Review

Clinical Cancer Research

Cancer Immunotherapy: A Future Paradigm Shift in the Treatment of Non–Small Cell Lung Cancer

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Abstract

Emerging evidence on the role of the antitumor activity of the immune system has generated great interest in immunotherapy even for tumors that were historically considered as nonimmunogenic. Immunotherapy is emerging as a major modality in non–small cell lung cancer (NSCLC) treatment focusing on vaccine approaches to elicit specific immune responses and development of inhibitors of the molecular mediators of cancer-induced immunosuppression (immune checkpoints) to boost antitumor immune responses. Amplification of the host response against evolving tumors through vaccination is being investigated in ongoing clinical trials with tumor cell vaccines; however, the clinical efficacy of these agents has been limited. Blocking inhibitory pathways such as the CTL antigen 4 (CTLA-4) and programmed cell death 1 (PD-1) checkpoint pathways with mAbs has generated antitumor immune responses that are transforming cancer therapeutics. PD-1 and programmed cell death ligand 1 (PD-L1) antibodies have shown durable responses in NSCLC, with a favorable safety profile and manageable side effects. The activity of immune checkpoint inhibitors is currently been assessed in treatment-naïve patients with PD-L1–positive advanced NSCLC. Combinatorial approaches with other immune checkpoint inhibitors, chemotherapy, or targeted agents are being explored in ongoing clinical trials, and may improve outcome in NSCLC. *Clin Cancer Res; 21(5); 976–84. ©2015 AACR.*

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Learning Objectives

Upon completion of this activity, the participant should have a better understanding of the basic principles of immunotherapy, including the mechanism of action, clinical efficacy, associated toxicities, and caveats related to such approaches, as illustrated in combinatorial checkpoint blockade immunotherapy in non–small cell lung cancer.

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Introduction

Current treatment strategies for non–small cell lung cancer (NSCLC) include chemotherapy regimens based on histology and targeted agents for patients who carry specific genomic alterations (1). The advent of molecularly targeted therapies has dramatically improved outcomes in the metastatic setting for patients with lung adenocarcinomas that harbor somatically activated oncogenes such as *EGFR* and translocated *ALK* (2). However, even with these therapies, the majority of patients with NSCLC do not attain prolonged disease control, and 5-year survival rates remain low (3). Thus, therapies that obtain long-lasting disease control without significant side effects are urgently needed. Early efforts of nonspecific immune stimulation–based therapies have yielded equivocal results. The ability of lung cancer to evade immunosurveillance is a result of production of immunosuppressive chemokines by the tumor cells, loss of MHC antigen expression, and higher numbers of T-regulatory (T_{reg}) cells in the tumor microenvironment (4, 5). Despite the initial limited efficacy of immunotherapy in treatment of NSCLC, novel approaches, including

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therapeutic vaccines and immune checkpoint inhibitors, have gained interest as a potential treatment paradigm, particularly in light of successes in castration-resistant prostate cancer and melanoma, leading to FDA approval of ipilimumab and sipuleucel-T. In this article, we review the basic mechanisms of antitumor immune responses and discuss clinical trials as well as the caveats associated with such approaches in treatment of NSCLC.

Cancer Immunosurveillance

The immune system plays a dual role in cancer progression; it can suppress tumor growth by eliminating cancer cells but also promote tumor growth by selecting for cancer cells that can evade surveillance. Immunoediting occurs in three phases: elimination, equilibrium, and escape, which involve the activation of innate and adaptive immune mechanisms (6, 7). Cancer cells express antigens that differentiate them from nontransformed cells. Human tumor antigens include mutational (i.e., p53), overexpressed cellular (i.e., HER2), viral (i.e., HPV), and cancer/testis (i.e., MAGE) antigens (8). In the elimination phase, transformed cells are destroyed by a competent immune system (9); cancer cells that survive immunosurveillance enter the equilibrium phase. Equilibrium represents a functional state of dormancy in which tumor outgrowth is controlled by adaptive immunity (7, 10). Tumor cells that have acquired the ability to circumvent immune recognition can become clinically apparent (11). Moreover, tumor cells that escape immunosurveillance can induce an immunosuppressive state through production of cytokines and growth factors such as VEGF and TGFβ, as well as by recruiting T_{reg} cells and myeloid-derived suppressor cells (7, 9, 12).

