demonstrated immunomodulatory effects. Zhang *et al.* analyzed 55 peripheral blood samples of NSCLC patients who had undergone paclitaxel-based chemotherapy [51]. Following treatment, there was a selective reduction in the quantity and percentage of CD4⁺CD25⁺FOXP3⁺ Tregs. In line with preclinical findings, paclitaxel preferentially induced apoptosis of Tregs rather than Teff cells. Paclitaxel simultaneously increased the production of helper T-cell type 1 cytokines and the activity of Teff cells.

Based on the above studies, low-dose paclitaxel showed an immunomodulatory effect by increasing cytotoxic immunostimulative factors and inhibiting immunosuppressive factors. Therefore, it may be advantageous to specifically combine low-dose paclitaxel with immunotherapy; however, the optimal immunotherapeutic agent to maximize synergistic effect is still unclear.

Gemcitabine

Gemcitabine is a nucleoside analog that exerts its antitumor effects through the inhibition of DNA replication and DNA chain termination, thereby preventing DNA synthesis and repair, which ultimately leads to apoptosis [52].

Preclinical studies show that gemcitabine has a powerful downregulation effect on the immunosuppressive microenvironment. Suzuki *et al.* showed that gemcitabine was able to specifically reduce the number of MDSCs found in the spleen of animals bearing large tumors without reducing the quantity of other immune cells such as CD4⁺ T cells, CD8⁺ T cells, B cells, NK cells, and Mφ [21]. The loss of MDSCs was accompanied by an increase in the antitumor activity of CD8⁺ T cells and activated NK cells. In another study using the TC-1 cell line in a murine model, multiple injections of gemcitabine resulted in a decrease in the proportion of MDSCs, Tregs, and Mφ in both splenic and tumor tissue compared with controls injected with only phosphate-buffered saline [54]. The authors proposed that gemcitabine alters the tumor microenvironment by decreasing the quantity of immunosuppressive cells, thereby facilitating the eradication of tumor cells.

Gemcitabine has also been shown to affect cell surface adhesion molecule expression. In a murine model of oral cancer, gemcitabine administration demonstrated enhanced tumor-specific T-cell responses with the upregulation of tumor cell surface expression of several immune accessory and cellular adhesion molecules (CAMs) including CD80, CD86, CD40, ICAM-1, VCAM-1 and P-selectin [53]. Suzuki *et al.* found that gemcitabine showed significant antitumor effects even in mice bearing gemcitabine-resistant cell lines [55]. They showed that sensitivity to gemcitabine *in vivo* correlates with tumor immunogenicity as measured by the prophylactic immunity test. The apparent immunomodulatory effects of gemcitabine support the need for further investigation into combination regimens using immunotherapy.

In clinical studies where investigators analyzed peripheral blood samples of pancreatic cancer patients who received gemcitabine therapy, there was a downregulation in inhibitory immune cells, such as MDSCs, Tregs, and molecules (e.g., TGF-β1), without inhibiting the activity of effector cells [22]. Plate *et al.* reported that gemcitabine treatment is not immune depleting based on peripheral blood cell counts before and after treatment in pancreatic cancer patients [56]. They also found that the percentages of CD3⁺ and CD45RO⁺ memory lymphocytes increased, which suggests an increase in naive T-cell activation. While additional studies support the findings that gemcitabine therapy can reduce the percentage and quantity of Tregs in pancreatic cancer patients, the clinical response to gemcitabine-based chemotherapy has not been shown to correlate with the observed reduction in Tregs [57]. In a study of 40 patients with NSCLC who had received gemcitabine plus cisplatin chemotherapy, there was a significant reduction in the percentages of CD4⁺CD25⁺FOXP3⁺ and CD8⁺CD28⁻ Tregs following treatment [58].

Based on the above studies, it appears that gemcitabine has an inhibitory effect on immunosuppressive cells and molecules rather than a stimulatory effect on cytotoxic immune cells. Therefore, there may be an additive effect when gemcitabine is combined with T-cell therapy, especially given the apparent gemcitabine-induced increase in adhesion molecule expression on tumor cell surfaces.

