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Lung Cancer – Vaccines

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Abstract

In lung cancer, early attempts to modulate the immune system via vaccine based therapeutics have to date, been unsuccessful. An improved understanding of tumor immunology has facilitated the production of more sophisticated lung cancer vaccines. It is anticipated, that it will likely require multiple epitopes of a diverse set of genes restricted to multiple haplotypes to generate a truly effective vaccine that is able to overcome the various immunologic escape mechanisms that tumors employ. Other issues to overcome include optimal patient selection, which adjuvant agent to use and how to adequately monitor for an immunological response. This review discusses the most promising vaccination strategies for non small cell lung cancer including the allogeneic tumor cell vaccine belagenpumatucel-L, which is a mixture of 4 allogeneic non small cell lung cancer cell lines genetically modified to secrete an antisense oligonucleotide to TGF- β 2 and three other target protein-specific vaccines designed to induce responses against melanoma-associated antigen A3 (MAGE-A3), mucin 1 (MUC1) and epidermal growth factor (EGF).

Keywords

lung cancer; vaccines

Introduction

Lung cancer remains the leading cause of cancer related mortality and up to one third of cancer-related deaths in the United States can be attributed to this disease¹. Non small cell lung cancer (NSCLC) accounts for 85% of newly diagnosed lung cancer cases and the majority of patients present with advanced or metastatic disease². Surgical resection is the only truly curative therapy, but with relapse rates greater than 40% after definitive resection, improved therapeutics in the adjuvant setting are required³. In advanced stages of disease, systemic chemotherapy and/or localized irradiation can produce objective responses and palliation of symptoms; however, these therapies offer only modest improvements in survival. Two-year survival rates for stages IIIB and IV non-small cell lung cancer (NSCLC) are 10.8% and 5.4% respectively; 5-year survival rates are 3.9% and 1.3% respectively⁴. In theory stimulating the immune system by inducing a cellular immune response that harnesses CD4⁺ and CD8⁺ cytotoxic T lymphocytes (CTLs) capable of selectively destroying cancer cells by targeting tumor-associated antigens (TAAs) should potentially result in tumor eradication. Efforts to date have met with limited success for a

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variety of reasons. These include ineffective priming of tumor-specific T cells, lack of high-avidity of primed tumor specific T cells, and physical or functional disabling of primed tumor-specific T cells by the primary host and or tumor related mechanisms^{5, 6}. In NSCLC a high proportion of the tumor-infiltrating lymphocytes are immunosuppressive T regulatory cells (CD4⁺ CD25⁺) that secrete transforming growth factor- β (TGF- β) and express a high level of cytotoxic T-lymphocyte antigen-4 (CTLA-4)⁷. These cells impede immune activation by facilitating T-cell tolerance to tumor associated antigens rather than cross-priming CD8⁺ T cells resulting in the nonproliferation of killer T cells that recognize the tumor without attacking it⁷⁻⁹. Elevated levels of interleukin (IL-10) and TGF- β are found in patients with NSCLC. Preclinical models have shown immune suppression is mediated by these cytokines serving as a defense for malignant T cells against the body's immune system¹⁰⁻¹². Other tumor escape mechanisms include decrease or loss of tumor specific antigen, down-regulation of expression of human leukocyte antigen (HLA) molecules, increased expression of immunosuppressive factors by cancer cells including regulatory T cells and tolerant dendritic cells.

Current vaccine approaches focus on coupling immunogenic adjuvant agents to tumor antigens. The adjuvant agents are typically mixed with tumor cells or tumor antigens and the admixture is then used to vaccinate patients. Adjuvant agents enhance the APC response to the vaccine. The APCs then present antigen to T cells resulting in activation of tumor specific T cells. Attempts have been made to strengthen the immune response by incorporating naturally secreted immune modulators into vaccines. Strategies include genetically modifying autologous tumor cells or allogeneic cell lines to secrete cytokines and/or co-stimulatory molecules. Another way to boost the immune system is by expressing the antigen in a viral vector, which can also be designed to encode co-stimulatory molecules or cytokines. Other approaches include priming the immune system by using vaccines containing autologous DCs loaded with tumor antigen to elicit a tumor-specific CTL response.

