Review Article

Immunotherapy combination strategies (non-chemotherapy) in non-small cell lung cancer

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Abstract: Immune checkpoint inhibitors enhance the activation and antitumor activity of the immune system, resulting in durable response rates in a select group of patients. Cytotoxic T lymphocyte antigen 4 (CTLA4) inhibitors target the inhibitory interaction between CTLA4 and CD80 or CD86. Programmed death 1 (PD1) inhibitors target the interaction between PD1 receptors on T-cells and PD-ligand 1 (PD-L1) and PD-ligand 2, blocking the inhibitory signaling and resulting in activation of T-cell effector function. These therapeutic drugs were originally evaluated in patients with metastatic melanoma before expansion to all tumor types, including non-small cell lung cancer (NSCLC) with promising results. The PD1 inhibitors such as pembrolizumab have now received FDA approval in the first-line setting for patients with positive PD-L1 expression tumor types; however, only a portion of patients have shown objective and sustainable responses. To expand the number of patients with observed response to immunotherapeutic agents including patients with negative PD-L1 expression tumors, clinical trials are ongoing to assess the safety and efficacy of combination immune checkpoint inhibitors and combination immune checkpoint inhibitors with targeted therapy. Immune checkpoint inhibitors have been found to be a promising therapeutic drug class with sustainable response rates and a tolerable safety profile, and efforts continue to improve these drugs in patients with NSCLC.

Keywords: Immune checkpoint inhibitors; lung neoplasm; programmed death 1 (PD1) inhibitor

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Introduction

Immune checkpoint inhibitors are a new class of cancer therapies that enhance the antitumor activity of the adaptive immune system and immune activation (1,2). These inhibitors have shown sustainable antitumor activity with the potential for improving long-term survival of patients with non-small cell lung cancer (NSCLC). Ipilimumab is a cytotoxic T-lymphocyte antigen 4 (CTLA4) human immunoglobulin G1 (IgG1) monoclonal antibody (MAb) that targets the inhibitory interaction between CTLA4 and CD80 or CD86. It is also thought to deplete tumor-infiltrating regulatory T-cells through antibody-dependent cell-mediated cytotoxicity, producing elevated levels of cell surface CTLA4 (3,4). Programmed death 1 (PD-1) immune checkpoint inhibitors such as nivolumab are human immunoglobulin G4 (IgG4) MAb that bind to PD-1 receptors on T-cells and block inhibitory signaling, which is a result of PD-ligand 1 (PD-L1) and PD-ligand 2 activating the T-cell effector function (5,6).

Initial immunotherapy trials were conducted with ipilimumab (also known as MDX-010 and BMS-734016) in the setting of metastatic melanoma, in which an objective

response was shown in 3 patients, including 2 with complete response (7). These results led to an expanded trial evaluating treatment of 56 stage IV metastatic melanoma patients with the anti-CTLA-4 inhibitor, which observed an overall objective response rate (ORR) of 13% (8). Based on these early clinical successes with immune checkpoint inhibitors in melanoma, as well as promising clinical trials that included patients with metastatic NSCLC, clinical trials were expanded to target advanced NSCLC (9). Early phase I clinical trials found that immune checkpoint inhibitors were overall well-tolerated despite frequency of immune-related adverse events (irAEs), with a small portion of patients showing durable response.

Larger clinical trials were developed investigating immunotherapy in patients with NSCLC. In a cohort of heavily pretreated advanced-stage NSCLC patients, nivolumab given at 1, 3, and 10 mg/kg once every 2 weeks resulted in an ORR of 17%, median overall survival (OS) of 9.9 months, and estimated median response duration of 17 months in the 22 patients with an objective response (10). KEYNOTE 001 assessed 495 heavily pretreated advanced NSCLC patients with the anti-PD-1 inhibitor pembrolizumab and found an ORR of 19.4% with median OS of 12.6 months (11). Durvalumab, a highly selective IgG1 monoclonal antibody that inhibits PD-L1 binding to PD-1 and CD20, was initially evaluated in a phase I/II dose-escalation and expansion trial that observed ORR of 25% in 52 patients as of December 2015, but the study remains ongoing (ClinicalTrials.gov NCT01693562) (12). The POPLAR study compared the anti-PD-1 inhibitor atezolizumab (also known as MPDL3280A) versus docetaxel in a second-line setting in 285 advanced-stage NSCLC patients, and observed an ORR of 14.6% with a median OS of 12.6 months compared with 9.7 months in patients who received docetaxel (12,13). Anti-PD-1/PD-L1 monotherapy has shown sustainable responses and a tolerable safety profile (14-18). Treatment success with immune checkpoint inhibitors resulted in FDA approval of nivolumab and pembrolizumab (both PD-1 inhibitors) as second-line therapy in 2015 and then approval of atezolizumab in 2016. In 2016, pembrolizumab was approved by the FDA in the first-line setting for patients with NSCLC and high PD-L1 expression.

Immunotherapy combinations

PD-1 plus CTLA4

Despite the recent successes with immunotherapy in NSCLC, only a small portion of patients have shown a sustainable response with immune checkpoint inhibitor monotherapy. Combining therapeutic drugs with different but synergistic mechanisms of action has been the hallmark of anticancer therapies and has led to cures and improved responses (2,19). Therefore, the logical next step was to combine immune checkpoint inhibitors with other therapeutic modalities to improve overall and objective responses in patients.

The rationale for combining PD-1 and CTLA4 inhibitors is reasonable, as previous research found that PD-1 and CTLA4 inhibit T-cell activation via distinct and non-redundant synergistic pathways (2,20-23). CD28 (a protein expressed on T-cells and involved in T-cell activation) competes with CTLA4 to bind with CD80/CD86 ligands, and binding by CTLA4 results in the inhibition of CD28 co-stimulatory signal required for T-cell activation and function, whereas PD-1 and PD-L1 inhibit T-cell activation and its cytotoxic activity (24). Combining PD-1/PD-L1, and CTLA4 inhibitors resulted in significantly increased objective responses in melanoma, and currently being evaluated in other tumor types including NSCLC (25).