Immune Checkpoints in Cancer Immunotherapy

Immune checkpoints refer to inhibitory pathways crucial for maintaining self-tolerance; tumors use certain checkpoint pathways to escape immune surveillance (13). Inhibitory ligands and receptors that regulate T-cell effector functions are commonly overexpressed in tumor cells or in the tumor microenvironment (14). The blockade of immune checkpoints releases the breaks on the immune system resulting in antigen-specific T-cell responses. The most studied immune checkpoint receptors are the inhibitory receptors CTLA-4 and PD-1 (Fig. 1). CTLA-4 is expressed on T cells and regulates the early stages of T-cell activation; it counteracts the activity of the T-cell costimulatory receptor CD28 by competing for its ligands B7.1 (CD80) and B7.2 (CD86; refs. 15, 16). CTLA-4 primarily regulates $CD4^+$ T cells and enhances the immunosuppressive activity of T_{reg} cells (17). In contrast with CTLA-4, PD-1 mediates immune resistance in the tumor microenvironment by downregulating the activity of effector T cells in peripheral tissues in the setting of an inflammatory response (18). PD-1 is expressed on tumor-infiltrating lymphocytes (TIL; mainly $CD4^+$ T cells) as well as on B cells, natural killer cells, monocytes, and dendritic cells (DC; ref. 19). Upon binding to its ligands (PD-L1 and PD-L2), PD-1 inhibits kinases that are involved in T-cell activation (20, 21); PD-1 is highly expressed on T_{reg} cells and may enhance

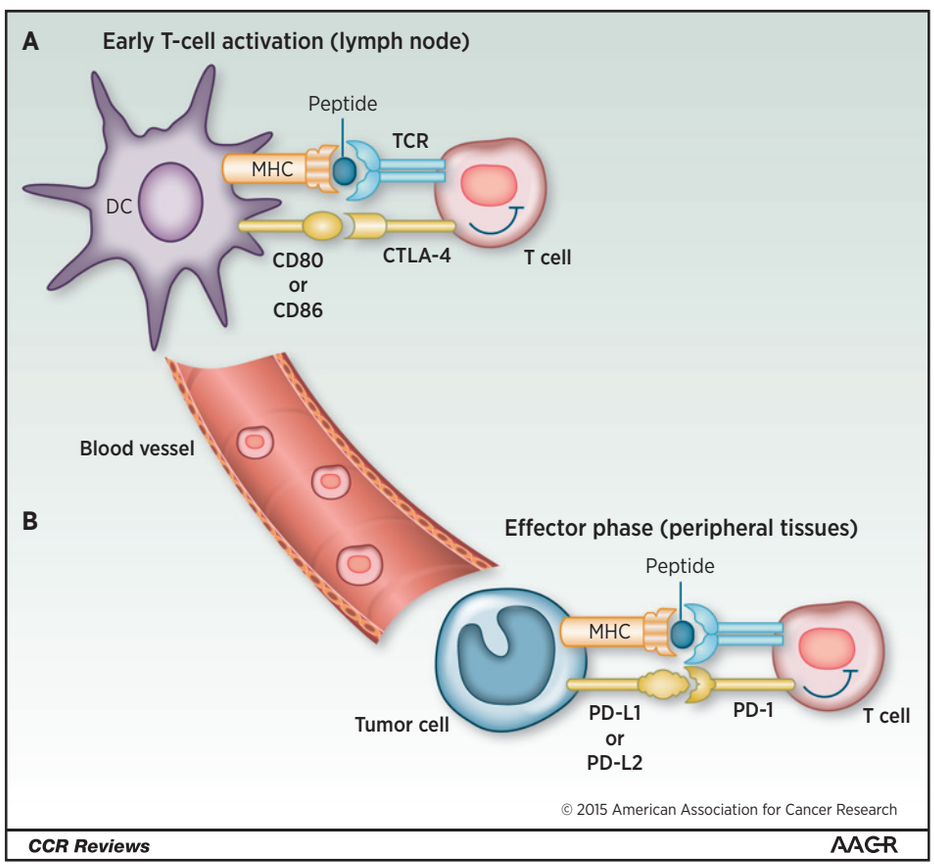

Figure 1.
CTLA-4 and PD-1/PD-L1 checkpoint blockade. A, after antigen recognition occurs, CTLA-4 expressed on T cells at the time of the initial response to antigen binds to B7.1 (CD80) and B7.2 (CD86) and regulates the early stage of T-cell activation by inhibiting PI3K signaling, binding phosphatases SHP-2 and PP2A, and blocking microcluster formation. B, PD-1 expressed on activated T cells binds to PD-L1 (B7-H1/CD274) and PD-L2 (B7-CD/CD273), thus limiting T-cell activation in peripheral tissue after inflammatory response by recruiting inhibitory phosphatase SHP-2, decreasing expression of antiapoptotic protein BCL-XL, and inhibiting PI3K/AKT.