Docetaxel

Docetaxel, an analog of paclitaxel, was originally created through a semisynthetic approach using the needles of the European yew *Taxus baccata* [59]. As a taxane, the tumoricidal function of docetaxel has been attributed to its ability to prevent cytoskeletal microtubule depolymerization to free tubulin, which ultimately arrests neoplastic cell proliferation [60].

Preclinical studies have shown the multiple effects of docetaxel via inhibition of immunosuppressive immune cells, increase in cytotoxic cells, and increase in the expression of CAMs. Kodumudi *et al.* found that docetaxel inhibits the suppressive effect of MDSCs and restores the functional activity of CD4⁺ and CD8⁺ T cells in 4T1- ν tumor-bearing mice [61]. Furthermore, they discovered that docetaxel polarizes MDSCs to an M1-like M ϕ phenotype, in both *in vitro* and *in vivo* models, leading to enhanced antitumor immunity. One report describes a massive infiltration of mononuclear lymphoid cells in four out of ten murine tumors that responded to treatment with docetaxel [62]. In another report, lymphocyte infiltration was found to be a result of a docetaxel-induced influx of helper/inducer T cells and NK cells that enhanced subsequent tumor response to radiotherapy [63]. In the human colon carcinoma cell line HT-29, low concentrations of docetaxel (1×10^{-9} to 3×10^{-9} M) augment cell-to-cell contact by increasing the expression of CAMs such as lymphocyte function associated antigen-3, ICAM-1, CD44s, CD44v6, CD15, CD13, VLA-4, VLA-5, and VLA-6 [20]. Additionally, when cocultured with tumor cells, peripheral blood lymphocytes and lymphokine-activated killer cells produced more TNF- α and IFN- γ in response to docetaxel treatment compared with untreated controls [63].

Clinically, in a study of 40 NSCLC patients, Li *et al.* investigated the effect of docetaxel on 3 subsets of Tregs that were isolated from peripheral blood samples including activated Tregs, resting Tregs, and nonsuppressive Tregs [64]. These subsets were all reduced following docetaxel treatment *in vitro*. Interestingly, only activated Tregs were found to significantly alter the expression of cytokines with increased IFN- α and decreased TGF- β following treatment ($p < 0.05$). In 17 samples, all 3 subsets of Tregs were reduced following four cycles of chemotherapy ($p < 0.05$). In patients with advanced breast cancer, docetaxel treatment increased the mixed lymphocyte reaction values, NK and lymphokine-activated killer cell cytotoxicity, and IL-6, GM-CSF, and IFN- γ levels while decreasing IL-1, TNF, and prostaglandin-E2 levels [65].

Pemetrexed

Pemetrexed is an antifolate that directly affects the enzymes involved in cell activation and cell division [66]. Its role in the immune system has not been fully documented and studies on the immunologic effects of pemetrexed chemotherapy are limited. Available information is from combination studies that will be discussed in the following section.