The initial combination immunotherapy clinical trials in melanoma resulted in improved responses, but also increased toxicities. In Checkmate 069, nivolumab and ipilimumab (n=95) versus ipilimumab alone (n=47) was assessed in a multicenter, double-blind, randomized controlled phase II trial in patients with advanced melanoma (26). The 2-year OS was 63.8% (95% CI, 53.3–72.6%) in the combined cohort versus 53.6% (95% CI, 38.1–66.8%) with ipilimumab alone (26). However, grade 3/4 treatment-related adverse events were observed in 54% of patients who received nivolumab plus ipilimumab versus 20% of patients who received ipilimumab alone (26).

With dual checkpoint inhibition as an accepted treatment option for patients with metastatic melanoma (26-28), combination immunotherapy trials have subsequently been expanded to NSCLC (25-27). At 7-month median follow-up in 51 patients, anti-PD-1 antibody, pembrolizumab, plus CTLA4 inhibitor, ipilimumab, in the second-line setting for NSCLC (29) resulted in ORR of 24% with two CR and nine PR. In addition, 40% of patients were observed to have stable disease (SD) and 18% had progressive disease (29). The median PFS was 6 months, and OS was 17 months; however, 67% of patients had significant treatment-related adverse events, with 11 patients (24%) having grades 3–5 toxicities (29).

Antonia et al assessed durvalumab, an anti-PD-L1 inhibitor, and tremelimumab, an anti-CTLA-4 inhibitor, in a multicenter, non-randomized, open-label, phase Ib clinical trial in NSCLC (ClinicalTrials.gov NCT02000947) (30). The study enrolled 102 patients in the dose-escalation phase and allowed enrollment of patients with negative PD-L1 expression (30). The maximum tolerated dose was exceeded in those who received durvalumab 20 mg/kg plus tremelimumab 3 mg/kg every 4 weeks (30). Of 102 patients, 82 (80%) had one irAE (mostly diarrhea, fatigue, and pruritus) and 29 (28%) required treatment withdrawal due to irAEs (30). Serious adverse events were noted in 37 patients (36%) (30). During the study, 22 patients died, with 3 deaths related to treatment (30). In 63 patients evaluated for clinical response with greater than 24 weeks of follow-up (30), 11 patients (17%; 95% CI, 9–29%) showed objective responses and 18 patients (29%; 95% CI, 18–41%) showed SD (30). Of the 11 patients with objective responses, median time to response was 7.1 weeks (interquartile range, 7.1–15.0 weeks) and median duration of response was not reached (interquartile range, 16.7 to not reached) (30). At the time of evaluation, response remained ongoing in 9 of 11 patients (30). Durvalumab 20 mg/kg plus tremelimumab 1 mg/kg every 4 weeks was concluded as the expansion phase dose (30). Interestingly, an objective response was observed in 5% of patients who had PD-L1-negative tumors, suggesting that PD-L1 expression may not predict response to durvalumab plus tremelimumab combination versus durvalumab alone (30).

The safety and efficacy of nivolumab and ipilimumab were assessed in the first-line setting in patients with chemotherapy naive advanced NSCLC in a phase Ib three-arm study (CheckMate-012, NCT01454102) (31). Of the six arms evaluated in the study, the two arms with nivolumab 3mg/kg every 2 weeks with ipilimumab every 6 weeks versus ipilimumab every 12 weeks were observed to be potential regimens for further clinical investigation (31). Fifty-two patients were randomized to receive monotherapy nivolumab 3 mg/kg every 2 weeks, 39 patients were randomized to receive nivolumab every 2 weeks in combination with ipilimumab every 6 weeks, and 38 patients were randomized to receive nivolumab every 2 weeks in combination with ipilimumab every 12 weeks (31). The primary endpoint was ORR and 24-week PFS. The median PFS was 8.1 months for patients receiving nivolumab plus ipilimumab every 12 weeks versus 3.9 months for patients receiving nivolumab plus ipilimumab every 6 weeks (31). The median PFS was 3.6 months in patients who received nivolumab alone (31). The 1-year OS was 69% in patients receiving nivolumab plus ipilimumab every 6 weeks *vs.* 73% in the monotherapy group. However, 1-year OS was not reached in the group receiving nivolumab plus ipilimumab every 12 weeks (31). Confirmed objective response was observed in 18 patients (47%; 95% CI, 31–64%) in the ipilimumab every 12-week group compared to 15 patients (38%; 95% CI, 23–55%) in the ipilimumab every 6 weeks group with all responses only partial responses. The study did not observe any patient with a complete response (31). The median PFS and ORR in patients with ≥1% PD-L1 expression was 8.1 months and 57% with nivolumab plus ipilimumab every 12 weeks versus 10.6 months and 57% with nivolumab plus ipilimumab every 6 weeks (31). Median PFS of 3.5 months was observed in the nivolumab monotherapy group (31), with ORR of 28%. For patients with EGFR-mutant NSCLC, ORR was 50% in the combination arm versus 41% in those with EGFR wild-type NSCLC (31). The ORR was 14% in EGFR-mutated patients (n=7) *vs.* 30% in EGFR wild-type NSCLC patients (n=30) receiving nivolumab monotherapy (31). As seen with other combination immunotherapy trials, all grade adverse events were similar across all study arms, with 82% in the 12-week combination, 72% in the 6-week combination, and 71% in the monotherapy groups (31). As expected, there was a higher incidence of grade 3/4 irAEs in the combination arms (37% for 12-week combination, 33% for 6-week combination) versus 19% in the monotherapy arm (31). No treatment-related deaths were observed; however, 12 patients (32%) in the ipilimumab every 12 week arm and 11 patients (28%) in the ipilimumab every 6 weeks arm experienced serious adverse events (31). Overall, the study found that nivolumab plus ipilimumab in the first-line setting was tolerable and resulted in improved and sustainable response rates (31). A phase III clinical trial is ongoing to evaluate nivolumab alone and in combination with ipilimumab 1 mg/kg every 6 weeks or platinum-doublet chemotherapy in the front-line setting with OS as the primary endpoint and plans to enroll 1,980 patients (144Tip—CheckMate 227, NCT02477826) (32).

Another combination trial that is on-going is the ARTIC trial. This global phase III randomized, open-label multicenter clinical trial is assessing different treatment regimens, including durvalumab plus tremelimumab versus durvalumab monotherapy or tremelimumab monotherapy versus standard of care in patients with negative PD-L1 expression in NSCLC (NCT02352948) (33).

