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Lung cancer vaccines: a review of three ongoing trials

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Every year, carcinoma of the lung is responsible for more deaths than any other cancer, with an overall 5-year survival of less than 16%. Despite advances in surgery, radiation and chemotherapy, recurrence rates are still high and outcomes are unacceptably poor. Novel approaches are currently being investigated and promising data exists to support the use of immunotherapy in the treatment of non-small-cell lung cancer. We present a review of three current Phase III clinical trials using vaccines to target and treat non-small-cell lung cancer. Although these trials are still ongoing, they represent an important milestone in the treatment of this disease. We will discuss the identification of targets that are relevant to lung cancer and the promising results of these vaccines that have been obtained in early-phase trials.

Keywords: adjuvant • antigen-specific cancer immunotherapeutic • cancer/testis antigens • melanoma antigen gene • mucin 1 • TGF-β

Cancer immunotherapy is based on the premise that an intact immune system can distinguish healthy cells from malignant cells. Strategies that use a host's immune mechanisms to produce selective antitumor effects are known as active immunotherapy. Such an approach is attractive in lung cancer following surgery or chemotherapy and radiation, when the burden of disease is reduced. The goal of such therapy is to target residual microscopic disease after resection and consolidate clinical responses to definitive therapy [1]. Novel treatment options are greatly needed in lung cancer, where the overall 5-year survival is less than 16% [101,102].

Although vaccines have been explored in cancer therapy for many years, application of this form of treatment to lung cancer is relatively new. An important component of any vaccine is identification of an appropriate target. Such a target would be present and overexpressed only on cancer cells and would be able to induce broad immune responses and T-cell recognition [2]. In solid tumors, in general, this is a difficult task. Non-small-cell lung cancer (NSCLC) is a heterogeneous disease and there may be many genetic alterations in any given lung tumor [3]. These genetic changes often lead to the expression of aberrant proteins. Identifying vaccine targets expressed by the majority of lung tumors is difficult owing to the genetic variability observed in these tumors. The field, however, is moving forward with the identification of potential new targets, improvements in vaccine manufacturing and better co-stimulatory adjuvants. We have limited this review to three vaccines currently in ongoing Phase III trials. We acknowledge that there are other vaccines in development, but they are presently in earlier stages. We will discuss the targets, review prior experience and provide a brief overview of these ongoing trials.

BLP25 liposomal vaccine

The target of the BLP25 liposomal vaccine (L-BLP25) is mucin 1 (MUC1), a transmembrane mucin that is overexpressed and underglycosylated in NSCLC. It is normally expressed on glandular and ductal epithelial tissues, exists as a large, heavily glycosylated protein, and may have roles in lubrication and protection from external

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damage [2]. It has been found on human epithelial adenocarcinomas, including breast, pancreas, ovary, lung and prostate, where it is overexpressed and underglycosylated. Tumor-associated MUC1 serves as a ligand for intercellular adhesion molecules and is integral for cell–cell communication [4,5]. The underglycosylation of MUC1 in malignant cells promotes the exposure of certain core peptides of the extracellular domain that remain masked in normal cells [2]. These peptides can act as a potential target for antibody-mediated therapy, with a goal of eliciting an antitumor response. MUC1 is also an attractive vaccine target because of its role in the spread of cancer. By promoting the adhesion of MUC1-expressing cancer cells to endothelial cells, MUC1 is believed to enhance the first step of establishing metastasis [6].

Data from a mouse model have shown that liposomal MUC1 peptides are able to elicit strong immune responses, although different responses were seen with different liposomal formulations [4]. Peptide antigens encapsulated in liposomes selectively induced T-cell responses, while peptides displayed on liposomal surfaces were successful in activating humoral responses as well. The T-cell response in the immunized mice included increased secretion of IFN-γ, indicative of a T-helper type-1 response. The humoral response was detected by high titers of IgG and IgM. These findings support the notion that delivery systems can be tailored to preferentially induce a cellular or humoral immune response.