Herein we discuss the most advanced therapies that are in the latter stages of development for NSCLC including the whole-cell vaccine, belagenpumatucel-L, and target protein-specific vaccines against: melanoma-associated antigen A3 (MAGE-A3), epidermal growth factor (EGF) and mucin 1 (MUC-1).

Biologic Rationale for using vaccines in non-small cell lung cancer

The primary aim of vaccine therapy is to provoke an adaptive antitumor immune response^{13, 14}. The first step in the cellular immunity cascade begins with the uptake of antigen, delivered by the vaccine, by antigen-presenting cells (APCs) such as dendritic cells (DCs) or macrophages. Once internalized, short peptide sequences of the antigen are presented on the extracellular surface of the APC in conjunction with the major histocompatibility complex (MHC) molecules. DCs then move from the periphery to the lymph nodes where they meet naïve T lymphocytes¹⁵. Complex reactions involving the specific T-cell receptor and the APC MHC-peptide molecule, as well as activation of co-stimulatory molecules such as B7.1 and B7.2, are required to activate CD8⁺ CTLs¹⁶. Once activated, these CTLs circulate and recognize cells that display the complementary peptide-MHC class I molecule on the cell surface. Cell death occurs via a combination of either granule exocytosis or expression of the FAS ligand, which ultimately leads to apoptosis¹⁷. Activation of CD4⁺ T cells leads to the secretion of a wide range of cytokines including interleukin-2 (IL-2), IL-12 and interferon (IFN)- γ , which in turn facilitate the activation of cognate CD8⁺ cytotoxic T cells which recognize the target peptide bound to MHC class I receptor's. Activation of CD4⁺ T cells also enhances the killing activity of natural killer

cells (NK) cells and the phagocytic activity of macrophages and triggers a humoral immune response leading to antibody production.

Unfortunately most cancer cells can evade the immune system by a variety of different mechanisms^{18, 19}. These include down-regulation of the expression of target antigens, MHC or co-stimulatory molecules, such as B7.1 or B7.2, upon initial antigen presentation leading to a failure of T cell recognition. Tumor cells also evade the immune system by down-regulating immune cytokines and developing resistance to CTLs. Lung cancer cells have been shown to produce a variety of immunosuppressive molecules including TGF- β , prostaglandin E2, IL-10, and cyclooxygenase-2 that can affect DC processing and presentation, as well as the acquisition and expression of CTL effector cell function^{18–20}. Strategies to develop effective immunotherapy for lung cancer have in the past failed. Recent successes in identifying T cell responses to tumor-specific antigens in patients with lung cancer suggest this goal may now be achievable²¹. The identification of lung tumor-associated antigens and presenting them in the optimal context may enable the immune system to generate anti-lung tumor effector cells²².

Allogeneic Tumor Cell Vaccines

Belagenpumatucl-L (Lucanix™)

Transforming growth factor-beta (TGF- β) has a tumor-associated immunosuppressive role via its inhibition of both NK cells and DC's^{23, 24}. Many tumors, including lung cancer, produce high levels of latent and active TGF- β and elevated levels have been identified as a poor prognostic factor in NSCLC^{25, 26}. A number of T cell- and DC-associated mechanisms have been proposed to explain the activity of TGF- β , including impairment of high affinity IL-2 receptor function and expression, leading to inhibition of CTL activation²⁷. TGF- β 2 is also able to convert naïve T cells to T regulatory cells by inducing the transcription factor FOXP3²⁸. T regulatory cells specifically prevent immune activation²⁹. DCs play a crucial role in the induction of antitumor immunity in tumor-bearing hosts by a process of antigenic cross-presentation³⁰. Immature DCs are unable to stimulate potent immune responses. TGF- β 2 can block maturation of DCs and inhibit their ability to present antigens³¹.