Combination chemoimmunotherapy

Preclinical studies

In a preclinical study of EL4 lymphoma-bearing mice, researchers investigated the combination of cisplatin with methyl gallate, an inhibitor of Treg migration, and demonstrated enhanced anticancer effects compared with methyl gallate or cisplatin alone ($p < 0.001$) [67]. In a study evaluating the combined efficacy of paclitaxel with DC therapy, lung cancer-bearing mice received a low-dose injection of paclitaxel (2 mg/kg) followed by an intratumoral injection of DCs (group 4) [68]. This combination significantly inhibited tumor growth compared with the control groups (group 1: saline alone; group 2: paclitaxel alone [intraperitoneal injection of 2 mg/kg]; and group 3: DCs alone [intratumoral injection of 2×10^6 DCs per mouse], $p < 0.05$). In group 4, there was an increased infiltration of CD4⁺ and CD8⁺ T cells with elevated levels of IFN- γ in the draining lymph nodes. Hsu *et al.* found that lymphoma-bearing mice pretreated with a single, low dose of paclitaxel (10 mg/kg) have improved antitumor efficacy following adoptive T-cell transfer (ACT) with 5×10^6 CD8⁺ T cells [48]. A study that investigated combination therapy with gemcitabine and SOD mim, a superoxide dismutase mimetic that targets immunosuppressive MDSCs in the tumor microenvironment, found significantly prolonged survival of tumor-bearing mice compared with the control and individual treatment groups (gemcitabine vs SOD mim plus gemcitabine; $p < 0.01$; phosphate-buffered saline vs SOD mim plus gemcitabine; $p < 0.001$; SOD mim vs SOD mim plus gemcitabine; $p < 0.001$) [69]. Moreover, gemcitabine, in combination with FGK45 (CD40 inhibitor), has been shown to increase the expression of class I human leukocyte antigen on malignant cells, increase tumor infiltration of CD4⁺ and CD8⁺ cells [70], and enhance the cross-presentation of tumor antigens to CD8⁺ T cells [71]. As for pemetrexed, the combination of pemetrexed and Treg blockade in murine malignant mesothelioma was associated with decreased tumor infiltrating Tregs, increased IL-2 production, DC maturation, and increased CD3⁺CD8⁺IFN- γ ⁺ tumor-infiltrating T cells compared with mice treated with pemetrexed or Treg blockade alone. Prolonged survival indicates a synergistic antitumor effect of pemetrexed combined with Treg blockade [72].

Table 2. Clinical trials with published data for combination of chemioimmunotherapy in non-small-cell lung cancer.

Trial name	Study group	Control group	Phase	Setting	N	Treatment type	Primary end point	Result, p-value	Ref.
CheckMate 012	Nivolumab (PD-1 antibody) + platinum doublet	–	I	Stage IIIB/IV NSCLC	56	First-line	Safety and tolerability	–	[82]
	Ipilimumab + Carb + Tax (phased)	–	I	Stage IIIB/IV NSCLC	12	First-line	Identify the recommended dose	–	[83]
KEYNOTE-021	Pembrolizumab (PD-1 antibody) + Carb + Pem	Carb + Pem	II	Stage IIIB or IV, non-squamous NSCLC without targetable <i>EGFR</i> mutation or <i>ALK</i> translocation	60: 63	First-line	Objective response rate	55 vs 29%; p = 0.0016	[73]
	Ipilimumab (CTLA-4 antibody) + Carb + Tax (phased and concurrent)	Placebo + Carb + Tax	II	Stage IIIB/IV NSCLC	61: 59: 62	First-line	irPFS	Phased vs control 5.7 vs 4.6 months, p = 0.05, concurrent vs control 5.5 vs 4.6 months, p = 0.13	[74]
	TG4010 + Cis + Gem	Placebo + Cis + Gem	IIB	Stage IIIB [wet] or IV NSCLC MUC1 expression ≥25%	74: 74	First-line	Six month PFS >40% or higher in the experimental group	Experimental 43.2 vs primary end point 40%, p = 0.01; experimental 43.2 vs control 35.1%, p = 0.307	[75]
TIME	TG4010 (MVA/MUC1/IL-2 vaccine) + platinum doublet	Placebo + platinum doublet	IIB/III	Stage IV NSCLC, MUC1 expression 50%	111: 111	First-line	PFS	5.9 vs 5.1 months, p = 0.019	[76]
Carb: Carboplatin; Cis: Cisplatin; Gem: Gemcitabine; irPFS: Immune-related progression-free survival; MVA: Modified vaccinia virus Ankara; NSCLC: Non-small-cell lung cancer; Pem: Pemetrexed; PFS: Progression-free survival; Tax: Paclitaxel.									

Table 3. Ongoing clinical trials for combination of checkpoint blockade with chemotherapy in non-small-cell lung cancer.

