New Strategies in Lung Cancer: Translating Immunotherapy into Clinical Practice

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Abstract

Recent breakthroughs in translating the early development of immunomodulatory antibodies into the clinic, notably with the anti–cytotoxic T-lymphocyte antigen-4 antibody, ipilimumab, have led to durable benefits and prolonged survival for a subgroup of patients with advanced melanoma. Subsequent studies have shown that related immune checkpoint antibodies, specifically those targeting the programmed death-1 pathway, have activity in non–small cell lung cancer. Non–small cell lung cancer is the commonest cause of cancer death worldwide and this exciting avenue of clinical investigation carries with it great promise and new challenges. In this article, we discuss recent developments in lung cancer immunotherapy, reviewing recent findings from therapeutic vaccine studies and in particular we focus on the refinement of immunomodulation as a therapeutic strategy in this challenging disease. Clin Cancer Res; 20(5); 1067–73. ©2014 AACR.

Background

The global burden of lung cancer is increasing rapidly with over 1.6 million new cases diagnosed annually and a majority of cases now occurring in developing countries (1). In the United States, because of its high incidence and mortality rate, lung cancer accounts for approximately one third of all cancer-related deaths and more than breast, prostate, and colorectal cancers combined (2). Approximately 85% of lung cancers are of non–small cell histology and when localized these tumors may be cured in 20% to 40% of cases by surgical resection (3). Although recent advances in computed tomographic screening may ultimately lead to earlier diagnoses, the majority of patients currently present with incurable metastatic disease and until recently lung cancer was treated almost exclusively with a platinum-based doublet (2–4).

Median survival for patients with advanced non–small cell lung cancer (NSCLC) ranges between 8 and 10 months for those with squamous tumors to 10 to 14 months for molecularly unselected patients with adenocarcinoma enrolled in the most recent phase III clinical trials of modern chemotherapy regimens (5–7). The VEGF antibody, bevacizumab, is approved in combination with chemotherapy for the treatment of patients with advanced nonsquamous NSCLC (8). In addition, the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI), erlotinib and afatinib, and the anaplastic lymphoma kinase–directed crizotinib have significant activity for specific molecular subsets of NSCLC that harbor driver mutations (9–11).

Despite these recent advances, there remains an urgent need for new treatment options for patients with squamous histology and the majority of patients with nonsquamous histology. Resistance to pemetrexed-based chemotherapy or selective inhibitors of driver mutations typically develops within months, and sustained responses lasting more than a year are uncommon. Immunotherapy has potential efficacy across the boundaries of histology and driver mutational status and perhaps most importantly may lead to sustained remissions for those patients who achieve a response. This article reviews recent attempts to harness the immune system in the treatment of lung cancer, including recently reported large vaccine trials and promising data on the use of immune checkpoint inhibitors for this challenging disease.

Harnessing the Immune System to Treat NSCLC

The presence of a functioning immune system, whereby tumor antigens are recognized as foreign and eliminated, is fundamental to the prevention of cancer development and progression. Immunoediting has been proposed as a mechanism by which the immune system deals with nascent tumors that have escaped intrinsic tumor suppressor mechanisms such as apoptosis (12). The three phases of immunoediting are elimination (continuous process whereby the host’s innate and adaptive immunity destroys subclinical tumor cells), equilibrium (growth and metastasis of surrounding cells are controlled by the immune system), and escape (growth of resistant clones that become clinically apparent; ref. 12). Cancer immunotherapy aims to induce (or re-induce) a cellular immune response consisting of CD4+ and CD8+
cytotoxic T lymphocytes (CTL) that can selectively destroy cancers by targeting tumor-associated antigens (TAA; ref. 13). Cellular immunity begins with the recognition and internalization of antigens by antigen-presenting cells (APC), including dendritic cells. After uptake, short peptide sequences of the antigen are presented on the surface of the APC in association with the major histocompatibility complex (MHC) molecules. Mobilization of dendritic cells from the periphery to lymph nodes then occurs, followed by complex interactions with naïve T cells that lead to the activation of costimulatory molecules and activation of CD8+ CTLs (14). Activated CD8+ CTLs can then circulate, recognize, and destroy cells displaying the complementary peptide-MHC class I molecule through granule exocytosis or Fas ligand expression, leading to apoptosis (15). During this process memory lymphocytes are produced, which are capable of inducing a rapid T-cell response when confronted with the same antigen in the future, whereas cytokines may also modulate the response in a positive or negative fashion (16).

Lung tumor cells produce numerous immunosuppressive molecules, including prostaglandin E2, interleukin-10, TGF-β, and cyclooxygenase-2, that collectively downregulate the immune response to tumor (17–20). After resection of NSCLC, high-level CD4+/CD8+ T-cell infiltration in the tumor is associated with improved survival, whereas higher levels of regulatory T-cell infiltration predicts a higher risk of relapse (21, 22). More recently, Fas ligand polymorphisms have been shown to affect immune-mediated resistance to NSCLC growth and metastasis, whereas variations in cytokine haplotypes may also affect the innate immune response to nascent NSCLC (17, 23).

