

Lung Cancer Immunotherapy

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Recent insights into anti-tumor immunotherapy have led to a wave of clinical trials involving immunotherapy for lung cancer. Vaccines have evolved from nonspecific immune stimulants, like Bacillus Calmette-Guerin (BCG), to much more specific and potent strategies, some of which generate active immune responses against tumor-associated antigens. Understanding the mechanisms of anti-tumor immunity and identifying target antigens will likely improve these therapeutic strategies and provide them with a niche in the future of lung cancer therapy.

Keywords: Immunotherapy, Non-small cell lung cancer, Small cell lung cancer, Tumor immunology, Tumor vaccines

Lung cancer is the leading cause of cancer mortality, resulting in more than 160,000 deaths per year in the United States.¹ Despite aggressive treatment with surgery, radiation and chemotherapy, the long-term survival for lung cancer patients remains low. Even those with “early stage” disease frequently succumb to lung cancer due to development of metastases. As a result, systemic therapy has become a requisite component of lung cancer management.

So far, systemic therapy has been limited to chemotherapy and biologic response modifiers. While novel agents like docetaxel, pemetrexed and erlotinib have recently demonstrated efficacy in treating patients with advanced lung cancer, clinical responses to treatment and improved survival have been modest. The limited successes of systemic chemotherapy have underscored the need to develop new therapeutic strategies like anti-tumor immunotherapy.

For several decades, effective immune-based anti-tumor therapy has been sought. There has been tantalizing evidence that the immune system, when properly stimulated, can eradicate cancer cells. Modest successes have been realized with nonspecific immune stimulants, such as interferon (IFN)- α and interleukin (IL)-2 for melanoma² and renal cell carcinoma,³ and impressive anti-tumor activity has been observed with Bacille Calmette-Guerin (BCG) for non-invasive bladder cancer.⁴

Building on enthusiasm generated by nonspecific immunotherapy, antigen-specific anti-tumor agents have been tested in animal models and in humans with advanced carcinomas. These novel strategies have been shown to generate anti-tumor immune responses in prostate cancer,⁵ melanoma⁶⁻⁸ and non-Hodgkin’s lymphoma,⁹ although clinical benefit has rarely been observed. For lung cancer, immunotherapy has only recently advanced from nonspecific to antigen-specific vaccination.

Early Studies of Immunotherapy for Lung Cancer

Lung cancer immunotherapy before 1990 focused on nonspecific immune stimulants including BCG, thymosin¹⁰ and *Corynebacterium parvum*.¹¹ Small cell lung cancer (SCLC) was commonly investigated because it was feasible to treat patients with minimal residual disease following curative chemotherapy and radiation.

Einhorn et al.¹² reported a series of 58 SCLC patients treated during the 1970s with chemotherapy, radiation and BCG immunotherapy. Five of 19 patients (26%) with limited stage disease were long-term survivors. Two larger series of patients with lung cancer treated with BCG found improvements in survival compared to historical controls, as well as a survival benefit for intrapleural BCG injection for patients with pleural effusions.^{13,14} Patients with adenocarcinomas were reported to respond better than those with squamous cell carcinomas.¹⁵ Excitement about nonspecific agents was tempered, however, by a randomized study of BCG as adjuvant therapy for SCLC demonstrating

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no impact on overall survival.¹⁶ In addition, a study of preoperative intra-tumoral injection of BCG found no impact on disease-free or overall survival,¹⁷ however, both of these randomized trials were underpowered to detect small differences. The role of BCG in lung cancer immunotherapy is now primarily as an adjuvant immune stimulant with autologous tumor or antigen-specific vaccine strategies.¹⁸

T cell growth factor thymosin, another nonspecific immune stimulant, was found to have activity in combination with chemotherapy, especially among patients whose immune systems were not activated at the time therapy was initiated.¹⁰ Similarly, early studies using the bacterium *Corynebacterium parvum* as a systemic anti-tumor agent for non-small cell lung cancer (NSCLC) demonstrated dose-related tumor responses, but no effect on overall survival.¹¹