The MYSTIC trial (NCT02453282) is an open-

label, global, multicenter, randomized, phase III study, evaluating the safety and efficacy of durvalumab plus tremelimumab in stage IV NSCLC patients in the first-line setting (34). Patients were randomized 1:1:1 to receive durvalumab plus tremelimumab versus monotherapy durvalumab versus standard of care with platinum-based chemotherapy (34). Patients were stratified based on PD-L1 expression status and histology (34). A press release from AstraZeneca reported that the combination of durvalumab and tremelimumab did not meet the primary endpoint of improved PFS compared with standard of care with platinum-based chemotherapy, but the study remains ongoing to evaluate OS (35).

In the NEPTUNE trial (NCT02542293), a randomized phase III trial in patients with advanced or metastatic EGFR and ALK wild-type NSCLC, durvalumab plus tremelimumab was compared versus standard of care with platinum-based doublet chemotherapy (36), with a primary endpoint of OS. The study is currently recruiting patients, with plans to also evaluate OS, PFS, and ORR in patients with negative PD-L1 expression (36). In a similar group of patients at first-line setting, the ongoing POSEIDON trial (NCT03164616), an open-label, global, multicenter, randomized, phase III trial, is assessing the efficacy and safety of durvalumab plus tremelimumab combined with platinum-based chemotherapy versus durvalumab monotherapy plus platinum-based chemotherapy versus platinum-based chemotherapy alone (randomized 1:1:1). Enrollment of 801 patients is planned, with primary endpoint being PFS.

PD-1 and other immuno-oncology agents

Recent advances in understanding the cancer immunity cycle and novel immunomodulatory targets have revealed multiple additional potential targets for immuno-oncology therapies (24,37). Several agents are in early clinical development for many of these targets, although none have yet been validated in larger randomized trials or gained regulatory approval, in contrast to many of the PD-1 and CTLA4 pathway inhibitors discussed above. Here, we discuss the agents that have data available, although we note a significant number of ongoing clinical trials that are also investigating these various targets. A summary of ongoing trials enrolling patients with NSCLC is presented in *Table 1*.

The targets discussed below are largely being studied in combination with PD-1/PD-L1 inhibition. The rationale for use of anti-PD-1 pathway therapy as a backbone in combination immunotherapy is due to its favorable efficacy and toxicity profile when used as monotherapy compared with inhibitors of CTLA4. Given the vast array of novel targets within cancer immunology that are the focus of investigational agents discussed below, it will be increasingly important to create methodologies to select rational combinations for further development and clinical testing.

Other immune checkpoint targets

In addition to the PD-1/PD-L1/PD-L2 and CTLA4-CD80-CD86 pathways, several additional cell surface protein members of the immunoglobulin superfamily have been shown to play an important role in regulating T-cell responses in cancer.

LAG3 is a checkpoint protein and functions as a negative regulator of T-cell responses to MHC class II restricted antigen presentation (38). IMP321 (developed by Immutep) is a soluble LAG3-Fc fusion protein that has demonstrated tolerability and clinical activity in phase I trials in renal cell, breast, and pancreatic cancers (39-41). BMS-986016 (developed by Bristol-Myers Squibb), an IgG4 monoclonal antibody targeting LAG3, has produced clinical responses in patients with melanoma who had progressed on or after prior PD-1/PD-L1 therapy in a phase I combination trial with nivolumab. This agent was also relatively well-tolerated, with treatment-related grade 3/4 adverse events occurring in 9% of patients (42). IMP321 and BMS-986016 have not been evaluated in trials specifically for NSCLC, although multiple phase I studies combining these agents with anti-PD-1/PD-L1 agents in a variety of tumor types are currently ongoing.

Killer inhibitory receptors, transmembrane glycoproteins expressed in natural killer (NK) cells, regulate the cytolytic function of NK cells through recognition of self-MHC class I molecules (43). Lirilumab (IPH2102/BMS-986015, developed by Innate Pharma, licensed to Bristol-Myers Squibb) is a fully human monoclonal antibody targeting major killer inhibitory receptors; it was well tolerated in a phase I study across various malignancies, with the most common adverse events being fatigue (30%), pruritus (20%), infusion-related reaction (14%), and headache (11%) (44). When used in combination with nivolumab, a preliminary report from a phase I/II trial noted promising early activity in squamous cell carcinoma of the head and neck, with about 25% having an objective response (45). Combination immunotherapy trials using lirilumab in hematologic and solid malignancies are in progress.

Table 1 Selected ongoing combination/novel immunotherapy clinical trials with targets beyond PD-1 and CTLA-4

Target	NCT #	Status	Title	Phase	Investigational drugs	Setting
LAG-3	NCT01968109	Recruiting	Safety study of anti-LAG-3 with and without anti-PD-1 in the treatment of solid tumors	I	BMS-986016, nivolumab	1st line/2nd line
	NCT02750514	Recruiting	Investigational immuno-therapy study to test combination treatments in pts with advanced NSCLC (FRACTION-Lung)	II	BMS-986016, nivolumab	Any
	NCT02460224	Recruiting	LAG525 single agent and in combination With PDR001 in pts with advanced malignancies	I/II	LAG525, PDR001 (anti-PD-1)	At least 1 prior therapy
TIM-3	NCT02817633	Recruiting	TSR-022, an anti-TIM-3 monoclonal antibody, in patients with advanced solid tumors	I	TSR-022, anti-PD-1 antibody	At least 1 prior therapy
	NCT03099109	Recruiting	LY3321367 alone or with LY3300054 in participants with advanced relapsed/refractory solid tumors	I	LY3321367 (anti-TIM3), LY3300054 (anti-PD-L1)	Any
	NCT02608268	Recruiting	MBG453 as single agent and in combination with PDR001 in patients with advanced malignancies	I/II	MBG453(anti-TIM3), PDR001 (anti-PD-1)	At least 1 prior therapy
B7-H3	NCT02381314	Recruiting	Enoblituzumab (MGA271) in combination with ipilimumab in refractory cancer	I	Enoblituzumab, ipilimumab	At least 1 prior therapy
	NCT02475213	Recruiting	Enoblituzumab (MGA271) in combination with pembrolizumab in refractory cancer	I	Enoblituzumab, pembrolizumab	At least 1 prior therapy
VISTA	NCT02671955	Recruiting	JNJ-61610588 in participants with advanced cancer	I	JNJ-61610588	At least 1 prior therapy
	NCT02812875	Recruiting	CA-170 (oral PD-L1, PD-L2 and VISTA checkpoint antagonist) in advanced tumors and lymphomas	I	CA-170	Refractory to standard therapy
KIR	NCT01714739	Recruiting	Anti-KIR antibody lirilumab in combination with nivolumab and nivolumab plus ipilimumab in patients with advanced solid tumors	I/II	Lirilumab, nivolumab, ipilimumab	At least 1 prior therapy
4-1BB	NCT02253992	Recruiting	Urelumab in combination with nivolumab in solid tumors and B-cell non-hodgkin lymphoma	I/II	Urelumab	At least 1 prior therapy
	NCT02554812	Recruiting	Avelumab in combination with other cancer immunotherapies in advanced malignancies (JAVELIN medley, Arm A or Arm D)	II	Utomilumab PF-04518600 (anti-OX40) avelumab	Any
	NCT02315066	Recruiting	OX40 agonist PF-04518600 alone and in combination with 4-1BB agonist PF-05082566 (Utomilumab)	I	PF-04518600 utomilumab	Refractory to standard therapy