The vaccine belagenpumatucl-L (Lucanix™) is a mixture of 4 allogeneic NSCLC cell lines genetically modified to secrete an antisense oligonucleotide to TGF- β 2. The rationale for using a cell cocktail vaccine is based on the observation that NSCLC tumor cell lines share immunogenic epitopes with primary tumors. MHC class I-restricted CTLs generated against a specific human lung adenocarcinoma cell line have exhibited demonstrable cytotoxicity against other lung cancer cell lines. To increase the array of tumor antigens presented in the vaccine, belagenpumatucl-L employs 4 cell lines that express low levels of TGF- β due to prior transfection with a TGF- β 2 antisense plasmid. It is anticipated that down-regulation of TGF- β expression in the vaccine will mitigate a major source of immune suppression at the vaccine injection site. The hypothesis is that injecting allogeneic tumor cells with down-regulated TGF- β 2 will enhance local immune recognition and activation of effector cells, leading to a systemic immune response capable of targeting the patient's native tumor.

In a randomized phase II trial³², 75 patients with stage II to IV NSCLC were randomized to intradermal injections of 1 to 3 dose levels (1.25×10^7 cells/injection, 2.5×10^7 cells/injection, or 5.0×10^7 cells/injection) on a monthly or every other month schedule to a maximum of 16 injections. No significant adverse events were reported, and a dose-related survival difference was demonstrated in patients who received $\geq 2.5 \times 10^7$ cells/injection ($p = 0.0155$). Analysis suggested that cohorts 2 and 3, who received doses $\geq 2.5 \times 10^7$ cells/injection, had a significant survival advantage compared to cohort 1 ($p = 0.0069$). Of the 61 patients with late-stage NSCLC (IIIB/IV), 15% had a partial response. The estimated

probabilities of surviving 1 and 2 years were 68% and 52% respectively for the higher-dose groups combined, and 39% and 20% respectively for the low-dose group. The estimated median survival for patients receiving $\geq 2.5 \times 10^7$ cells/injection was 581 days, compared with 252 days for patients receiving 1.25×10^7 cells/injection ($p = 0.0186$). The difference in overall survival between dose cohorts 1, 2, and 3 in stage III/IV patients was not statistically significant ($p = 0.5148$). Increased cytokine production (IFN- γ , $p = 0.006$; IL-6, $p = 0.004$; and IL-4, $p = 0.007$) was observed among clinical responders (partial response or stable disease), who also displayed an elevated antibody-mediated response to the vaccine ($p = 0.014$). The results of this early phase clinical trial are intriguing. The phase II trial results certainly appear promising but the small number of patients and the possibility of selection bias suggest that the early results need to be compared to a control arm in a randomized trial.

A phase III investigation (STOP trial) is ongoing at present evaluating the vaccine as a maintenance therapy in patients with unresectable stage III/IV NSCLC who have responded to or have stable disease after first-line platinum-based chemotherapy. The primary endpoint is to compare the overall survival of subjects treated with belagenpumatucel-L vs placebo. The course of therapy in the treatment arm is best supportive care (BSC) plus monthly intradermal (ID) injections of belagenpumatucel-L consisting of 25,000,000 cells in a volume of 0.40 mL once a month for 18 months and then once at 21 and 24 months in the absence of disease progression or unacceptable toxicity. The study commenced in July 2008 and is expected to enroll 506 patients by October 2012. The estimated primary completion date is June 2012.

Autologous Tumor Cell Vaccines

Cell suspension of whole tumor transfected with GM-CSF

GVAX® is a vaccine composed of whole tumor cells genetically modified to secrete GM-CSF. The initial vaccine used autologous irradiated tumor cells transfected with a non-replicating adenoviral vector engineered to secrete GM-CSF³³. In the phase I trial involving patients with stage IV NSCLC, the vaccine was well tolerated, with toxicities restricted to grade 1 and 2 local skin reactions. The vaccine demonstrated activity, with 5 patients having stable disease and 2 patients with no demonstrable disease (having had surgical resection of all known metastatic sites prior to vaccination) achieving prolonged remissions of more than 40 months. Based on these promising results, a larger phase I/II trial was reported in 2004³⁴. This trial consisted of 2 cohorts: patients with stage IB or II NSCLC and patients with stage III or IV disease. Only 43 of 83 patients who underwent tumor harvest were treated, of whom 33 had advanced disease. Three of the 33 advanced-stage patients (2 of whom had bronchioloalveolar carcinoma) had durable complete responses lasting 6, 18, and ≥ 22 months. Interestingly, secretion of GM-CSF after vaccination was shown to correlate with outcome. A longer survival was observed in patients receiving vaccines secreting GM-CSF at more than 40 ng/24 h/ 10^6 cells (median survival = 17 months; 95% CI 6 to 23 months) than in patients receiving vaccines secreting less GM-CSF (median survival = 7 months; 95% CI 4 to 10 months) ($p = 0.028$). The results of this study suggested a vaccine dose-related survival advantage.