Immunotherapy agent	Chemotherapy agent	Clinicaltrials.gov identifier	Trial name	Phase	Setting	Treatment type
PD-1 antibody						
Nivolumab	Carb + Nab-Tax	NCT02309177		I	Stage IIIB or IV NSCLC	First-line
	Irinotecan + capecitabine	NCT02423954		I/II	Advanced NSCLC, pancreatic cancer, renal cell cancer, colorectal carcinoma, endometrial cancer and uterine cancer	Not specified
	Standard chemoradiotherapy	NCT02434081	NICOLAS	II	Stage IIIA/B NSCLC	First-line
	Platinum doublet	NCT02944396		II	Stage IIIB/IV NSCLC	First-line
	Nab-Tax	NCT02967133		II	Stage IIIB/IV NSCLC	Second-line
	Decitabine/tetrahydrouridine	NCT02664181		II	Stage IIIB/IV NSCLC	Second-line
Pembrolizumab	Platinum doublet	NCT02477826	CheckMate 227	III	Stage IV or recurrent NSCLC	First-line
	Cis/Carb + Pem, Carb + Tax/Nab-Tax	NCT01840579	KEYNOTE-011	I	Stage IIIB/IV NSCLC	First-line
	Carb + Tax + radiotherapy	NCT02621398		I	Inoperable stage II disease requiring chemoradiation therapy or stage IIIA or IIIB NSCLC	First-line
	Cis + Etoposide	NCT02987998		I	Stage IIIA NSCLC	Neoadjuvant
	Carb + Nab-Tax	NCT02382406		I/II	Stage IIIB/IV NSCLC	First-line
	Gem	NCT02422381		I/II	Stage IIIB/IV NSCLC	Second- or third-line
	Carb + Tax	NCT02581943		II	Stage IIIB/IV NSCLC	Second- or third-line
	Nab-Tax	NCT02684461		II	Stage IV NSCLC following first-line induction chemotherapy that does not include a taxane	Maintenance of first-line
	Platinum + Pem	NCT02578680	KEYNOTE-189	III	Stage IV nonsquamous NSCLC	First-line
	Carb + Tax/Nab-Tax	NCT02775435	KEYNOTE-407	III	Stage IV squamous NSCLC	First-line
PD-L1 antibody						
Atezolizumab	Carb + Tax + radiotherapy	NCT02525757		II	Stage IIIB/IV NSCLC	First-line
	Carb + Nab-Tax	NCT02716038	MAC	II	Stage IB-IIIa NSCLC	Neoadjuvant
	Carb + Nab-Tax	NCT02367781	IMpower 130	III	Stage IV nonsquamous NSCLC	First-line
	Carb + Tax/Nab-Tax	NCT02367794	Impower 131	III	Stage IV squamous NSCLC	First-line
	Platinum + Pem	NCT02657434	Impower 132	III	Stage IV nonsquamous NSCLC	First-line
	Carb + Tax + Bev	NCT02366143	Impower 150	III	Stage IV nonsquamous NSCLC	First-line
	Cis + Doc	NCT02572843		II	Stage IIIA (N2) NSCLC	Neoadjuvant
CTLA-4 antibody						
Ipilimumab	Carb + Tax	NCT02279732		III	Stage IV or recurrent NSCLC	First-line
	Carb/Cis + Tax	NCT01820754		II	Stage IB-III squamous NSCLC	Neoadjuvant

Bev: Bevacizumab; Carb: Carboplatin; Cis: Cisplatin; Doc: Docetaxel; Gem: Gemcitabine; Nab-Tax: Nanoparticle albumin-bound paclitaxel; NSCLC: Non-small-cell lung cancer; Pem: Pemetrexed; Tax: Paclitaxel.

Table 3. (cont.). Ongoing clinical trials for combination of checkpoint blockade with chemotherapy in non-small-cell lung cancer.

Immunotherapy agent	Chemotherapy agent	Clinicaltrials.gov identifier	Trial name	Phase	Setting	Treatment type
Combination of PD-1/PD-L1 antibody with CTLA-4 antibody						
Durvalumab + Tremelimumab	Carb/Cis + Gem/Pem	NCT03057106		II	Stage IV NSCLC	First-line
Nivolumab + Ipilimumab	Platinum doublet	NCT02659059	CheckMate 568	II/III	Stage IV NSCLC and recurrent stage IIIB	First-line
Nivolumab + Ipilimumab	Platinum + Pem	NCT02864251	CheckMate722	III	Stage IV or recurrent NSCLC with <i>EGFR</i> mutation, T790M negative who failed first-line <i>EGFR</i> TKI therapy	Second-line

Bev: Bevacizumab; Carb: Carboplatin; Cis: Cisplatin; Doc: Docetaxel; Gem: Gemcitabine; Nab-Tax: Nanoparticle albumin-bound paclitaxel; NSCLC: Non-small-cell lung cancer; Pem: Pemetrexed; Tax: Paclitaxel.