their proliferation (22). PD-1 ligands are frequently upregulated in human cancers, including NSCLC (15), and there are two mechanisms of expression of immune checkpoint ligands on tumor cells: through oncogenic signaling independent of inflammatory signals in the tumor microenvironment (innate immune resistance; refs. 23, 24) or through induced response to inflammatory signals produced by an active antitumor immune response (adaptive immune resistance; refs. 14, 25). Inhibition of the CTLA-4 and PD-1 pathways has been shown to enhance intratumoral immune responses in numerous preclinical studies (26, 27), and blockade of immune checkpoints has introduced a new era in cancer treatment. Given that tumor cells express multiple inhibitory ligands and TILs express a variety of inhibitory receptors, antitumor immune responses can be enhanced through multilevel blockade of immune checkpoints.

Checkpoint Inhibitors

CTLA-4 inhibitors

Ipilimumab. Ipilimumab is a fully humanized IgG1 anti–CTLA-4 mAb that blocks binding of CTLA-4 to its ligand. A randomized phase II clinical trial of paclitaxel and carboplatin with or without ipilimumab in treatment-naïve stage IV NSCLC showed improvement in immune-related progression-free survival (irPFS) with ipilimumab, when ipilimumab was given after chemotherapy (5.7 vs. 4.6 months, $P = 0.05$; ref. 28). The rationale for administration of chemotherapy before ipilimumab (phased regimen) was to allow antigen release to occur before initiation of immune modulation with ipilimumab. There was a trend toward improved overall survival (OS) in patients who received phased chemotherapy with ipilimumab compared with chemotherapy alone (12.2 vs. 8.3 months, $P = 0.23$). Progression-free survival (PFS) and OS benefit was more prominent for squamous cell carcinomas (HR, 0.55; 95% CI, 0.27–1.12 and HR, 0.4; 95% CI, 0.2–1.03, respectively). Common toxicities included anemia, diarrhea, and fatigue; grade 3/4 immune-mediated toxicities (colitis, transaminitis, and hypophysitis) occurred more commonly in patients receiving ipilimumab. A phase III confirmatory trial is ongoing (NCT01285609; Table 1).

Tremelimumab. A phase II study of tremelimumab as maintenance therapy compared with observation in patients with stable or responding disease after first-line chemotherapy failed to show an improvement in PFS (29). Clinical trials combining the anti–PD-L1 antibody, MEDI4736, with tremelimumab are ongoing in NSCLC (NCT02000947; Table 1).

Table 1. Ongoing and recently completed clinical trials of CTLA-4 and PD-1 immune checkpoint inhibitors in NSCLC