Clinical development of immunotherapy for NSCLC has involved two broad classes of agents, allogeneic vaccines and immune checkpoint inhibitors. Vaccines currently in development attempt to stimulate a cellular immune response to antigens differentially expressed on NSCLC and other tumors [e.g., MAGE-A3 (melanoma-associated antigen A3) ref. 24]. Immune checkpoints are molecules expressed on the surface of T lymphocytes and other immune cells that modulate the immune response to antigen through inhibitory or stimulatory signaling to T cells (Fig. 1; ref. 25). Early-phase clinical trials of antibodies targeting the co-inhibitory immune checkpoints, cytotoxic T-lymphocyte antigen-4 (CTLA-4), and programmed death-1 (PD-1), have shown promise, and these agents are currently undergoing phase III evaluation for the treatment of NSCLC, either in combination with chemotherapy or as single agents (26, 27).

**On the Horizon**

**Vaccines for NSCLC**

Currently six different vaccines are undergoing or have completed late-phase clinical development for NSCLC: liposomal BLP25 (L-BLP25) vaccine and MAGE-A3 vaccine for earlier-stage disease and EGF vaccine, belagenpumatucel-L, tegopenpumatucel-L, and TG4010 vaccine for advanced disease (28–32). Despite phase II studies of these agents failing to demonstrate improved survival for vaccinated patients, several phase III studies have nonetheless been undertaken. To date, phase III results involving L-BLP25 and belagenpumatucel-L have been reported and these have been negative (28, 31).

**Liposomal BLP25**

MUC1 is a glycoprotein aberrantly expressed on the cellular membranes of a variety of tumors, including NSCLC (33). L-BLP25 vaccine consists of a liposomal formulation of 25 amino acids from the immunogenic variable number of tandem repeats region of MUC1 (34). In a randomized phase II study, 171 patients with stage IIIB/IV NSCLC were randomly assigned to L-BLP25 or best supportive care (BSC) after initial chemoradiation (CRT) or chemotherapy with the primary endpoint of overall survival (OS; ref. 35). This study failed to demonstrate a statistically significant improvement in OS for L-BLP25 (17.2 months vs. 13 months for BSC alone, HR = 0.745, 95% CI, 0.533–1.042); however, based on non-prespecified subgroup analyses suggesting that stage IIIB patients may have benefited from the vaccine the phase III START (Stimulating Targeted Antigenic Responses To NSCLC) was undertaken (36).

START randomized 1,313 patients with stage IIIB NSCLC postdefinitive CRT to L-BLP or placebo with BSC (28). This study failed to demonstrate improved OS with L-BLP25 (25.6 m vs. 22.3 m for placebo, P = 0.123), although in a predefined subgroup analysis patients who received concurrent as opposed to sequential CRT seemed to benefit from the vaccine (median OS 30.8 m vs. 20.6 m, P = 0.016). Although several other studies involving L-BLP25 in NSCLC are ongoing, the development of biomarkers of response to L-BLP25 has yet to be reported, making it difficult to define a group of patients who might benefit from its use (37, 38).

**MAGE A3**

Between 20% and 50% of NSCLC cases express MAGE-A3, and expression is associated with an aggressive disease course (24). MAGE-A3 vaccine contains purified MAGE-A3 recombinant protein in a liposomal formulation (39). In the recently reported final results of a randomized phase II study, MAGE-A3 vaccine failed to prolong disease-free interval versus placebo when administered as adjuvant therapy after resection of MAGE-A3-positive stage IB–II NSCLC (39). MAGE-A3 positivity in tumor cells was analyzed using quantitative RNA PCR. No significant difference in the secondary endpoints of disease-free survival or OS was noted between the two arms in this study. A predictive 84-gene signature has been evaluated in patients with resected NSCLC treated with MAGE-A3 plus AS02a immunostimulant (40). Treated gene signature–positive patients showed a favorable disease-free interval compared with placebo-treated gene signature–positive patients (HR = 0.42; 95% CI, 0.17–1.03; P = 0.06), whereas among gene signature–negative patients, no such difference was found (HR = 1.17; 95% CI, 0.59–2.31; P = 0.65). The genes identified were mainly immune related, involving IFN-γ pathways and...
specific chemokines. The authors have suggested that the expression of these genes influences the tumor's immune microenvironment and the patient's clinical response.

The results of the MAGRIT trial (MAGE-A3 as Adjuvant NSCLC Immunotherapy), a large international phase III study are awaited. This trial has now completed accrual of 2,270 patients with resected MAGE-A3-positive stage IB to IIA NSCLC (41).