Clinical studies

Clinical trials with published data for combination chemoimmunotherapy in NSCLC are summarized in Table 2. The KEYNOTE-021 trial compared pemetrexed and carboplatin, with or without pembrolizumab, for advanced nonsquamous NSCLC [73]. In the pembrolizumab plus chemotherapy group, 33 of 60 patients achieved an objective response (55%; 95% CI: 42–68) compared with 18 of 63 patients (29%; 95% CI: 18–41) in the chemotherapy alone group (estimated treatment difference 26% [95% CI: 9–42]; $p = 0.0016$). These results have demonstrated that the addition of pembrolizumab to pemetrexed and carboplatin chemotherapy improves efficacy in patients with advanced non-squamous NSCLC. Additionally, a study of stage III/IV NSCLC demonstrated that phased ipilimumab – two doses of paclitaxel/carboplatin followed by four doses of ipilimumab plus paclitaxel/carboplatin – prolongs immune-related PFS (irPFS) and PFS relative to the control group that received up to six doses of only paclitaxel/carboplatin (irPFS: 5.7 vs 4.6 months; $p = 0.05$; PFS: 5.1 vs 4.2 months; $p = 0.02$). This effect was not seen in the concurrent ipilimumab therapy (four doses of ipilimumab plus paclitaxel/carboplatin followed by two doses of paclitaxel/carboplatin; irPFS: 5.5 vs 4.6 months; $p = 0.13$; PFS: 4.1 vs 4.2 months; $p = 0.25$) [74].

A Phase IIB clinical trial investigated the 6-month PFS of a viral vector based cancer vaccine (TG4010) plus cisplatin and gemcitabine in a group of 148 patients with advanced NSCLC that expressed the cell surface glycoprotein mucin-1 (MUC1). TG4010 is a targeted immunotherapy based on the modified vaccinia virus, Ankara, that codes for MUC1 tumor associated antigen and IL-2. The 6-month PFS in this study was 43.2% in the TG4010 plus chemotherapy group compared with 35.1% in patients who had received chemotherapy alone [75]. In a Phase IIB/III study, TG4010 plus platinum doublet showed longer PFS than platinum doublet alone in stage IV NSCLC patients whose MUC1 expression was higher than 50% (5.9 vs 5.1 months; $p = 0.019$) [76]. Antonia *et al.* reported 29 patients with extensive stage small cell lung cancer who were treated with a p53 cancer vaccine combined with cisplatin-based chemotherapy [77]. One patient showed a clinical response to the vaccine alone. In contrast, the highest rate of objective clinical response to chemotherapy was shown in patients immediately following vaccination (61.9%). Clinical response to subsequent chemotherapy was closely associated with induction of a p53-specific immune response to vaccination. In a study of 48 metastatic breast cancer patients, docetaxel was investigated in combination with a therapeutic cancer vaccine (PANVAC) and found to be potentially beneficial (PFS 7.9 vs 3.9 months; $p = 0.09$) [78]. Another study enrolled 28 androgen-insensitive prostate cancer patients and demonstrated that in those who previously received a cancer vaccine, there was a longer duration of response to docetaxel compared with those patients who received docetaxel alone (PFS 6.1 vs 3.7 months; p -value unspecified) [79]. Ongoing clinical trials on the combination of checkpoint blockade or other immunotherapy with chemotherapy are summarized in Tables 3 and 4.

Conclusion & future perspective

There is a growing body of evidence that has demonstrated the immunomodulatory effects of chemotherapeutic agents on NSCLC. This review shows that chemotherapeutic agents can exert a variety of influences on multiple immune cells, cytokines, CDs, CAMs, and MHCs resulting in an alteration of the immunosuppressive tumor microenvironment and enhancing the cytotoxicity of immune cells (Table 1). Combining chemotherapy with

Table 4. Clinical trials for other combination of chemoimmunotherapy in non-small-cell lung cancer.