A subsequent trial evaluated unmodified tumor cells combined with an allogeneic bystander cell line (K562, human erythroleukemia cell line) genetically modified to secrete higher levels of GM-CSF. The combination is referred to as bystander GVAX®. This vaccine does not require genetic modification of individual autologous tumor cell preparations and provides substantially higher and more consistent GM-CSF secretion. Forty-nine patients with stage IIIA to IV NSCLC received vaccine, but unlike in the previous study, none achieved a partial or complete tumor response³⁵. There is speculation that derivation from a bystander cell line may detract from GM-CSF's ability to boost immune responses against

the TAA by the induction of myeloid suppressor cells and associated impairment of antigen-specific T-cell responses. It has been concluded that GM-CSF has to be secreted by the tumor cell itself or as recombinant purified protein.

Recent data from phase III clinical trials utilizing this approach in other cancer settings have not demonstrated improved survival compared with standard chemotherapy leading to uncertainty of the future of this platform.

Protein-specific Vaccines

Melanoma-associated antigen (MAGE)-A3

Melanoma-associated antigen (MAGE) is a TAA expressed in cancer cells. MAGE is not expressed in normal tissue, except in male germ cell lines, which are devoid of MHC molecules and are therefore unable to present MAGE-A antigens^{36, 37}. MAGE-A3 is the most silent gene in germ line cells within the MAGE-A family and is considered a promising immunotherapy target³⁸. MAGE-A3 is expressed in 35% of NSCLC, and the rate of expression increases as the disease spreads (30% of stage I patients and 50% of stage II patients express MAGE-A3 in their primary tumors). MAGE-A3 expression is thus associated with poor prognosis^{39, 40}.

A randomized phase II trial in 182 patients with completely resected stage IB or II MAGE-A3⁺ NSCLC comparing postoperative injections of MAGE-A3 recombinant protein combined with an adjuvant system (q3w × 5 followed by q3m × 8) vs. placebo has been reported (Table 1)⁴¹. After a median follow-up of 28 months, disease-free survival and overall survival hazard ratios (HR) were 0.73 (95% CI 0.45 to 1.16) and 0.66 (95% CI 0.36 to 1.20), respectively, in favor of the MAGE-A3 group. None of the outcome endpoints reached statistical significance; however, the signal with respect to survival benefit was considered strong enough to warrant a phase III evaluation. A recent paper reported an analysis of gene expression profiling of tumors prior to treatment to identify a predictive signature that correlates with clinical activity of MAGE-A3 antigen-specific immunotherapy⁴². In the overall population with resected stage IB/II NSCLC, MAGE-A3 treatment decreased the relative risk of recurrence by 25% (HR 0.75; 95% CI 0.46 to 1.23). In the population with the predictive gene signature, MAGE-A3 treatment decreased the relative risk of recurrence by 43% (HR 0.57; 95% CI 0.25 to 1.34).

The MAGRIT (MAGE-A3 as Adjuvant non-small cell lung cancer ImmunoTherapy) study is currently recruiting in 33 countries, with 2,270 patients with resected stage IB to IIIA MAGE-A3⁺ NSCLC expected to enroll. Following adjuvant chemotherapy, patients are randomly assigned to receive 13 intramuscular injections of vaccine (GSK1572932A) or placebo over a 27-month period, with disease-free survival as the primary endpoint. The trial registry does not define the type of adjuvant chemotherapy. The study commenced in October 2007 and is ongoing.