Table 1 *(continued)*

Table 1 *(continued)*

Target	NCT #	Status	Title	Phase	Investigational drugs	Setting
OX40	NCT02554812	Recruiting	Avelumab in combination with other cancer immunotherapies in advanced malignancies (JAVELIN Medley, Arm B or Arm D)	II	PF-04518600 (anti-OX40), utomilumab, avelumab	Any
	NCT02315066	Recruiting	OX40 agonist PF-04518600 alone and in combination with 4-1BB agonist PF-05082566	I	PF-04518600, PF-05082566	Refractory to standard therapy
	NCT03241173	Not yet recruiting	INCAGN01949 in combination with immune therapies in advanced or metastatic malignancies	I	INCAGN01949, nivolumab, ipilimumab	Progression after available therapies
	NCT02410512	Recruiting	MOXR0916 and atezolizumab in participants with locally advanced or metastatic solid tumors	I	MOXR0916, atezolizumab	Progression after available therapies
	NCT02221960	No longer recruiting	MEDI6383 alone and in combination with MEDI4736 in adult subjects with select advanced solid tumors	I	MEDI6383 (anti-OX40), MEDI4736 (durvalumab)	Progression after available therapies
GITR	NCT02598960	Recruiting	BMS-986156, given by itself or in combination with nivolumab in patients with solid cancers or cancers that have spread	I/II	BMS-986156, nivolumab	At least 1 prior therapy
	NCT03126110	Recruiting	INCAGN01876 combined with immune therapies in advanced or metastatic malignancies	I/II	INCAGN01876, nivolumab, ipilimumab	Progression after available therapies
	NCT02740270	Recruiting	GWN323 alone and in combination with PDR001 in patients with advanced malignancies and lymphomas	I	GWN323, PDR001 (anti-PD-1)	Any
	NCT01239134	Recruiting	TRX518 (anti-GITR mAb) in stage III or IV malignant melanoma or other solid tumors	I	TRX518	Progression after available therapies
	NCT02583165	Recruiting	MEDI1873 (GITR agonist) in adult subjects with select advanced solid tumors	I	MEDI1873	Any
CD27	NCT02335918	Recruiting	Anti-CD27 (varlilumab) and Anti-PD-1 (nivolumab) in advanced refractory solid tumors	I/II	Varlilumab, nivolumab	Any

Table 1 *(continued)*

Table 1 *(continued)*

Target	NCT #	Status	Title	Phase	Investigational drugs	Setting
IDO	NCT02178722	Recruiting	Pembrolizumab in combination with epacadostat in subjects with selected cancers (KEYNOTE-037/ECHO-202)	I/II	Epacadostat, pembrolizumab	At least 1 prior therapy
	NCT02318277	Recruiting	Epacadostat (INCB024360) in combination with durvalumab in subjects with selected advanced solid tumors (ECHO-203)	I/II	Epacadostat, MEDI4736 (durvalumab)	At least 1 prior therapy
	NCT02327078	Recruiting	Epacadostat administered in combination with nivolumab in select advanced cancers (ECHO-204)	I/II	Epacadostat, nivolumab	Prior platinum-based chemotherapy
	NCT02959437	Recruiting	Azacitidine combined with pembrolizumab and epacadostat in subjects with advanced solid tumors (ECHO-206)	I/II	Epacadostat, pembrolizumab, azacitidine	Progression after available therapies
	NCT03085914	Recruiting	Epacadostat in combination with a PD-1 inhibitor and chemotherapy in subjects with advanced or metastatic solid tumors (ECHO-207)	I/II	Epacadostat, nivolumab or pembrolizumab, chemotherapy	Progression after anti-PD-1/PD-L1 therapy
	NCT02460367	Recruiting	Tergenpumatucel-L (HyperAcute lung) immunotherapy in combination with indoximod and docetaxel in pts with advanced previously treated NSCLC	I/II	Indoximod, tergenpumatucel-L, docetaxel	1-3 prior lines, including platinum-based chemo
	NCT02658890	Recruiting	BMS-986205 in combination with nivolumab and in combination with both nivolumab and ipilimumab in cancers that are advanced or have spread	I/II	BMS-986205, nivolumab, ipilimumab	At least 1 prior therapy
ADORA2A	NCT02655822	Recruiting	CPI-444 as single agent and in combination with atezolizumab in patients with selected incurable cancers	I/Ib	CPI-444 (anti-ADORA2A), CPI-444 + atezolizumab	1-4 prior lines of therapy
	NCT02403193	Recruiting	Phase I/Ib trial of single agent PBF-509 and in combination with PDR001 for patients with advanced NSCLC	I/Ib	PBF-509 (anti-ADORA2A), PDR001 (anti-PD-1)	At least 1 prior therapy
TGF-β	NCT02423343	Recruiting	Galunisertib administered in combination with nivolumab in advanced refractory solid tumors (phase 1b) and in recurrent or refractory NSCLC or hepatocellular carcinoma (Phase 2)	I/II	Galunisertib (anti-TGFβ), nivolumab	1 prior line of therapy
Daratumumab	NCT03098550	Recruiting	Nivolumab combined with daratumumab in participants with advanced or metastatic solid tumors	I/II	Nivolumab, daratumumab	1 prior line of therapy
	NCT03023423	Recruiting	Daratumumab administered in combination with atezolizumab compared with atezolizumab alone in subjects with previously treated advanced or metastatic NSCLC	I/II	Daratumumab, atezolizumab	Previously treated advanced/metastatic