During the 1990s nonspecific immunotherapy was attempted with IL-2 and other cytokines or inflammatory mediators. For example, Schiller et al.¹⁹ reported a series of 15 patients with advanced lung cancer treated with IL-2 and tumor necrosis factor- α . Cardiopulmonary toxicity was common, and there were no significant tumor responses. Similarly, a combination of IL-2 and IFN- α demonstrated no benefit.²⁰ A combination of IL-2 and melatonin, on the other hand, demonstrated a clinical benefit (20% partial response, 50% stable disease) in a study of 20 patients.²¹

Targeting Lung Tumors with Passive Immunotherapy

Monoclonal antibodies, first produced more than 30 years ago, have only recently been applied to human anti-tumor therapy. “Humanized” antibodies targeting specific tumor-associated antigens are now widely embraced agents for therapy of lymphoma (e.g., rituximab) and breast cancer (e.g., trastuzumab). So far, however, passive immunotherapy has not made an impact on lung cancer.

Trastuzumab (anti-HER2 antibody) has been evaluated for patients with advanced NSCLC. In a phase II study of 24 patients with HER-2 overexpressing tumors, only one patient had a partial clinical response to therapy.²² In another study, trastuzumab was tested in combination with chemotherapy for advanced NSCLC.²³ None of the 13 HER2-positive patients in this cohort responded to targeted therapy.

Another agent that has been developed recently is an antibody targeting the ganglioside fucosyl GM-1. In preclinical studies, this drug was shown to decrease formation of metastatic lung cancer via antibody-dependent cell-mediated cytotoxicity.²⁴

Antigen-specific radioimmunotherapy has been attempted for SCLC by utilizing bispecific monoclonal antibodies. This was felt to be a promising treatment strategy because SCLC is highly radiosensitive. In one study, anti-carcinoembryonic antigen (CEA) antibody was attached to a radionucleotide-binding antibody; 3 of 12 patients responded to this treatment.²⁵

Generating Active Anti-tumor Immunity Against Lung Cancer

While these “passive” immunotherapy strategies may lead to development of highly effective anti-tumor agents, the more potent therapeutic tools will likely be active immunotherapy agents. Lung cancer, although not very “immunogenic,” may provide an accessible target for the properly primed immune system.²⁶

The identification of tumor-associated antigens has enabled development of vaccines that prime potent, antigen-specific immune responses. Such antigens were first identified for melanoma, initially by evaluating tumor-infiltrating lymphocytes. Melanoma has been considered an “immunogenic” tumor based on the presence of anti-tumor immune cells within tumor tissue, as well as the therapeutic benefits observed with nonspecific immune stimulatory agents like IL-2. Lung tumors have fewer tumor-infiltrating lymphocytes revealing that an effective immune response is not typically primed (not necessarily that it is primed and rendered ineffective).²⁷ In lung cancer, as in melanoma, antigens targeted by tumor-infiltrating lymphocytes have been shown to induce tumor-specific cytolytic T cell responses.^{28,29} Identifying lung tumor antigens and presenting them in the optimal context may enable the immune system to generate anti-lung tumor effector cells that are usually absent.

Understanding how antigen-specific immune responses are generated has been a major focus of tumor immunology. As depicted in figure 1, antigen-presenting cells, including macrophages and dendritic cells, ingest parts of tumor cells, process their proteins and display them as short peptides bound to major histocompatibility complexes (MHC) class I and MHC class II. The T-cell receptor on naïve T cells binds to specific MHC/antigen complexes, leading to the exquisite specificity seen in cellular immunity. This T-cell receptor binding of the MHC/antigen complex, in conjunction with binding of costimulatory molecules B7.1 and B7.2 on the antigen-presenting cells, leads to activation of T cells. T cells that encounter their cognate antigen without proper costimulation (e.g., on a non-antigen-presenting cell) may be rendered tolerant, thus precluding generation of an immune response.