Epidermal growth factor (EGF)-based vaccines

Epidermal growth factor (EGF) and its cell membrane receptor (EGFR) are known to be over-expressed in many epithelial tumors, including lung cancer. In the last decade several small molecule inhibitors of EGFR-associated tyrosine kinases and monoclonal antibodies targeting EGFR have been developed⁴³. Strategies targeting EGF using immunotherapeutics are also currently in development.

A vaccine consisting of human recombinant EGF conjugated to the carrier protein P64K, derived from *Neisseria meningitidis*, was evaluated in 3 pilot clinical trials and a pooled analysis accruing a total of 83 patients with stage IIIB/IV NSCLC was performed (Table

1) ⁴⁴. Alum and Montanide™ ISA51 have been used as vaccine adjuvants. In one study, a single dose of cyclophosphamide (200 mg/m²) was administered 3 days prior to treatment. After EGF vaccination, 83% of vaccinated patients at least doubled their baseline anti-EGF antibody titers (seroconversion) and 49% increased their baseline levels up to 4-fold. Patients with titers > 1:4000 were considered good antibody responders (GARs). Survival was better in patients who seroconverted (mean 11.06 months, median 8.4 months) compared to patients who did not (mean 5.67 months, median 3.5 months). There was an additional survival advantage for GARs (mean 12.2 months, median 8.37 months) compared to poor antibody responders (mean 8.07 months, median 8.07 months). No significant toxicities were noted.

Neninger Vinageras et al. conducted a randomized phase II controlled trial of a human recombinant EGF-based vaccine (CIMAavax®) in patients with stage IIIB/IV NSCLC who had completed first-line chemotherapy⁴⁵. Eighty patients were randomly assigned to receive best supportive care or EGF vaccine. The vaccine dose contained 50µg equivalents of EGF and the vaccine was administered on days 1, 7, 14, 28 and then monthly thereafter. Serum EGF concentrations were reduced in 64.3% of patients receiving the vaccine indicating an immune mediated decrease in circulating EGF. When comparing vaccinated patients (n=37) with controls (n=37), the median overall survival showed a non significant trend toward a survival advantage (6.5 months vs. 5.3 months; *p* = 0.098). In the subset of vaccinated patients ≤ 60 years of age (n=22) versus controls (n=28), survival was significantly longer (11.6 months vs. 5.3 months; *p* = 0.0124). The vaccination was safe and well tolerated. There was a direct correlation between GAR and survival and between decreased serum EGF and survival. Both of these parameters have been proposed as markers to optimize vaccine dosage and schedules in future clinical trials. Clinical trials to reevaluate this approach in a larger patient series are ongoing in Cuba.

MUC1

Mucin 1 (MUC1) is a mucinous transmembrane glycoprotein that is over-expressed and under- or aberrantly glycosylated in many human malignancies. It is normally restricted to the apical surface of polarized epithelial cells, including those of the respiratory tract⁴⁶. In many epithelial malignancies, MUC1 is over-expressed and loses its polarity of expression⁴⁷. MUC1 has a large NH₂-terminal ectodomain that becomes under- or aberrantly glycosylated, with shortened carbohydrate side chains, unmasking epitopes on its peptide core which can act as tumor-associated neo-epitopes. The precise role of MUC1 in promoting tumor cell growth and survival is unclear, but it is thought to be involved in tumorigenicity, tumor cell migration, and increased resistance to stress-induced apoptosis of chemotherapeutic agents⁴⁸⁻⁵¹.

High serum levels of MUC1 are associated with immune suppression and poor prognosis in patients with advanced adenocarcinoma^{52, 53}. It is thought that cells that over-express tumor-associated MUC1 may escape a strong host immune response, making MUC1 an attractive target for cancer immunotherapy.

BLP25 liposome vaccine (L-BLP25) targets the exposed core peptide of the MUC1 TAA. L-BLP25 is a lyophilized preparation consisting of BLP25 lipopeptide, immunoadjuvant monophosphoryl lipid A, and 3 lipids (cholesterol, dimyristoyl phosphatidylglycerol, and dipalmitoyl phosphatidylcholine), forming a liposomal product⁵⁴. The vaccine is designed to induce a cellular immune response that may lead to immune rejection of tumor tissues that express MUC1 antigen.