Agent	Phase	Design and description	Study population	Primary endpoint	Enrollment	NCT
CTLA-4 inhibitors						
Ipilimumab	III	Ipilimumab + paclitaxel/carboplatin vs. placebo + paclitaxel/carboplatin	Squamous cell, stage IV or recurrent NSCLC	OS	920	NCT01285609
Tremelimumab	Ib	Dose-escalation and dose-expansion study of tremelimumab with MEDI4736	Advanced NSCLC	MTD, safety based on rate of AEs, SAEs	208	NCT02000947
PD-1 inhibitors						
Nivolumab	II	Single-arm nivolumab after failure of >2 prior systemic regimens	Squamous cell, advanced or metastatic NSCLC	ORR	100	NCT01721759
	III	Nivolumab vs. docetaxel	Squamous cell, previously treated or metastatic NSCLC	OS	264	NCT01642004
	III	Nivolumab vs. docetaxel	Nonsquamous previously treated metastatic NSCLC	OS	574	NCT01673867
	III	Nivolumab vs. investigator's choice chemotherapy	Stage IV or recurrent PD-L1$^+$ NSCLC	PFS	495	NCT02041533
	I	Multiarm safety study of nivolumab + gemcitabine/cisplatin, pemetrexed/cisplatin, erlotinib, carboplatin/paclitaxel, ipilimumab or bevacizumab maintenance	Stage IIIb/IV NSCLC	Safety based on rate of AEs, SAEs, and laboratory abnormalities	412	NCT01454102
	I	Dose-escalation study of safety and efficacy of nivolumab + anti–LAG-3 mAb (BMS-986016)	NSCLC progressing while on- or after receiving anti–PD-1 or anti–PD-L1 antibodies	Safety based on rate of AEs, SAEs, deaths, and laboratory abnormalities	168	NCT01968109
Pembrolizumab	II/III	Low-dose or high-dose pembrolizumab vs. docetaxel	Previously treated NSCLC	PFS, OS, rate of AEs, and study discontinuation	920	NCT01905657
	III	Pembrolizumab vs. platinum-based chemotherapy	Treatment-naïve PD-L1$^+$ metastatic NSCLC	PFS	300	NCT02142738
	III	Single-agent pembrolizumab vs. platinum-based chemotherapy	Treatment-naïve PD-L1$^+$ advanced or metastatic NSCLC	OS	1,240	NCT02220894
	II	Single-agent pembrolizumab	NSCLC with brain metastases	ORR	54	NCT02085070
	I/II	Safety, tolerability, and efficacy of pembrolizumab + chemotherapy or immunotherapy	Stage IIIb/IV treatment-naïve or recurrent (>1 year after adjuvant therapy) stage I–IIIA NSCLC	PFS, ORR, recommended phase II dose for pembrolizumab	320	NCT02039674

Abbreviations: AE, adverse event; KIR, killer-cell immunoglobulin-like receptor; LAG-3, lymphocyte activation gene-3; MTD, maximum tolerated dose; ORR, objective response rate; SAE, serious adverse event.

PD-1 and PD-L1 inhibitors

Nivolumab. Nivolumab is a fully humanized monoclonal IgG4 antibody targeting PD-1. Since the first-in-human phase I clinical trial of an anti–PD-1 antibody showing activity in NSCLC (30), subsequent studies have demonstrated that PD-1 pathway blockade confers durable tumor responses (31). A phase I trial in heavily pretreated NSCLC demonstrated a 17% objective response rate (ORR) with a median response duration of 74 weeks and ongoing response in 55% of patients. OS was 42% and 14% at 1 and 2 years, respectively (32, 33). Common toxicities were fatigue, decreased appetite, and diarrhea, with a treatment-related grade 3/4 toxicity rate of 14%. Pneumonitis was reported in 7% of patients with NSCLC, with 3 patient deaths reported. Ongoing phase III clinical trials are testing nivolumab monotherapy versus docetaxel (NCT01642004 and NCT01673867) in the second-line treatment setting. A phase III first-line trial of nivolumab versus standard chemotherapy in PD-L1–positive metastatic NSCLC is currently recruiting (NCT02041533).

Preclinical data showed that dual CTLA-4 and PD-1 blockade significantly increased antitumor immune responses (34), and a phase I trial of nivolumab and ipilimumab demonstrated a 40% ORR in melanoma (35). Combination of nivolumab with ipilimumab is being tested in an ongoing phase I clinical trial in NSCLC (NCT01454102); interim analysis revealed an ORR of 22% (36). Any-grade treatment-related adverse events were reported in 39 (85%) patients, and 3 treatment-related deaths were noted. This clinical trial is a multicohort study that will also evaluate the safety and efficacy of nivolumab as a first-line single agent and in combination with standard chemotherapy in stage IIIB/IV NSCLC. Interim results from the nivolumab monotherapy cohort revealed an ORR of 30%; response rate (RR) was 67% in PD-L1–positive patients, whereas no responses were observed in PD-L1–negative patients (37). In an interim analysis of the chemotherapy plus nivolumab arm, nivolumab combined with platinum-based regimens demonstrated antitumor activity, with 1-year OS of 59% to 87% and an acceptable tolerability profile (38). Those observations—although encouraging—are limited by small sample size, and longer follow-up is required to confirm their generalizability. Nivolumab is also being combined with an anti–LAG-3 mAb in a phase I trial (NCT01968109).