Table 1 *(continued)*

Table 1 *(continued)*

Target	NCT #	Status	Title	Phase	Investigational drugs	Setting
PI3K inhibitor	NCT02646748	Recruiting	Pembrolizumab combined with itacitinib (INCB039110) and/or pembrolizumab combined With INCB050465 in advanced solid tumors	I	Pembrolizumab, itacitinib (JAK inhibitor), INCB050465 (PI3K-δ inhibitor)	No more than 2 lines of prior therapy
	NCT02637531	Recruiting	IPI-549 monotherapy and in combination with nivolumab in subjects with advanced solid tumors	I	IPI-549 (PI3K-γ inhibitor), nivolumab	Advanced solid tumors including NSCLC
PD-1 + vaccines	NCT02466568	Not yet recruiting	A randomized phase i/ii study of nivolumab in combination with GM.CD40L vaccine in adenocarcinoma of the lung	I/II	GM.CD40L vaccine, nivolumab	Chemotherapy naïve or prior adjuvant chemo
	NCT02897765	Recruiting	NEO-PV-01 + adjuvant with nivolumab in patients with melanoma, NSCLC or transitional cell carcinoma of the bladder	I	NEO-PV-01 vaccinenivolumab adjuvant chemotherapy	No more than 1 prior line of therapy
	NCT02439450	Recruiting	Viagenpumatucel-L (HS-110) in combination with multiple treatment regimens in patients with NSCLC (the "DURGA" trial)	I/II	Viagenpumatucel-Lnivolumab	At least 1 prior line of therapy, metastatic
	NCT02955290	Recruiting	EGF vaccine CIMAvax in combination with the anti-PD-1 nivolumab in patients with previously treated advanced NSCLC	I/II	Nivolumab, recombinant human EGF-rP64K/ montanide ISA 51 vaccine	Stage IIIB or stage IV NSCLC
	NCT03164772	Not yet recruiting	Phase 1/2 study of combination immunotherapy and mRNA vaccine in subjects with NSCLC	I/II	Durvalumab, tremelimumab BI 1361849 (mRNA Vaccine)	Any
	NCT02460367	Recruiting	Tergenpumatucel-L (HyperAcute lung) immunotherapy in combination with indoximod and docetaxel in pts with advanced previously treated NSCLC	I/II	Tergenpumatucel-L, indoximod, docetaxel	1–3 prior lines, including platinum-based chemotherapy
ACT	NCT03215810	Not yet recruiting	Nivolumab and tumor infiltrating lymphocytes (TIL) in advanced NSCLC	I	TIL (including conditioning chemotherapy and IL-2), Nivolumab	PD-1/PD-L1 inhibitor naïve
	NCT02070406	Recruiting	Gene-modified T cells, vaccine therapy, and ipilimumab in treating patients with locally advanced or metastatic Malignancies	I	NY-ESO-1 TCR PBMC infusion; dendritic cell vaccine therapy; ipilimumab	No approved alternative therapies, NY-ESO-1 + by IHC

Table 1 *(continued)*

Table 1 *(continued)*

Target	NCT #	Status	Title	Phase	Investigational drugs	Setting
VEGF	NCT02443324	Recruiting	Ramucirumab plus pembrolizumab in participants with gastric or GEJ adenocarcinoma, NSCLC, transitional cell carcinoma of the urothelium, or biliary tract cancer	I	Ramucirumab, pembrolizumab	0-3 prior lines of systemic therapy
	NCT02572687	Recruiting	Ramucirumab (LY3009806) plus MEDI4736 in participants with advanced gastrointestinal or thoracic malignancies	I	Ramucirumab, MEDI4736 (durvalumab)	1–3 prior lines of systemic therapy
HDAC inhibitor	NCT02805660	Recruiting	HDAC inhibitor, mocetinostat, in combination with PD-L1 inhibitor, durvalumab, in advanced or metastatic solid tumors and NSCLC	I/II	Mocetinostat, durvalumab	Advanced/ metastatic solid tumors including NSCLC
	NCT02437136	Recruiting	Entinostat in combination with pembrolizumab in patients with NSCLC, with expansion cohorts in patients with NSCLC, melanoma, and mismatch repair-proficient colorectal cancer	I/II	Entinostat, pembrolizumab	1 prior line of therapy
	NCT02638090	Recruiting	Pembrolizumab and vorinostat in patients with immune therapy naïve and immune therapy pretreated stage IV NSCLC	I/II	Vorinostat, pembrolizumab	1 prior line of therapy
	NCT02954991	Recruiting	A parallel phase 2 study of glesatinib, sitravatinib or mocetinostat in combination with nivolumab in advanced or metastatic NSCLC	II	Mocetinostat, nivolumab	Prior therapy with platinum based doublet and checkpoint inhibitor
DNMT-inhibitor	NCT02664181	Recruiting	Nivolumab, alone or in combination with oral decitabine/ tetrahydrouridine as second line therapy for NSCLC	II	Nivolumab, decitabine/ tetrahydrouridine	1 or more prior systemic therapies
	NCT02959437	Recruiting	Azacitidine combined with pembrolizumab and epacadostat in subjects with advanced solid tumors (ECHO-206)	I/II	Azacitidine, epacadostat, pembrolizumab	Progression after available therapies

NCT #, clinicaltrials.gov identifier; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; NSCLC, non-small cell lung cancer; SoC, standard of care; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; LAG-3, lymphocyte-activation gene 3; TIM-3, T-cell immunoglobulin and mucin-domain containing-3; VISTA, V-domain Ig suppressor of T cell activation; KIR, killer-cell immunoglobulin-like receptors; GITR, glucocorticoid-induced TNFR-related protein; IDO, indoleamine 2,3-dioxygenase; ADORA2A, adenosine A2A receptor; TGF-β, transforming growth factor beta; PI3K, phosphoinositide 3-kinase; ACT, adoptive cellular therapy; VEGF, vascular endothelial growth factor; HDAC, histone deacetylase; DNMT, DNA methyltransferase.