A randomized phase IIB trial of L-BLP25 in patients with stage IIIB/IV NSCLC after stable disease or response to primary chemotherapy has been completed⁵⁴. L-BLP25 was given

weekly for 8 weeks (administered at 4 different sites to improve vaccine uptake in draining lymph nodes), with the option to proceed to maintenance therapy consisting of vaccination every 6 weeks starting in week 13. All patients received a single infusion of cyclophosphamide 300 mg/m² 3 days before vaccine administration, which has been shown to reduce the activity of suppressor T cells. The study was powered to detect a 5-month difference in survival (HR = 0.583), with a power of 80% and one-sided $p < 0.25$. There were 88 patients in the vaccination arm and 83 in the best supportive care (BSC) arm. Treatment was well tolerated, with 96.6% of patients in the vaccine arm completing the planned 8 injections and 69.3% proceeding to the maintenance phase. The most common adverse events were grade 1 flu-like symptoms, events related to cyclophosphamide administration, and mild injection-site reactions. T-cell proliferation assays were performed at baseline and during immunization. Of 78 samples evaluated, 16 demonstrated an antigen-specific T-cell response. The median overall survival was 17.4 months for the vaccination arm vs. 13.0 months with BSC, a difference that did not reach statistical significance ($p = 0.066$, unadjusted Cox). The 2-year survival rate was 43.2% for the L-BLP25 arm vs. 28.9% for the BSC arm. The greatest difference in survival was observed in patients with stage IIIB loco-regional disease (adjusted HR = 0.524; 95% CI, 0.261 to 1.052; $p = 0.069$). In this posthoc analysis by stage, patients with stage IIIB disease with malignant pleural effusion and stage IV disease (60.2%) showed overlapping survival curves. The reason why vaccine treatment seems to benefit only patients with locoregional IIIB (39.8%) disease is probably related to immune resistance mechanisms that are more active in advanced disease, although this hypothesis needs to be confirmed. In a recently reported study with a median follow-up of 53 months, the updated observed 2-year survival rate for patients with stage IIIB loco-regional disease was 60% (median survival 30.6 months) for the L-BLP25 arm vs. 36.7% for the BSC arm (median survival 13.3 months) ($P=0.16$)⁵⁵. Although this represents a subgroup analysis with a non-significant P value, the magnitude of the difference and its durability over an extended period of time suggests that further studies are warranted.

A multicenter, phase III, randomized, double-blind, placebo-controlled study of the cancer vaccine Stimuvax® (L-BLP25 or BLP25 liposome vaccine) in NSCLC subjects with unresectable stage III disease is currently recruiting. The START (Stimulating Targeted Antigenic Responses To NSCLC) trial is expected to recruit 1,476 patients who have had a response or have stable disease after at least 2 cycles of definitive platinum based chemotherapy. The study commenced in December 2006 and is expected to reach its estimated enrollment by September 2014. Following randomization, subjects in the investigational arm will receive, within 3 days of their treatment assignment, a single I.V. infusion of 300 mg/m² (to a maximum of 600 mg) cyclophosphamide three days before the first L-BLP25 vaccination. Subjects then receive eight consecutive weekly subcutaneous vaccinations with 930µg of L-BLP25 (primary treatment phase) at weeks 0; 1; 2; 3; 4; 5; 6 and 7 followed by vaccinations with L-BLP25 at 6-week intervals, commencing at week 13 (maintenance treatment phase) until disease progression.

IDM-2101: 10-epitope CTL vaccine

Tumors express a wide variety of TAAs and have a potential for TAA loss. In addition, patients have differing T-cell repertoires, suggesting that a successful vaccine may need to incorporate a wide selection of CTL specificities. The IDM-2101 vaccine was designed to overcome these potential difficulties by inducing CTL responses against 5 TAAs frequently over-expressed in NSCLC: carcinoembryonic antigen (CEA), p53, HER2/*neu*, and MAGE-2 and -3⁵⁶. IDM-2101 is composed of 10 synthetic peptides from these TAAs, 9 of which represent CTL epitopes. The tenth is a pan-DR epitope designed to augment the CTL response⁵⁷.