Immunotherapy agent	Description of immunotherapy	Chemotherapy agent	Clinicaltrials.gov Identifier	Phase	Setting	Treatment type
Vaccine						
K562	Allogeneic tumor cell vaccine	Cyclophosphamide + celecoxib	NCT01143545	I	Lung cancer, esophageal cancer, malignant pleural mesothelioma, sarcoma; thymic carcinoma with NED or MRD	Adjuvant
Adjuvant tumor lysate vaccine	H1299 cell lysate/iscomatrix vaccine	Cyclophosphamide + celecoxib	NCT02054104	I/II	Lung cancer, esophageal cancer, malignant pleural mesothelioma, sarcoma, thymic carcinoma with NED, MRD	Adjuvant
ADX511-001	HPV-E7 vaccine	Pem maintenance	NCT02531854	II	HPV + nonsquamous NSCLC following first-line induction chemotherapy	Maintenance of first-line
HS-110	Allogeneic cell based cancer vaccine	Low-dose cyclophosphamide	NCT02117024	II	Stage IIIB or IV non-small-cell lung adenocarcinoma	Third-line
CG8123	GM-CSF gene-modified autologous tumor vaccine	Low-dose cyclophosphamide	NCT00089726	II	Stage IIIB or IV NSCLC	Not specified
Allogeneic tumor cell vaccine	Active intralymphatic immunotherapy with IFN-treated cells	Cyclophosphamide	NCT00002475	II	Stage IIIB or IV lung cancer, malignant pleural mesothelioma, pancreatic cancer, breast cancer, colorectal cancer and kidney cancer	Not specified
OSE2101	A multi-epitope therapeutic cancer vaccine	Doc/Pem	NCT02654587	III	Stage IIIB or IV NSCLC	Second- or third-line
Immune cell immunotherapy						
Autologous ROR1-CAR-T cells	Targeting ROR1 with chimeric antigen receptor-modified T cells	Fludarabine phosphate + cyclophosphamide	NCT02706392	I	Stage IV NSCLC and other cancer ROR1 expression in >20%	Second-line
DCVAC/LuCa	Autologous DCs pulsed with tumor cells	Carb + Tax	NCT02470468	I/II	Stage IV NSCLC	First-line
D-CIK	Dendritic activated cytokine-induced killer cell	Cis + Gem	NCT02651441	I/II	Stage IIIB or IV NSCLC	First-line
Genetically modified T cells	Autologous WT1-TCR α 4 gene-transduced CD8-positive Tcm/Tn lymphocytes	Cyclophosphamide	NCT02408016	I/II	Stage III or IV NSCLC, pleural mesothelioma must express HLA-A*0201, evidence of WT1 tumor expression	Second-line
Anti-NY ESO-1 mTCR PBL	T-cell receptor immunotherapy targeting NY-ESO-1	Cyclophosphamide + fludarabine	NCT01967823	II	Stage IIIB or IV NSCLC, synovial sarcoma, breast cancer, hepatocellular cancer expresses ESO, must be HLA-A*0201 positive	Second-line or more

Carb: Carboplatin; CAR-T: Chimeric antigen receptor T cell; Cis: Cisplatin; CTL: Cytotoxic T lymphocyte; D-CIK: Dendritic activated cytokine-induced killer cell; DC: Dendritic cell; Doc: Docetaxel; Gem: Gemcitabine; HPV: Human papillomavirus; MRD: Minimal residual disease; Nab-Tax: Nanoparticle albumin-bound paclitaxel; NED: No clinical evidence of active disease; NSCLC: Non-small-cell lung cancer; PBL: Peripheral blood lymphocyte; Pem: Pemetrexed; Tax: Paclitaxel; TCR: T-cell receptor; TLR9: Toll-like receptor 9.

Table 4. (cont.). Clinical trials for other combination of chemoimmunotherapy in non-small-cell lung cancer.