Pembrolizumab. Pembrolizumab (MK-3475), an anti–PD-1 humanized IgG4 antibody, was FDA approved in September 2014 for previously treated melanoma. In NSCLC, a phase I clinical trial in patients who had failed two systemic regimens showed an ORR of 24% (39). Pembrolizumab is now being tested versus docetaxel (NCT01905657) and platinum-based combinations for PD-L1–positive NSCLC (NCT02142738) in previously treated and metastatic treatment-naïve NSCLC, respectively. A phase III trial comparing pembrolizumab with platinum-doublet chemotherapy in treatment-naïve PD-L1–positive patients is ongoing (NCT02220894). The activity of pembrolizumab in untreated brain metastases from NSCLC will be assessed in a phase II trial (NCT02085070). The efficacy of pembrolizumab in combination with chemotherapy, targeted agents, or ipilimumab will be determined in an ongoing phase I/II trial in stage IIIb/IV treatment-naïve NSCLC (NCT02039674). Ongoing clinical trials with PD-1 inhibitors are summarized in Table 1.

PD-L1 inhibitors. BMS-936559 was the first IgG4 mAb targeting PD-L1 that reported activity in NSCLC. The phase I trial of BMS-936559 showed an ORR of 10% in 49 patients with NSCLC, and 12% of patients had stable disease at 6 months; PFS at 24 weeks was 31%, and the RR was independent of histology (40).

Various other anti–PD-L1 antibodies have shown activity in NSCLC. An RR of 21% was reported in a phase I trial of the engineered human IgG1 anti–PD-L1 inhibitor MPDL-3280A (41). Further trials are evaluating MPDL-3280A compared with docetaxel (NCT01903993 and NCT02008227). A phase II trial of MPDL-3280A monotherapy in PD-L1–positive NSCLC is ongoing (NCT01846416) and efficacy of combinatorial approaches with erlotinib (NCT02013219), bevacizumab (NCT01633970) and the MEK inhibitor cobimetinib (NCT01988896) are evaluated in phase I trials.

MEDI4736 is an engineered human IgG1 mAb that blocks PD-L1 binding to PD-1, thus allowing T cells to recognize and eliminate tumor cells; MEDI4736 has shown activity in NSCLC (42). Interim results from the NSCLC cohort of an ongoing phase I study in advanced solid tumors (NCT01693562) demonstrated early and durable activity in NSCLC with an ORR of 13% at 12 weeks. MEDI4736 was well tolerated, with no treatment discontinuations, drug-related colitis, or grade 3/4 pulmonary toxicities (43). A phase III clinical trial of MEDI-4736 following concurrent chemoradiotherapy in patients with unresectable stage III disease is ongoing (NCT02125461). Multiple clinical trials are evaluating the efficacy of MEDI-4736 as single agent (NCT02087423) or in combination with tremelimumab (NCT02000947) and gefitinib (NCT02088112). Anti–PD-L1 agents in clinical trials are summarized in Table 2.

Caveats associated with immune checkpoint blockade

Clinical endpoints. Response patterns with immunotherapy differ from those of cytotoxic agents, and ORR and PFS traditionally used as clinically meaningful endpoints seem to be limited in their ability to predict outcome. Immune-related response criteria (irRC) have been introduced to characterize patterns of response (44) and novel clinical endpoints such as the irPFS, defined as the time of initiation of treatment to immune-related progression or death, are used, however, are not universally accepted. Given the late plateau in survival curves with immunotherapy driven by long-term responders, OS remains the golden standard primary endpoint (45).

Predictive biomarkers. Patient selection for immunotherapy and identification of predictive biomarkers for immune therapies are currently active areas of research. PD-L1 expression by immunohistochemistry has been found to predict response to PD-1 and PD-L1 inhibitors, conferring increased ORR to pembrolizumab (46), MPDL-3280A (47), and MEDI-4736 (43). However, in each trial, PD-L1–negative tumors, albeit at a lower rate, can still respond to these antibodies. The use of PD-L1 expression as a companion diagnostics platform for immune therapies has been limited by the lack of a standardized assay, different cutoff points to determine PD-L1 positivity, variability in intervals between biopsy and treatment, as well as sample preservation.