Helper T cells (CD4+) recognize their cognate antigens (MHC class II molecules) which are found only on antigen-presenting cells, whereas CD8+ cytolytic T cells recognize their cognate antigens (MHC class I molecules) that are found on all somatic cells including nonhematopoietic cells. Activation of CD4+ T cells leads to secretion of cytokines such as IFN- γ and IL-12, which in turn augment the stimulation of active CD8+ T cells. Helper T cells also augment the killing activity of natural killer cells, the phagocytic activity of macrophages and amplify antigen-specific immunity by local secretion of IL-2.

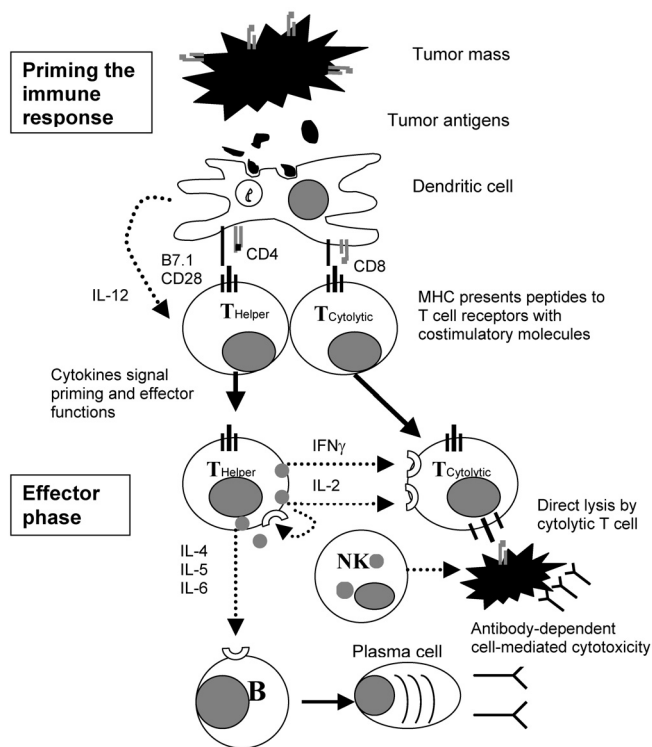


Figure 1. Anti-tumor immune priming and response.

Priming anti-tumor immunity, therefore, requires knowledge of specific antigens, as well as delivery of appropriate immune signaling components, including costimulatory molecules and cytokines. Since some tumor-associated antigens have been identified for lung cancer, the field is now poised to develop potent anti-tumor vaccines that incorporate these other factors. Some that have been evaluated are discussed below.

Antigen-specific Vaccination

Now that tumor antigens like the ganglioside fucosyl GM-1 have been identified, targeted active immunotherapy strategies have become feasible (table 1). Krug et al.³⁰ reported a clinical trial using a synthetic version of fucosyl GM-1 conjugated to the immune stimulant keyhole limpet hemocyanin in patients completing primary treatment for SCLC. Among 16 patients evaluated in this dose finding study, eight generated IgM responses. Another ganglioside, GD-3, has been tested in an active immunization strategy.³¹ BEC2-BCG, a vaccine that induces anti-ganglioside GD3 antibodies, was tested in SCLC patients after chemotherapy or combined chemotherapy and radiotherapy.¹⁶ In a large randomized international phase III trial, this agent provided no survival benefit.³²

Polysialic acid, a long polymer of negatively charged sialic acid residues that binds to a neural cell adhesion molecule, has been found on the surface of SCLC tumor cells. Because this molecule has a role in embryonic development, immune tolerance is normally developed. To overcome this, polysialic acid was modified by N-propionylation. In a series of six

patients vaccinated with the modified molecule, five demonstrated antibody responses against the target tumor antigen.³³

Cancer-associated mucins are another potential target for immunotherapy. These molecules are thought to promote metastases by facilitating adhesion of malignant cells to the endothelial cell surface. They exhibit unique glycosylation patterns, making them tumor-specific immunogens.³⁴ In a phase I clinical trial using MUC1 peptide in stage III/IV NSCLC,³⁵ safety and tolerability of this agent was established. Five of 12 patients (42%) had immunologic responses, and 4 of 12 patients (33%) achieved stable disease. In a randomized phase II clinical trial including 65 patients with stage IIIB NSCLC, Murray et al.³⁶ reported a 54% 2-year survival compared to a median survival of 13 months in the control group (best supportive care).