**EGF**

The EGF pathway is fundamental to the development of NSCLC and has been successfully targeted with TKIs including erlotinib and afatinib (9, 10). Investigators in Cuba have developed a vaccine that contains human recombinant EGF complexed with a *Neisseria meningitidis*–derived carrier protein (42). This vaccine is cytostatic and prevents EGF binding to its receptor through development of antibody-mediated immunity to EGF. In a randomized phase II study, patients with advanced NSCLC who received the EGF vaccine after first-line chemotherapy had a trend toward improved survival (6.5 m vs. 5.3 m, \( P = 0.098 \); however this was not statistically significant (43). Subsequently, enrollment has been completed on a phase III study in the same disease setting with results awaited (30).

**Belagenpumatucel-L.**

Defective TGF-\( \beta \) signaling is associated with immunosuppression and shortened survival in advanced NSCLC (44). Belagenpumatucel-L is an allogeneic whole-cell vaccine that transfects cells with a TGF-\( \beta 2 \) antisense gene thus downregulating TGF-\( \beta 2 \) (44). In a phase II study in advanced NSCLC, 15% of patients responded to vaccination and 59% of patients were free from tumor progression after 4 months (45). In a phase III study of 532 patients with stage IIIA–IV NSCLC, belagenpumatucel-L failed to prolong OS when administered as consolidation after first-line chemotherapy (31). Predefined subgroup analyses from this study suggest that patients with non-adenocarcinoma

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**Figure 1.** Selected immune checkpoints for which modulating molecules are in late preclinical or clinical development. B7RP1, B7-related protein-1; ICOS, inducible T-cell costimulator; KIR, killer cell immunoglobulin–like receptor; LAG3, lymphocyte–activation gene 3; GAL9, galectin-9; TIM3, T-cell immunoglobulin domain and mucin domain 3; OX40L, OX40 ligand.
tumors and those who were randomized to vaccination within 12 weeks of completing chemotherapy may have benefited from the vaccine.

**Tergenpumatucel-L**

Data have recently been reported regarding the potential sensitizing effect of an allogeneic vaccine, tergenpumatucel-L, on response to subsequent lung cancer chemotherapy (46). Tergenpumatucel-L consists of three allogeneic lung tumor cell lines that are modified to express a gene encoding the α galactosyl transferase (αGAL) enzyme, which may potentiate immune recognition of tumor cells. In a phase II study 28 patients with advanced NSCLC received a series of eight vaccines given 2-weekly, immunogenicity measures were monitored including serum anti-αGAL and IFN-γ. Median OS was 11.3 months whereas 8 patients had stable disease lasting at least 16 weeks. IFN-γ responses were noted in 11 patients, and this cohort attained OS of 21.9 months. In addition, 31% of patients who received the vaccine responded to subsequent chemotherapy. Tergenpumatucel-L is currently undergoing phase III comparison with second-line chemotherapy for advanced NSCLC (47).

**TG4010**

This vaccine utilizes an attenuated Ankara virus that has been engineered to express MUC1 and interleukin-2 (48). In a phase II study the addition of TG4010 to first-line chemotherapy for advanced NSCLC followed by maintenance TG4010 crossed a predefined statistical boundary for efficacy when compared with chemotherapy alone (48). In this study, 43% of vaccinated patients were free from tumor progression at 6 months (48). Accrual is continuing to a phase III study that included patients with advanced NSCLC, the ORR in patients, and this cohort attained OS of 21.9 months. In addition, 31% of patients who received the vaccine responded to subsequent chemotherapy. Tergenpumatucel-L is currently undergoing phase III comparison with second-line chemotherapy for advanced NSCLC (47).

**Immune Checkpoint Inhibition for NSCLC**

Regulation of the T-cell response to antigen is mediated through a balance between costimulatory and coinhibitory signaling molecules known as immune checkpoints (49). T-cell activation induces expression of coinhibitory checkpoints and dampens the immune response to antigens including tumor antigens (49). In addition, tumors may induce expression of coinhibitory checkpoints on tumor cells and infiltrating lymphocytes, thus blocking the innate immune response (49). Agents targeting the coinhibitory checkpoints, CTLA-4, PD-1, and the ligand of PD-1, programmed death ligand 1 (PD-L1), have shown promise in early-phase clinical trials that included patients with advanced NSCLC. Reports of prolonged responses and stable disease with these antibodies are of particular interest given that the median progression-free survival (PFS) with cytotoxic chemotherapy in this disease setting is 4 to 7 months (5, 8, 10).