Smoking history was associated with an increased ORR to pembrolizumab (48), and this was consistent with an RR of 25% for smokers versus 16% for never smokers seen with MPDL-3270A (47). A similar pattern was observed for active/former smokers treated with nivolumab and MPDL-3280A (41, 49); the underlying mechanism remains unknown; however, it is possible that smoking status is a surrogate marker for mutational density (50). Somatic

Table 2. Ongoing clinical trials of PD-L1 inhibitors in NSCLC

Agent	Phase	Design and description	Study population	Primary endpoint	Enrollment	NCT
MPDL-3280A	II	Single-arm study evaluating safety and efficacy of MPDL-3280A	PD-L1$^+$ locally advanced or metastatic NSCLC	ORR	128	NCT01846416
	II	MPDL-3280A vs. docetaxel after failure of platinum-based chemotherapy	Advanced or metastatic NSCLC	OS	287	NCT01903993
	III	MPDL-3280A vs. docetaxel after failure of platinum-based chemotherapy	Advanced or metastatic NSCLC	OS	850	NCT02008227
	II	Single-arm study evaluating safety and efficacy of MPDL-3280A	PD-L1 + locally advanced or metastatic NSCLC	ORR	128	NCT01846416
	Ib	Safety and tolerability of MPDL-3280A + erlotinib	EGFR TKI treatment-naïve advanced NSCLC	Rate of dose-limiting toxicities	32	NCT02013219
	Ib	Safety and efficacy of MPDL-3280A + bevacizumab and/or + chemotherapy	Stage IIIB/IV or recurrent NSCLC	Rate of AEs, MTD	180	NCT01633970
	Ib	Safety and tolerability of MPDL-3280A + cobimetinib	Locally advanced or metastatic NSCLC	Rate of dose-limiting toxicities, AEs	90	NCT01988896
MEDI4736	III	MEDI4736 vs. placebo following concurrent chemoradiotherapy	Stage III unresectable NSCLC	OS, PFS	702	NCT02125461
	II	MEDI4736 after failure or >2 prior systemic treatment regimens	Locally advanced or metastatic NSCLC	ORR	210	NCT02087423
	Ib	Safety and tolerability of MEDI4736 + tremelimumab	Advanced NSCLC	Rate of AEs and SAEs, MTD	208	NCT02000947
	I	Safety, tolerability, and antitumor activity of MEDI4736 + gefitinib after failure of standard treatment	Advanced or metastatic NSCLC	Safety based on rate of AEs, SAEs, and laboratory abnormalities	47	NCT02088112

Abbreviations: AE, adverse event; SAE, serious adverse event; TKI, tyrosine kinase inhibitor.

mutational density reflecting neoantigen generation has been shown to confer enhanced antigenicity and response to ipilimumab in melanoma (51); this development remains to be demonstrated in NSCLC.

There is emerging evidence linking epithelial–mesenchymal transition (EMT) and suppression of antitumor immunity as a causative mechanism of metastasis through miR-200 loss that controls PD-L1 expression on lung cancer cells (52). EMT was found to be associated with higher levels of immune-modulatory molecules in lung cancer; however, that observation has not translated to a clinical response to immune checkpoint blockade to date (53).

Therapeutic Vaccines

Therapeutic vaccines have been used to prime the host immune system to recognize tumor antigens and augment antitumor T-cell responses; two types of vaccines are being evaluated in NSCLC: tumor cell and antigen-based vaccines (54). Immunization against tumor epitopes is achieved by injection of recombinant tumor antigen proteins, peptides, or gangliosides that in turn activates humoral and cellular immune responses against tumor antigens (Fig. 2; ref. 55). Vaccines are most likely more effective in patients with minimal residual disease after definitive treatment and could result in long-lasting therapeutic effects. Unfortunately, the clinical efficacy of vaccines is limited as the majority target tumor-associated as opposed to tumor-specific antigens and have been unable to circumvent the multiple immunosuppressive mechanisms in the tumor microenvironment (56).

Antigen-specific vaccines

Tecemotide (Liposomal BLP25). Tecemotide (L-BLP25) is a mucin 1 (MUC1) antigen-specific peptide vaccine capable of inducing a T-cell response to MUC1, a glycoprotein that is overexpressed in NSCLC (57). A phase III clinical trial of tecemotide in patients with unresectable stage III NSCLC who completed chemoradiotherapy with confirmed stable disease or objective response did not show a survival benefit for patients receiving the vaccine (58). Ongoing trials are summarized in Table 3.

Melanoma-associated antigen 3. Melanoma-associated antigen (MAGE) is a family of tumor-specific antigens, and MAGE-A3 is overexpressed in 35% to 55% of cases of NSCLC (59). A phase II clinical trial failed to demonstrate a PFS benefit for stage IB/II MAGE-A3–positive NSCLC treated with recombinant MAGE-A3 protein (60); however, an 84-gene expression signature was shown to identify patients that would benefit from a MAGE-A3 vaccine highlighting the need of development of companion diagnostic assays for such treatments (61). A phase III trial of MAGE-A3 vaccine versus placebo in patients with MAGE-A3–positive IB/IIIA NSCLC was initiated (NCT00480025); however, the trial was stopped in April 2014 as it did not meet its endpoints (62).