Epidermal growth factor (EGF), now a well-established target for biologic therapy, is also a potential tumor antigen. Preclinical studies have established the antigenicity and anti-tumor activity of EGF protein administered to animals.³⁷ In two randomized phase II studies,³⁸ recombinant EGF conjugated to *Neisseria meningitidis* P64K protein was administered to a total of 40 advanced NSCLC patients. Anti-EGF antibody responses were identified with a significant increase in survival for patients who maintained antibody response (9.1 months vs. 4.5 months). The same agent was tested in a larger randomized phase II clinical trial³⁸ which vaccinated 100 patients with stage IIIB or IV NSCLC who had progressed through first-line chemotherapy. Forty-five percent of vaccinated patients developed a strong anti-EGF antibody response. Compared to controls (best supportive care), those who received the treatment had significantly longer overall survival (8.5 vs. 4.3 months).

Wilm's tumor protein (WT1), a protein overexpressed in leukemias and several solid tumors, including lung cancer, has been demonstrated to be a target antigen for immunotherapy. Oka et al.³⁹ evaluated this vaccination strategy in 26 patients (10 with lung cancer). The patients received HLA-A*2402-restricted, natural or modified 9-mer WT1 peptide combined with Montanide ISA51 adjuvant. This study reported decreased tumor markers in 3 of 10 patients with lung cancer and identified a correlation between clinical response and anti-tumor CD8+ T cell activity. One responder continued to receive the treatment for 2 years without significant adverse effects.

MAGE-3, a tumor-associated antigen originally identified in melanoma, has also been found in non-small cell lung tumors.⁴⁰ In a clinical trial,⁴¹ nine NSCLC patients were vaccinated with the protein; 3 developed antibody responses. Seven of 8 patients who received MAGE-3 combined with adjuvant ASO2B generated antibodies against MAGE-3. Several of these patients also developed T cell responses to the protein. The authors concluded that vaccination with this recombinant protein provides an integrated immune response against tumor cells.

Table 1. Human lung cancer vaccine trials.

Immunotherapeutic Agent	Investigator (ref)	Type of Trial
<i>Antigen-specific vaccines</i>		
Fucosyl GM-1-KLH	Krug (30)	Phase I
BEC2-BCG	Giaccone (18)	Phase III
BLP25 (MUC1)	Palmer (35), Murray (36)	Phase II
EGF (conjugated with P64K)	Gonzales (37), Neningen (38)	Phase II
WT1 peptide	Oka (39)	Phase I
MAGE-3	Atanackovic (41)	Phase II
P53/dendritic cells	Gabrilovich (44)	Phase II
<i>Strategies that incorporate cytokines or costimulatory molecules</i>		
GVAX (GM-CSF)	Nemunaitis (52)	Phase I/II
ALVAC-CEA/B7.1	Ertl (61)	Phase II
Allogeneic tumor cell with B7.1	Raez (63)	Phase I
Dendritic cell	Hirschowitz (62)	Phase I

Dendritic cells have recently been investigated as a delivery mechanism for tumor-associated antigens. Both whole cell⁴² and peptide⁴³ strategies have been reported. In one study, 18 patients with metastatic gastrointestinal or lung cancer and HLA-A24 were treated with autologous dendritic cells pulsed with CEA-derived peptide. Immune reactions, measured by skin testing and *in vitro* T cell assays, were observed in most of the patients. Although no objective clinical responses were reported, some patients had stable disease while receiving this immunotherapy.

Another study of dendritic cell immunotherapy was reported by Gabrielovich et al.⁴⁴ in which 22 patients with previously-treated extensive stage SCLC were treated with autologous dendritic cells transfected with adenovirus containing p53. Eleven of 20 patients (55%) demonstrated immunological response to vaccination. This study found few responders to the vaccine, but several patients responded to subsequent chemotherapy, suggesting a sensitizing effect.