A phase II study investigated the efficacy of IDM-2101 in 63 HLA-A2⁺ patients with metastatic NSCLC (Table 1)⁵⁶. One-year survival for the treated group was 60% and the median survival was 17.3 months. One patient had a complete response and one had a partial response. A phase III validation trial in patients with advanced NSCLC is anticipated.

Dendritic cell vaccines

Some investigators have suggested that targeting tumor antigens alone may not elicit a strong enough immune response to be of therapeutic benefit. The discovery of DCs as professional APCs heralded their use in the development of cancer vaccines⁵⁸. It has been proposed that manipulating DCs as a vaccine adjuvant may be an effective way to stimulate antitumor immunity and overcome tolerance. The principal purpose of DC-based immunotherapy is to induce an antigen-specific immune response⁵⁹. Once stimulated by maturation factors such as inflammatory cytokines, or via CD40, DCs up-regulate adhesion and co-stimulatory molecules to become terminally differentiated stimulators of T-cell immunity⁶⁰. The effector arms of this antitumor response are CD4⁺ and CD8⁺ cells, which can only become activated against antigen when presented by APCs⁵⁹.

Upon administration of a DC-based vaccine, the DCs should migrate to secondary lymphoid organs and induce an antigen-specific immune response^{61, 62}. In the small phase I/II trials of DC vaccines in patients with NSCLC that have been reported, the most common antigen used has been CEA^{63–68}, a glycoprotein that acts as an adhesion molecule and is over-expressed in 70% of NSCLC⁶⁹. Human leukocyte antigen (HLA)-restricted class I CEA peptides or altered peptides were used as antigens in these studies, as the CEA peptide must be modified for effective immunogenicity.

Hirschowitz et al. reported a study of 16 patients with NSCLC ranging from stage IA to IIIB who received DC-based vaccines⁷⁰. The objectives of the study were to evaluate tolerability and measure immunologic responses in a heterogeneous group of NSCLC patients. Autologous DCs generated ex vivo from monocyte precursors (CD14⁺) were loaded with apoptotic bodies from an allogeneic adenocarcinoma cell line that over-expressed the TAAs HER2/*neu*, CEA, Wilm's tumor 1, MAGE-2, and survivin. Two doses of vaccine consisting of 8 to 9×10^7 cells were administered intradermally one month apart. Immune reactivity measured in vitro showed 3 distinct patterns: 5 of 16 patients showed no measurable immune reactivity, 5 showed reactivity to autologous DCs in the absence of tumor antigen (tumor-independent response), and 6 showed a tumor-specific immune response. Immunologic responses were independent of stage and prior therapy. There was no correlation between immune response and clinical outcome. In a continuation of the study, 14 additional patients were given the vaccine⁷¹. This study evaluated immunologic responses to immature, antigen-pulsed autologous DC vaccines in 2 distinct groups of NSCLC patients. Seven patients had undergone surgical resection (stage I/II) with or without adjuvant therapy, and 7 had unresectable stage III disease and were treated with chemoradiation alone. Four of the 7 stage III unresectable and 6 of the 7 stage I/II surgically resected patients, including 3 of 3 resected patients who had also received adjuvant chemoradiation, demonstrated immune responses to the vaccine. No clear indication of therapeutic efficacy was noted.

In another study, patients with NSCLC were vaccinated with DCs loaded with MUC1 peptides or tumor lysate⁷². A survival advantage was observed in vaccinated patients whose tumors were found to express MUC1 compared with patients with MUC1-negative tumors (16.75 months vs. 3.8 months; $p = 0.0101$). In this study DCs from 14 patients with advanced or metastatic breast or lung cancer (9 positive for MUC1 and 5 negative for MUC1) were loaded with MUC1 antigens or tumor lysate and used for therapeutic vaccination. Clinically, effects such as reduction in tumor sizes or tumor marker levels or

disappearance of malignant pleural effusion were observed in 7 of the 9 MUC1-positive cases. All of the patients who exhibited partial responses were vaccinated with peptides rather than tumor lysate. Additional studies are required to fully elucidate the potential of this approach.