Additional inhibitory immune checkpoint targets of interest are also under active investigation, including T-cell immunoglobulin-mucin domain 3, V-domain immunoglobulin suppressor of T-cell activation, and B7-H3 (CD 276), for which antagonist drugs are currently under investigation (*Table 1*).

Co-stimulatory targets

The identification of several co-stimulatory targets in the tumor necrosis factor receptor (TNFR) superfamily has led to development of agents designed to stimulate the T-cell-mediated antitumor immune response. The rationale for combination with immune checkpoint inhibitors is that these agents may work synergistically to augment the immune response that is released by checkpoint inhibition.

41BB, also known as CD137, is a receptor expressed on T-cells and NK cells, as well as dendritic cells and granulocytes. Activation of 41BB through its ligand 41BBL results in co-stimulation of T-cells independent of CD28, as well as enhanced survival of activated T-cells (46). Urelumab (BMS-663513, developed by Bristol-Myers Squibb), an IgG4 monoclonal antibody targeting 41BB, demonstrated a high incidence of hepatic toxicity in early trials in melanoma. Subsequently, it was shown to be significantly more tolerable at lower doses with preserved pharmacodynamic activity (47). A phase Ib trial of adjuvant cetuximab combined with urelumab in squamous cell carcinoma of the head and neck suggested clinical benefit and enhanced markers of immune response (48). Utomilumab (PF-05082566, developed by Pfizer), a fully human IgG2 monoclonal antibody specific to 41BB, was recently evaluated in a phase Ib study in combination with pembrolizumab and demonstrated manageable toxicity and early evidence of clinical activity in patients treated with a variety of solid tumors, including NSCLC (49). Further clinical trials with both of these agents are currently ongoing for a variety of malignancies (*Table 1*).

Activation of OX40, a co-stimulatory receptor expressed on T-cells, through its ligand OX40L promotes T-cell activation. In addition, agonism of OX40 may inhibit regulatory T-cell function (46,50). Combining MOXR0916 (developed by Genentech), a humanized agonist IgG1 monoclonal antibody targeting OX40, and atezolizumab in patients with advanced solid tumors in a phase I trial was well tolerated, with only one related grade 3 event (pneumonitis responsive to corticosteroids) and at least some objective responses (8). PF-04518600 (developed by Pfizer), a fully human IgG2 agonistic monoclonal antibody against human OX40, was also evaluated as monotherapy in a phase I study of solid tumors and was well tolerated with some early evidence of clinical activity (51). MEDI6383 (developed by AstraZeneca), a human OX40 ligand fusion protein, has also entered early clinical development (52). Clinical trials evaluating each of these agents, some as monotherapy and some in combination with checkpoint inhibitors, are in progress in a variety of solid tumors, although not currently specifically for NSCLC.

Glucocorticoid-induced TNFR-related protein (GITR), also known as CD357, is upregulated in $CD4^+$ and $CD8^+$ T-cells on stimulation, although it is expressed constitutively in regulatory T-cells and in NK cells, dendritic cells, and monocytes. GITR can facilitate effector T-cell activity, at least in part by suppressing regulatory T-cell function (46). A phase I monotherapy trial of TRX-518 (developed by Leap Therapeutics), a fully humanized IgG1 GITR-targeting monoclonal antibody, was well tolerated in patients with solid tumors, with early activity assessment showing some patients with SD (53). BMS-986156 (developed by Bristol-Myers Squibb) is a fully human IgG1 GITR agonist monoclonal antibody. In a phase I/II solid-tumor trial with or without nivolumab, it was well tolerated, with some clinical activity observed in patients treated with the combination. The most common treatment-related adverse events with the combination were fever (30%), chills (16%), and fatigue (14%) (54). Recently, AMG-228 (developed by Amgen), an agonistic human IgG1 monoclonal antibody also targeting GITR, was investigated in a phase I study in multiple tumor types as monotherapy. It was well tolerated, although early efficacy assessment showed no patients with objective responses (55). Early-phase clinical trials involving TRX-518, BMS-986156, and other GITR-specific antibodies, as single agent or with anti-PD-1/PD-L1, are ongoing.

CD27 is another co-stimulatory molecule expressed on T-cells (as well as some B-cells, including plasma cells). Upon binding to its ligand (CD70), there is T-cell activation, proliferation, and effector function (46). In a single-agent phase I solid-tumor study of varlilumab (CDX1127, developed by Celldex Therapeutics), a first-in-class, agonist monoclonal antibody with specificity for CD27, a tolerable safety profile was shown, although one patient experienced asymptomatic grade 3 hyponatremia as a dose-limiting toxicity. Early response data showed one patient with metastatic renal cell carcinoma who had a PR, and several patients with SD lasting >3 months (56). A

phase II study is ongoing in multiple tumor types, although not in NSCLC.

Several other immune-stimulatory targets are being evaluated, including ICOS and various immune-stimulatory cytokines (*Table 1*).

Tumor microenvironment

Interactions between immune cells and the tumor microenvironment are also recognized as important mechanisms through which cancers can evade the host immune response. In addition to targeting immune cells directly through stimulatory and checkpoint pathways, a number of therapeutics are being developed with the aim of altering the tumor microenvironment to create more favorable conditions for anti-tumor immunity.