**CTLA-4**

Ipilimumab is a fully human monoclonal antibody (mAb) that augments antitumor immunity through CTLA-4 inhibition and has been approved for the treatment of advanced-stage melanoma (50). In a multi-arm phase II study that included patients with advanced NSCLC, the addition of concurrent and maintenance ipilimumab to standard first-line chemotherapy in a phased schedule (ipilimumab commencing after two of a planned four cycles of chemotherapy) led to prolonged immune-related PFS (irPFS) compared with chemotherapy alone (5.7 m vs. 4.6 m, P = 0.05; ref. 26). The use of irPFS as a primary endpoint instead of traditional Response Evaluation Criteria in Solid Tumors (RECIST) attempts to account for the unique patterns of response that have been reported with immune checkpoint inhibition (51). The study authors have suggested that the benefit of ipilimumab seemed to be confined to patients with squamous NSCLC; however, this inference should be treated with caution, given that only 28% (57/204) of patients in this phase II trial had squamous tumors and this analysis was not a predefined endpoint of the study. Nevertheless, enrollment has now commenced to an international phase III study in this histologic subgroup (52).

**PD-1**

Several mAbs that target PD-1 are in clinical development, and detailed data in NSCLC are available from a large phase I study of nivolumab, a fully human immunoglobulin G4 (IgG4) mAb directed against PD-1 (27). This study enrolled heavily pretreated patients with advanced melanoma, renal cell carcinoma, and NSCLC and responses have been noted in all 3 of these tumor types. Long-term follow-up data on 129 patients with NSCLC treated with nivolumab were presented at ASCO 2013 (53). The overall response rate (ORR) for patients with NSCLC was 17.2%, with a median duration of response of 17 months; response did not seem to differ by histologic subtype, and 55% of responses were ongoing at the time of the report. Drug-related adverse events (any grade) occurred in 71% of patients with NSCLC, with grade 3 and 4 drug-related adverse events reported in 14%. Drug-related pneumonitis occurred in 6% of patients, with 2% of these being grade 3 and 4, and 2 deaths in patients with NSCLC because of pneumonitis. Management algorithms for pneumonitis have been developed, and this toxicity seems to be mitigated by early recognition, aggressive workup, and prompt use of immunosuppression (54). Studies of nivolumab in advanced NSCLC are ongoing, including phase III studies as a single agent versus second-line chemotherapy and phase I combination studies with platinum doublet chemotherapy and several target-specific agents including ipilimumab (55–58).

MK-3475 is a humanized IgG4 mAb directed against PD-1 that has shown safety and preliminary efficacy in advanced renal cell carcinoma, melanoma, and NSCLC (59). In a phase I study of 38 patients with advanced NSCLC, this agent was associated with a 21% ORR; however, when stratified by PD-L1 expression, the ORR in tumors with high expression of PD-L1 was 57% compared with 9% in PD-L1-negative tumors (60).

Data on single-agent activity and combination immune checkpoint inhibition of anti-PD1 with ipilimumab in...
advanced melanoma have been particularly promising and additional data in NSCLC are awaited with interest (61).

PD-L1

PD-L1 is one of two ligands of PD-1 (the other being PD-L2) and several mAbs to PD-L1 are in clinical development. BMS-936559 demonstrated preliminary efficacy in a phase I study that included 49 patients with advanced NSCLC; ORR was 10% and 31% of patients were free from tumor progression at 6 months (62). MPDL3280a is an IgG4 mAb engineered to abrogate its antibody-dependent cell-mediated cytotoxicity function thus potentially avoiding the destruction of tumor-directed activated T cells (63). In a phase I study, 52 heavily pretreated patients with NSCLC were enrolled at doses of MPDL3280a up to 20 mg/kg with no maximum tolerated dose or dose-limited toxicities reported (63). PD-L1 expression by IHC was predictive of response with an ORR of 46% in those with intermediate PD-L1 expression and 83% in a small number of patients with high-level PD-L1 expression. Responses were durable with 24-week PFS of 46%. Importantly, no grade 3 to 5 pneumonitis was reported in this study, and studies of MPDL3280a in NSCLC and other solid tumors are planned.

Future Directions

Immune-based therapy for NSCLC has the potential to deliver prolonged disease responses, disease stability, and perhaps effective cure for subgroups of patients with advanced NSCLC. Significant efforts have been made to develop vaccines against NSCLC antigens, but experience in the clinic with these agents has so far been disappointing, although results from several phase III trials are awaited. Immune checkpoint inhibition targeting the PD1/PD-L1 axis delivers a moderate response rate by RECIST in advanced NSCLC; however, more importantly these responses are much more durable than those seen with non–immune-based therapeutics. Detailed immune profiling of tumor and patient response to PD1/PD-L1 inhibition is required to assist in the development of biomarkers of response. Combinatorial strategies including with chemotherapy, targeted agents and novel immune checkpoint mAbs are undergoing investigation, and toxicity and efficacy results from these studies will help define the optimal role for immune-based therapeutics in NSCLC.

Disclosure of Potential Conflicts of Interest

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