To achieve a greater immune response through better antigen presentation, researchers have used different mechanisms of delivering vaccine to APCs. Morse et al. added antigen directly to ex vivo-generated DCs via an engineered fowlpox virus that expressed full-length CEA and TRICOM, an adjuvant agent consisting of 3 co-stimulatory molecules: B7.1, intracellular adhesion molecule (ICAM)-1, and lymphocyte function-associated antigen (LFA)-3⁶⁶. Only 3 of 14 patients enrolled had NSCLC. T-cell responses were induced in 10 of 14 patients (71%), with 5 having stable disease for 3 months. The authors did not report the responders' underlying disease. Another study used a modified vaccinia virus engineered to express the entire MUC1 gene to vaccinate patients with advanced cancer expressing MUC1⁷³. Three of 14 patients enrolled had NSCLC. Of the 14, 4 had stable disease for 6 to 9 months and 5 had an induced T-cell response.

Conclusion

Until recently, NSCLC was considered non-immunogenic or poorly immunogenic. Several hypotheses explaining the lack of potency for vaccines in lung cancer include an ineffective priming of tumor-specific T cells, lack of high avidity of primed tumor-specific T cells, and physical or functional disabling of primed tumor-specific T cells by the primary host and or tumor related mechanisms. At present, there are limited data from trials showing a clear clinical benefit for vaccines in lung cancer. One explanation may be that a measurable immune response does not translate into a clinically meaningful response. Patient selection may also be a problem for ongoing clinical studies. The majority of these trials are focused on patients with advanced-stage disease, while the ideal candidates for lung cancer vaccines may be patients with stage I or II disease who are considered at high risk of recurrence post-resection. Selecting the optimal tumor antigens to target is also problematic. A truly effective therapeutic lung cancer vaccine may require multiple epitopes of a diverse set of genes restricted to multiple haplotypes in order to combat the various tumor escape mechanisms. Despite the multiple negative clinical trials, there are encouraging signs from several newer agents. Their role in the management of patients with NSCLC, will be defined by the results of ongoing randomized phase III trials. As with targeted agents it is likely that immunotherapy will only work for a select number of patients and as such the development of predictive biomarkers to allow for optimal patient selection is as important as developing effective vaccination strategies. It is unlikely that only one modality of treatment will be used in the future and a combination of multiple immunologically active agents in addition to conventional chemotherapy and targeted agents given at varying stages of the disease process may overcome some of the limitations experienced with using only one treatment method.

Abbreviations

NSCLC	non-small cell lung cancer
CTLs	cytotoxic T lymphocytes
TAA s	tumor-associated antigens
APC s	antigen-presenting cells
DC s	dendritic cells

MHC	major histocompatibility complex
MAGE	melanoma-associated antigen
EGF	epidermal growth factor
GARs	good antibody responders
MUC1	mucin 1
CEA	carcinoembryonic antigen
TGF-β	transforming growth factor-beta
NK cells	natural killer cells

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Table 1

Vaccines trials at an advanced stage in non-small cell lung cancer

	Vaccine	Patient Population	Clinical Trial
<i>Allogeneic Tumor Cell Vaccines</i>	Belagenpumatucel-L (Lucanix®)	IIIA/IIIB/IV	STOP trial – Phase III. Maintenance therapy for patients who have responded to or have stable disease following first-line, platinum-based combination chemotherapy (NCT00676507)
<i>Protein Specific Vaccines</i>	MAGE-A3	IB/II or IIIA	MAGRIT trial – Phase III. Adjuvant therapy in patients with resectable MAGE-A3 positive NSCLC (NCT00480025)
	EGF	IIIB/IV	A Phase II/III trial of recombinant human EGF-rP64K/Montanide ISA 51 vaccine administered to patients after conventional first line chemotherapy (NCT00516685)
	MUC-1	Unresectable stage IIIA/IIB	START TRIAL. Phase III trial of Stimuvax® (L-BLP25 or BLP25 Liposome Vaccine) in patients with stable disease or response following chemoradiation (NCT00409188)