Indoleamine 2,3-dioxygenase (IDO), a cytosolic enzyme that catalyzes the rate-limiting step in the catabolism of tryptophan, has been found to be produced by tumor cells as a mechanism of immune escape by suppressing T-cell activity and inducing differentiation of naïve T-cells into regulatory T-cells (57). In preclinical animal models, treatment with IDO-inhibitor agents was associated with increased antitumor immune responses, with synergistic responses to tumor vaccines and other immunotherapy (57). A phase I trial of indoximod (NLG-8189, a tryptophan analog IDO inhibitor developed by NewLink Genetics), as a single agent in advanced solid malignancies, demonstrated favorable toxicity and pharmacodynamic profiles, with early response data showing some patients having SD lasting >6 months (58). Epacadostat (INCB024360, developed by Incyte), a selective oral IDO inhibitor, was well tolerated as a single agent in a recent phase I study. Although no objective response was seen with monotherapy, SD lasting ≥16 weeks was observed in 7 of 52 patients (59). Epacadostat is also being evaluated in combination with pembrolizumab in a number of cancers, including NSCLC in the KEYNOTE-037 trial, where early evaluation demonstrated an ORR of 35% and disease control rate of 60% in NSCLC. The combination was well tolerated, with adverse events mostly being fatigue (19%), arthralgia (9%), and increased AST (9%). Discontinuation due to treatment-related adverse events occurred in <5% of patients (60). BMS-986205 (developed by Bristol-Myers Squibb), a selective IDO1 inhibitor, is being investigated as monotherapy and in combination with nivolumab in a variety of solid tumors; both good tolerability and a favorable pharmacodynamic profile were shown (61). Clinical trials targeting IDO using indoximod, epacadostat, and other IDO inhibitors (including BMS-986205), as well as peptide-based anti-IDO vaccination, are ongoing for a variety of tumor types, including NSCLC (62).

Phosphatidylserine is a membrane phospholipid exposed during apoptosis; it functions in preventing an inflammatory response to programmed cell death. It is also commonly expressed in tumor cells and tumor vasculature, serving as an additional mechanism for immune escape in tumors with a high rate of proliferation and cell turnover. Bavituximab (PGN401, developed by Peregrine Pharmaceuticals) is a phosphatidylserine-targeting monoclonal antibody that has been evaluated in a number of clinical trials, including in combination with docetaxel in a phase II randomized study in advanced non-squamous NSCLC (63). Results from this study, as well as from an interim analysis of the follow-up phase III SUNRISE trial, failed to show significantly improved OS. However, a follow-up analysis from SUNRISE recently demonstrated that patients in the bavituximab plus docetaxel treatment arm who received subsequent immunotherapy did not reach the median OS compared with 13.0 months in patients who received placebo plus docetaxel and subsequent immunotherapy (HR =0.43; P=0.005) (64).

Daratumumab, an anti-CD38 IgG1 monoclonal antibody originally developed for multiple myeloma treatment, has also garnered interest as a potential immune modulator after it was demonstrated to influence the tumor microenvironment by inhibiting T-regulatory cells, myeloid-derived suppressor cells, and regulatory B-cells, an important mechanism thought to contribute to anti-tumor response with this agent (65). Phase I/II trials combining daratumumab with either atezolizumab or nivolumab in patients with advanced NSCLC are ongoing. PI3K-γ has also emerged as a target involved in myeloid cell immune inhibitory effects within the tumor microenvironment; at least one clinical trial with a PI3K-γ inhibitor plus pembrolizumab in advanced solid tumors including NSCLC is ongoing (66).

Other mechanisms of immune evasion within the tumor microenvironment are also actively being investigated in immunotherapy development. Extracellular adenosine is commonly observed at high concentrations in the tumor microenvironment, in part influenced by a hypoxic environment. Adenosine, through binding and agonism of the adenosine receptor A2A (ADORA2A), has been shown to exert immunosuppressive effects through suppression of T-cell and NK cell activity (67). Tumor growth factor-β is a potent immunosuppressive cytokine implicated in evasion

of antitumor immunity and associated with angiogenesis and proliferation of stromal cells (68). Early clinical trials utilizing novel antagonists for these and other pathways, alone or in combination with checkpoint inhibitors, are in progress (*Table 1*).

PD-1 and vaccines

Vaccine strategies for advanced NSCLC treatment have included use of vaccines made from allogeneic cell lines or with MUC1, EGFR, MAGEA3, and NY-ESO-1 targets. Many have demonstrated promise, including the ability to prime and expand tumor antigen-specific T-cells. However, in NSCLC clinical studies, these vaccines were generally met with overall similar results, showing at best a very modest benefit or a benefit for only a subgroup of patients (69-75).

In a phase II study of TG4010 (a modified vaccinia virus expressing MUC1 and IL-2) combined with first-line chemotherapy in patients with stage IV untreated NSCLC, a small but statistically significant improvement in PFS from 5.1 to 5.9 months (P=0.019) was shown (74). Tecemotide (L-BLP25, a vaccine utilizing the MUC1-derived 25-amino acid L-nBLP25) was investigated in the START trial, in which patients with unresectable stage III NSCLC were randomized to vaccination versus placebo after treatment with platinum-based chemotherapy and radiation. A non-significant improvement in OS from 22.3 to 25.6 months (P=0.123) was observed. However, in a preplanned subgroup analysis of patients who received concurrent (rather than sequential) chemoradiotherapy, OS significantly improved from 20.6 to 30.8 months (P=0.016) (75).

Although clinical success with a vaccination approach has been limited, the signals of antitumor activity in certain subgroups of patients, coupled with new excitement surrounding checkpoint blockade as a means to augment the response to vaccination, have revived significant interest in this area of immunotherapy. Indeed, several ongoing clinical trials are combining these or other vaccinations with checkpoint inhibitors in advanced NSCLC (*Table 1*).

PD-1 and cellular therapy

Adoptive cellular therapy (ACT), involving the infusion of T-cells into patients with the aim of kindling a tumor-specific immune response, offers a promising means of trafficking an immune infiltrate into "poorly inflamed" tumors. Clinical experience to date with ACT has been mainly before the era of checkpoint blockade, although currently there is intense interest in combining these therapies to overcome the inhibitory factors thought to limit the efficacy of ACT in many patients (76).