We evaluated a novel strategy for priming immunity against tumor-antigens using heat shock proteins secreted by tumor cells.⁴⁵ These proteins are “chaperones” that carry self- and tumor-specific peptides (potential tumor antigens). Initial studies with the heat shock protein gp96, isolated from human tumors, provided encouraging results in human trials.^{46,47} Our group has also investigated a gp96-Ig fusion protein, a chimeric protein that becomes secreted instead of remaining in the endoplasmic reticulum. Murine studies demonstrated that tumor-secreted gp96-Ig is capable of generating both immune and clinical responses. A phase I clinical trial of this agent is forthcoming.

Engineering More Potent Immunotherapy

The benefit of incorporating cytokines into anti-tumor vaccines has been well established. The cytokine granulocyte-monocyte

colony stimulating factor (GM-CSF), a significant mediator of proliferation, maturation and migration of dendritic cells,^{48,49} has been shown to enhance the generation of potent, durable anti-tumor immunity.⁵⁰ In a clinical trial using autologous, irradiated lung tumor (NSCLC) cells engineered to secrete GM-CSF,⁵¹ 18 of 25 assessable patients demonstrated evidence of anti-tumor immunity. Three of 6 patients with metastases removed were found to have tumor necrosis and several patients in the study had long-term disease stability.

In a multicenter phase I/II trial, Nemunaitis et al.⁵² evaluated 43 lung cancer patients vaccinated with GVAX, a GM-CSF-secreting bystander cell admixed with autologous tumor lysate. The most common toxicity was a local injection-site reaction (93%). Three of 33 advanced-stage patients, 2 with bronchoalveolar carcinoma, had durable clinical responses. Longer median survival was observed in patients whose vaccines secreted more GM-CSF (17 months vs. 7 months), suggesting a cytokine dose-response relationship. However, measurements of immunologic response were not associated with clinical response or survival.

Another promising strategy is the incorporation of costimulatory molecules into tumor vaccines. Tumor cells transfected with B7.1 (figure 1) and HLA molecules have been shown to stimulate an immune response by direct antigen presentation and activation of T cells without intermediary cells.⁵³ Vaccine trials with B7.1-transfected allogeneic or autologous cells have shown activity in murine and human studies of renal cell carcinoma,⁵⁴⁻⁵⁶ bladder cancer⁵⁷ and breast cancer.⁵⁸ In another clinical trial,⁵⁹ an HLA-A21, HER2/*neu*-expressing allogeneic breast cancer cell line genetically modified to express B7.1 was administered as a vaccine to 30 women with previously-treated stage IV breast cancer. Immune responses were identified in

9 of 15 patients evaluated. Prolonged disease stabilization was observed in four patients, although no objective tumor regression was seen.

In a similar strategy, a non-replicating canarypox virus (ALVAC) was constructed to express both human CEA and the B7.1 costimulatory molecule.⁶⁰ In a phase I trial, ALVAC-CEA-B7.1 was given without evidence of significant toxicity or autoimmune reactions. Three patients experienced clinically stable disease that correlated with increasing CEA-specific precursor T cells, as shown by *in vitro* IFN- γ enzyme-linked immunoassay spot tests. These three patients underwent repeated vaccinations resulting in augmented CEA-specific T cell responses. This strategy is now being tested in a phase II trial.⁶¹

In an attempt to incorporate more tumor-associated antigens, Hirschowitz et al.⁶² reported a novel strategy using dendritic cells pulsed with apoptotic bodies from an allogeneic NSCLC cell line expressing HER2/*neu*, CEA, WT1, MAGE2 and survivin. The cells were administered as an adjuvant agent to 16 patients with stage IA to IIIB NSCLC after surgery, chemoradiation or multimodality therapy. Six of the patients developed antigen-specific immune responses.