TG4010. TG4010 is an antigen-based vaccine based on a poxvirus that codes for the MUC1 antigen and IL2. TG4010 was tested in combination with chemotherapy in a phase II trial; however, there was no significant difference in OS (63). A phase IIB/III trial of first-line chemotherapy with TG4010 in stage IV NSCLC is ongoing (NCT01383148; Table 3).

Recombinant human epidermal growth factor. The EGF vaccine is an antigen-based vaccine in which recombinant EGF is fused to a carrier protein. Upon administration it generates an anti-EGF antibody response that prevents endogenous EGF from binding to EGFR, thus inhibiting cancer proliferation (64). A phase II trial failed to demonstrate a survival benefit for patients with stage IIIB/IV NSCLC who had previously received first-line chemotherapy; however, patients with an antibody response had a better OS (65).

Racotumomab. Racotumomab (IE10) induces a humoral and cellular response to Neu-glycosylated sialic acid containing ganglioside. A phase II/III trial of maintenance racotumomab in patients with stage IIIB/IV disease achieving a response or stable

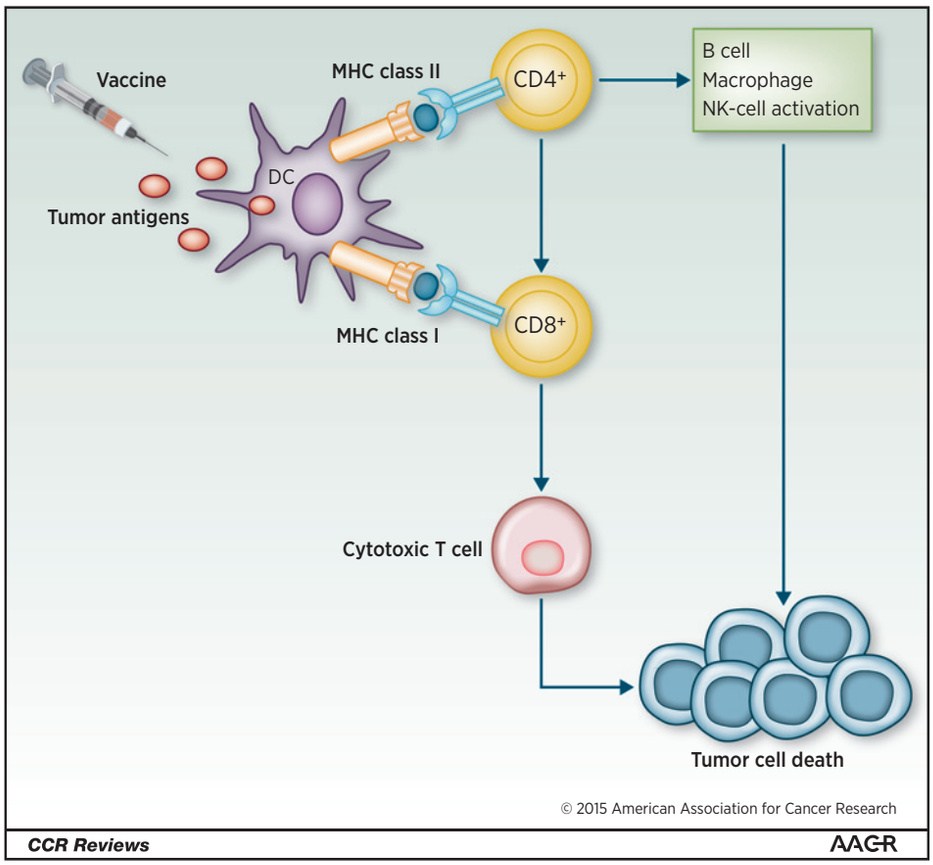

Figure 2.
Mechanism of action of cancer vaccines. Vaccines raise a T-cell– or B-cell–mediated antitumor response; once injected, components of the vaccine activate DCs, which in turn migrate to local lymph nodes. In the lymph node, activated DCs present antigens (bound to MHC) to T cells that are subsequently activated; $CD4^+$ T cells produce cytokines that promote $CD8^+$ T-cell maturation. $CD8^+$ T cells ultimately leave the lymph node and traffic in sites in which cells bearing the target antigen reside, initiating a cytotoxic antitumor response.

disease after first-line treatment is currently ongoing; interim results showed a slightly better OS in the vaccinated group (OS of 10.9 vs. 6.9 months for the vaccine and control group, respectively; $P = 0.002$; ref. 66). Results of the phase III part of the trial are awaited.