Immunotherapy agent	Description of immunotherapy	Chemotherapy agent	Clinicaltrials.gov Identifier	Phase	Setting	Treatment type
DC-CTL	Dendritic cytotoxic lymphocyte	Cis + Gem	NCT02766348	II	Stage III or IV NSCLC	First-line
DCVAC/LuCa	Autologous DCs pulsed with tumor cells	Carb + Pem	NCT02669719	II	Stage IV NSCLC with wild-type <i>EGFR</i> , <i>ALK</i> -negative	First-line
AGS-003-LNG	Autologous DC-based immunotherapy	Carb/Cis + Pem/Tax/nab-Tax	NCT02662634	II	Stage III (T1-3, N1-2, M0) NSCLC	Neoadjuvant
Other immunotherapy						
Bavituximab	Chimeric monoclonal antibody	Carb + Tax	NCT01160601	II	Stage IIIB or stage IV nonsquamous NSCLC	First-line
Bavituximab	Chimeric monoclonal antibody	Doc	NCT01138163	II	Stage IIIB or stage IV nonsquamous NSCLC	Second-line
CPG 7909	TLR9 agonist	Taxane + Platinum	NCT00070629	II	Stage IIIB or IV NSCLC	First-line

Carb: Carboplatin; CAR-T: Chimeric antigen receptor T cell; Cis: Cisplatin; CTL: Cytotoxic T lymphocyte; D-CIK: Dendritic activated cytokine-induced killer cell; DC: Dendritic cell; Doc: Docetaxel; Gem: Gemcitabine; HPV: Human papillomavirus; MRD: Minimal residual disease; Nab-Tax: Nanoparticle albumin-bound paclitaxel; NED: No clinical evidence of active disease; NSCLC: Non-small-cell lung cancer; PBL: Peripheral blood lymphocyte; Pem: Pemetrexed; Tax: Paclitaxel; TCR: T-cell receptor; TLR9: Toll-like receptor 9.

immunotherapy can induce cytolysis and inhibit cancer cell proliferation through varying pathways. Clinical trials have provided the proof-of-concept that combination chemotherapy with immunotherapy can be more beneficial than either therapy alone (Table 2). The results of larger studies on combination chemoimmunotherapy are awaiting analysis (Tables 3 and 4).

While designing rationale-based combination chemoimmunotherapy trials, there are several factors that need to be considered: the direct cytotoxicity of chemotherapeutic agents on immune cells; the innate immune status of cancer patients will influence their response to chemotherapy; and the dose and sequence of combined agents should be based on evidence from preclinical studies as well as clinical feasibility. Furthermore, some of the aforementioned studies were carried out with doublet or triplet chemotherapeutics and the exact effect of a single chemotherapeutic agent cannot be distinguished. Additionally, the immunomodulatory changes that were seen were detected either in tumor tissue, peripheral blood samples, or spleens of tumor-bearing mice; their correlation with clinical outcomes remains unknown. This stresses the need for further characterization of immune responses using correlative studies in clinical trials. Additionally, emerging therapies, such as chimeric antigen receptor T-cell therapy, for NSCLC provide an opportunity to promote tumor-infiltrating lymphocytes [80]; chimeric antigen receptor T-cell therapy needs to be investigated in combination with the above-mentioned chemoimmunotherapies [81].

Executive summary

Benefits of combination therapy

- Combination therapies, such as chemo-antiangiogenic therapy, chemo-targeted therapy, and targeted-antiangiogenic therapy, showed significant survival benefits for advanced non-small-cell lung cancer (NSCLC) patients.
- Benefits observed with single immunotherapy are limited to a population of patients for whom rationale-based combination chemotherapy with immunotherapy can be beneficial.

Immunomodulatory effects of chemotherapeutic agents

- Preclinical and clinical studies have demonstrated that conventional chemotherapeutic agents in NSCLC modulate the immune microenvironment following treatment.
- Mechanistic evidence exists that combination chemotherapy with immunotherapy enhances antitumor effects.

Conclusion & future perspective

- The synergistic effects of combining chemotherapy with immunotherapy are being investigated in larger clinical trials.
- Rationale-based combination chemoimmunotherapy trials with correlative studies on immune response monitoring can be the future trend of NSCLC treatment.

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