A more potent combination of target antigens may be delivered by an allogeneic whole cell-based vaccine. Such a vaccine could potentially target all the “shared” tumor-associated antigens and elicit a polyvalent cytolytic T lymphocyte response for each antigen, thus preventing tumor evasion by antigenic modulation. We developed an allogeneic lung cancer vaccine expressing the costimulatory molecule B7.1⁶³ to test the hypothesis that liberation of antigen in the context of the local inflammatory response will attract dendritic cells that uptake the antigen and the killed vaccine cells, and will cross-present the tumor-associated antigens through patient MHC to generate a tumor-specific cytolytic T lymphocyte response.

We treated 19 patients with irradiated allogeneic lung tumor (NSCLC) cells expressing the costimulatory molecule B7.1. Four patients experienced serious adverse events that were unrelated to vaccine. Another four patients experienced only minimal skin erythema. Among six clinically responding patients, immune responses were observed for as long as 150 weeks, long after administration of the vaccine. One patient had a partial clinical response and 5 had stable disease. Median survival for all patients was more than 18 months (90% confidence interval, 7 to 23 months), with estimated 1-year, 2-year and 3-year survivals of 52%, 30% and 30%, respectively. This study shows that a whole cell vaccine rendered immunogenic by transfection with B7 and HLA A1 or A2 can induce a potent immune response.

Challenges in Lung Cancer Immunotherapy

Despite the fact that many strategies have been evaluated, the most potent and effective lung cancer immunotherapy has not

yet been identified because most agents that have shown preclinical efficacy have not been effective in human trials. The optimal source of antigens (e.g., autologous or allogeneic tumor-associated antigens), the role of costimulatory molecules like B7.1 and the mode of delivery (e.g., viral gene transfer, dendritic cells or tumor cells), are just some of the questions under investigation.

Another challenge with these agents is the lack of correlation between immune response and clinical activity. Clinical responses to immunotherapy may be delayed compared to clinical responses to chemotherapy. Also, agents that provoke an increase in tumor size (sometimes interpreted as tumor progression) may be provoking an inflammatory reaction within the tumors. Perhaps the classic ways that clinical responses are assessed (e.g., RECIST criteria) during chemotherapy may not be the most appropriate for immunotherapy trials. Instead, progression-free survival or overall survival may be better measures of clinical activity in these studies, which is something important to consider when designing or evaluating immunotherapy trials.

So far immunotherapy seems to hold the greatest promise in the adjuvant setting, when tumor burden is low and there is only minimal chance of significant immune tolerance. Another challenge, therefore, will be to identify the optimal sequence of adjuvant therapeutic agents. Adjuvant chemotherapy currently has an established, proven role as an adjuvant modality for early stage lung cancer, therefore, it will be important to study whether tumor vaccines will be as effective after chemotherapy.

Some new directions in anti-tumor immunotherapy will come from new studies of immune modulation. For example, a subset of CD4⁺ CD25⁺ T lymphocytes (known as regulatory T cells) have been shown to play a major role in down-regulating immune responses to tumors, including lung cancer.^{64,65} Depleting these cells with targeted monoclonal antibodies like denileukin difitox may permit the generation of an anti-tumor immune response.⁶⁶ Similarly, agents that target tolerizing B cells (e.g., rituximab) are being studied in the setting of anti-tumor immunotherapy.

Conclusion

Immunotherapy for lung cancer is a burgeoning therapeutic modality, although its development lags several years behind that for other malignancies. “Passive” immunity strategies like monoclonal antibodies have not yet demonstrated effectiveness for lung cancer. Targeted “active” immunotherapy, on the other hand, now includes a series of novel agents capable of generating anti-tumor immunity against lung cancer. Several agents have demonstrated immune responses, and some have been found to have clinical efficacy. In the future, improved treatments will likely come from identification of other tumor-associated antigens, as well as a more solid understanding of antigen presentation and costimulatory molecules. Targeted agents, in sequence

with other adjuvant anti-tumor therapeutics, hold great promise for impacting the prognosis of lung cancer patients.

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