Whole-cell vaccines

Belagenpumatucel-L. Belagenpumatucel-L is an allogeneic tumor cell vaccine comprising of four NSCLC cell lines (H460, H520, SKLU-1, and RH2) transfected with a TGFβ2 antisense gene. A phase III trial evaluated Belagenpumatucel-L as maintenance therapy; however, improvement in OS was only reported in predefined subsets, with particular efficacy in patients with stage IIIB/IV nonadenocarcinomas. Patients who had received previous radiation also had improved OS with the vaccine (67).

Immunotherapy–Radiotherapy Combinatorial Therapeutic Approaches

Radiotherapy has been shown to enhance antitumor immune responses by causing inflammatory cell death, MHC I upregulation, and release of antigens taken up by DCs and presented to T cells that in turn migrate back to the tumor and provide local control, thus serving as an intrinsic vaccine priming adaptive immunity (68). High-dose ionizing irradiation has also been shown to upregulate PD-L1 expression, and PD-L1 blockade enhances the efficacy of radiation through a cytotoxic T-cell–dependent mechanism (69) . Anti–PD-1 and anti–CTLA-4 therapy in combination with radiation has been reported to cause an increase in tumor-specific T cells in the draining lymph nodes, and radiation-induced immune responses can have antitumor activity

Table 3. Ongoing clinical trials of therapeutic vaccines in NSCLC

Vaccine	Phase	Design and description	Study population	Primary endpoint	Enrollment	NCT
L-BLP25	III	L-BLP25 + BSC vs. placebo for patients with stable disease or objective response after chemoradiotherapy	Unresectable NSCLC in Asian population	OS	500	NCT01015443
	III	L-BLP25 vs. placebo for patients with stable disease or objective response after chemoradiotherapy	Unresectable stage III NSCLC	OS	1,002	NCT02049151
	II	BLP25 and bevacizumab after definitive chemoradiotherapy	Newly diagnosed stage IIIA/IIIB unresectable NSCLC	Safety	55	NCT00828009
TG4010	II/III	Efficacy and safety of first-line therapy with or without TG4010	Stage IV NSCLC	Phase II: PFS Phase III: OS	1,000	NCT01383148

Abbreviation: BSC, best supportive care.

outside the radiation field (70). Although evidence predominantly comes from preclinical studies, such an abscopal response to radiation and ipilimumab has been reported in NSCLC (71). Advances in the field have given birth to immunologically mediated, radiation-driven personalized systemic therapy, a concept that is being actively investigated in ongoing clinical trials. The efficacy of such approaches should be weighed against toxicities from combined immunotherapy and radiation, especially radiation pneumonitis.

Future Directions

Novel approaches incorporating checkpoint inhibitors have shown promising preliminary results and durable responses in NSCLC. Identification of the specific immune-checkpoint pathway(s) that drive immune resistance is imperative to guide personalized therapeutic choices. There is preclinical evidence that oncogenes drive immune escape. In particular oncogenic EGFR signaling has been shown to remodel the tumor microenvironment by upregulation of the immunosuppressive molecules PD-1, PD-L1, and CTLA-4 (72). Blockade of oncogenic pathways might enhance or deter antitumor immunity, which provides the rationale for combination approaches involving targeted agents and immunotherapy. Furthermore, priming of endogenous tumor response by tumor vaccines might induce upregulation of immune checkpoints that could subsequently be blocked as part of multimodality treatment strategies. Immune checkpoint blockade might be combined with new molecules such as anti-CD25 antibodies that deplete $CD4^{+}Foxp3^{+}$ T_{reg} cells (73), anti-CD27 antibodies that in combination with PD-1 blockade reverse $CD8^{+}$ T-cell exhaustion (74), and antibodies recognizing the TNF superfamily receptor (75). Additional studies are needed to explore the synergistic effect of immune, cytotoxic, or targeted therapies as well as developing robust companion diagnostic assays for such treatments.

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