ACT-employing tumor-infiltrating lymphocytes (TIL), which are expanded *ex vivo* from autologous tumor tissue, is a promising immunotherapy that has evolved over the past 60 years into a tenable treatment for select solid tumors, particularly melanoma. The National Cancer Institute has pioneered this treatment and reported response rates of 50% or greater in metastatic melanoma with 22% of patients achieving durable CRs, including responses of >9 years (77,78). Further studies have subsequently demonstrated that effective TIL can be generated from NSCLC and that treatment of NSCLC patients with this approach is feasible (79). One limitation to note is that, for TIL generation, patients must have a sufficiently large surgically resectable tumor, so that the cellular product is produced. Clinical trials investigating the addition of checkpoint inhibitors to TIL therapy are ongoing in melanoma and at least one such trial is planned in NSCLC (*Table 1*).

A similar but unique approach using engineered T-cells with tumor-antigen specificity or chimeric antigen receptor T-cells has been employed with significant success in hematologic malignancies, including acute lymphoblastic leukemia (80). Phase I clinical trials using engineered T-cells with specificity for a variety of antigens (including NY-ESO-1, ROR1, and MAGE-A4) have recently been developed for a variety of solid tumors, including NSCLC, although none to date employ a combination approach with checkpoint inhibitors.

PD-1 and epigenetic modulators

There is increasing recognition that regulation of epigenetic modifications plays a significant role in the interaction between cancer and the immune system. Mechanisms including DNA methylation and histone modification can result in regulation of immune checkpoints and tumor antigen expression, and a growing body of evidence suggests that therapies with epigenetic effects can be used to alter and potentially enhance response to immunotherapies (81).

Histone deacetylase (HDAC) inhibitors, which have an established role in treating hematologic malignancies including multiple myeloma and T-cell lymphoma, have been found to act through immunogenic effects in addition to suppression of cellular proliferation. Specifically, HDAC inhibitors can increase the expression of various

T-cell chemokines within tumors. In fact, experiments in multiple lung tumor models demonstrated a significant enhancement in the anti-tumor T-cell response generated by PD-1 inhibition when combined with an HDAC inhibitor (82). An early report from a phase I/II study of entinostat (an oral, class I selective HDAC inhibitor) and pembrolizumab in patients with advanced melanoma or NSCLC demonstrated good tolerability and a reduction in peripheral blood myeloid-derived suppressor cells (83). Several clinical trials investigating the combination of HDAC inhibitors and checkpoint blockade are ongoing (*Table 1*).

DNA methyltransferase (DNMT) inhibitors, which are approved for use in myelodysplastic syndrome and myeloid leukemias, have also been shown to influence the tumor-immune interaction by upregulating transcription of chemokines and tumor-associated antigens and through suppression of regulatory T-cell function (81,84,85). Recent translational research has highlighted these effects in patients with NSCLC treated with the DNMT inhibitor azacitidine, as well as shown increased expression of PD-L1 in tumor cells (84). A phase II randomized trial with the oral azacitidine CC-486 (developed by Celgene) plus pembrolizumab was evaluated in patients with stage IIIV/IV NSCLC with only 1 prior platinum based chemotherapy to pembrolizumab 200 mg every 21 days with either CC-486 300 mg on days 1–14 or placebo with primary endpoint of PFS (86,87). Fifty-one patients were randomized to pembrolizumab plus CC-486 and 49 patients were randomized to pembrolizumab plus placebo with patients stratified by histology (86,87). Median duration of treatment for pembrolizumab plus CC-486 was 14 weeks with median number of 5 cycles compared to mediation duration of 24 weeks with median number of 7 cycles in the pembrolizumab plus placebo arm (86,87). Patients on pembrolizumab plus CC-486 were found to have higher rates of dose interruptions and discontinuations with gastrointestinal events, fatigue, and elevated transaminase levels the most common treatment related adverse events (86,87). The study observed median PFS of 3.1 months in pembrolizumab plus CC-486 compared to 4.0 months in pembrolizumab plus placebo with ORR observed in 10 patients (19.6%) in the pembrolizumab plus CC-486 compared to 7 patients (14.3%) in the pembrolizumab plus placebo arm (86,87). The study concluded that the addition of CC-486 to pembrolizumab did not improve the primary endpoint of PFS compared to pembrolizumab plus placebo (86,87). PD-L1 expression did not appear to predict CC-486 treatment efficacy (86,87).

PD-1 and vascular endothelial growth factor (VEGF)

VEGF has also been shown to exert immune-suppressive effects through support of tissue remodeling and fibrosis and prevention of immune infiltration into tumors. Thus, VEGF inhibition has also emerged as a potential strategy to facilitate the antitumor immune response (88,89). An early trial exploring the combination of the VEGF receptor (VEGFR) tyrosine kinase inhibitor (TKI) sunitinib with tremelimumab in patients with melanoma was unfortunately met with unexpected dose-limiting toxicity in the form of acute kidney injury (90). A more recent investigation has shown that bevacizumab combined with ipilimumab was better tolerated, with a disease control rate of 67.4% and correlative analyses showing increased immune activation and lymphocyte trafficking (91). Agents targeting VEGF or VEGFR, including bevacizumab and ramucirumab, are already commonly used in NSCLC, although their use in combination with immunotherapy remains an investigational but promising application. Several clinical trials testing this combined approach are currently accruing patients (*Table 1*).

Conclusions

In a select group of patients, monotherapy with immune checkpoint inhibitors has provided durable responses. However, many patients do not respond to the presently available single-agent immune checkpoint inhibitors. Combining immunotherapies has shown promising results, including improvements in ORR and OS (29-31). Combination immune checkpoint inhibition may also target immune inhibitor pathways, allowing tumors to evade treatment and preventing resistance (2). Adverse events from combination immunotherapies have been observed to occur at increased frequency (30,31). As we continue to improve response rates from immunotherapeutic drugs, managing the adverse effect profiles will play an important role in their success. There are several ongoing clinical trials evaluating the efficacy and safety profile of immune checkpoint inhibitor combinations. Additional research on new therapeutic targets is underway preclinically and in early-phase clinical trials (24). CTLA4, PD-L1, and PD-1 are not the only receptors providing negative regulatory signals; there are several ongoing preclinical and phase I

clinical trials targeting new receptors (24). The addition of immunotherapy with targeted therapy has also shown promising results. As we improve our understanding of the immune inhibitory pathways allowing tumors to evade the immune system, future targets continue to be discovered and tested with the hope of increasing the number of patients benefiting from immune checkpoint inhibitors.

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Footnote

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