Mucin 1 vaccines have shown promising results in many cancer types. A Phase I study of their use in resected and locally advanced pancreatic cancer demonstrated the formation of MUC1 specific IgG antibody in five of 16 patients, possibly indicating activation of peptide-specific helper T-cells [7]. A MUC1 vaccine was also evaluated in nine patients with a history of breast cancer, and all patients demonstrated high titers of IgG and IgM after treatment [8]. Seven patients had evidence of IgM antibody binding to tumor cells. The L-BLP25 vaccine has also shown promise in hormone-naive prostate cancer patients after radical prostatectomy. A Phase II study in such patients demonstrated a prolongation of prostate-specific antigen doubling time and was well tolerated [9]. A recent study of epithelial ovarian cancer showed the addition of an anti-MUC1 monoclonal antibody to docetaxel greatly improved the efficiency of cell killing and apoptosis [10].

The L-BLP25 has been studied in early-phase trials in stage IIIB and IV NSCLC, and has been shown to be safe and effective in eliciting a T-cell response [11,12]. A Phase I trial to evaluate the safety and immunogenicity of the L-BLP25 was published in 2001 (Table 1) [12]. The vaccine consists of a 25-amino acid synthetic MUC1 lipopeptide, along with monophosphoryl lipid A as an adjuvant [9,13]. The study enrolled 17 patients, 12 of whom completed the vaccination protocol. Eligibility requirements included stage IIIB or IV NSCLC, age 18–75 years, Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1 or 2, and adequate hematologic parameters. Eight patients were treated with a 20-µg dose of the L-BLP25 vaccine, nine patients received a 200-µg dose. All patients were treated with cyclophosphamide 300 mg/m^2 3 days prior to the first vaccine dose, to inhibit suppressor T-cell function and enhance the immune response [14]. Vaccinations were dosed subcutaneously at weeks zero, two, five and nine. Four of the five patients who did not complete the vaccines were withdrawn due to progression of disease, as the study did not allow concomitant chemotherapy or radiotherapy.

Measurements of antibody production, cytotoxic T lymphocytes (CTLs) and proliferative T-helper cells from patients on this trial were used to gauge an immunological response. In five of 12 evaluable patients, immunological assays confirmed the generation of CTLs against MUC1-positive tumor cells (three in the 20 µg group and two in the 200 µg group). Five patients were not evaluable as they had measurable CTLs prior to vaccination. Little or no antibody against MUC1 was detected, which was not unexpected as the goal was to induce a T-cell response with an encapsulated peptide antigen, not a humoral response as would be seen with a surface-exposed peptide liposome. There was no significant anti-MUC1 T-cell lymphoproliferative activity noted in any participants, but this assay was not done routinely in all trial participants. Of the 12 patients who completed the vaccination protocol, eight exhibited tumor progression and four had stable disease at week 13. Median survival was 5.4 months in the 20 µg group and 14.6 months in the 200 µg group. This compares to historical controls of patients with stage IIIB and IV NSCLC, treated with chemotherapy, whose median survival was 9.1 and 7.8 months, respectively [15]. Notable toxicities of L-BLP25 included injection site erythema (grade 1 or 2, in nine patients), lymphopenia (grade 2, in two patients) and liver enzyme abnormalities (grade 1 or 2, in six patients). In summary, this Phase I trial showed the L-BLP25 to be well tolerated and capable of inducing a cellular immune response.

A Phase IIB trial evaluating the L-BLP25 in stage IIIB and IV NSCLC was published in 2005 [11]. Its purpose was to evaluate the effect of L-BLP25 on survival and toxicity, and secondary end points included health-related quality of life (QoL) and immune response to the vaccine. Eligibility requirements included stable or responding stage IIIB or IV NSCLC after any first-line

Table 1. Summary of clinical trials of BLP25 liposomal vaccine targeting mucin 1 in non-small-cell lung cancer.

	Palmer *et al.* (2001) [12]	Butts *et al.* (2005) [11]	START, currently enrolling [103]
Study designs			
Trial design	Phase I, open-label, safety and dose comparison study	Phase IIB, open-label, randomized (1:1) study to test the safety and efficacy of L-BLP25 plus BSC compared with BSC alone	Phase III, double-blind, randomized (2:1), placebo-controlled study to compare survival duration by treatment arm
Patient population	Stage IIIB or IV NSCLC	Stage IIIB or IV NSCLC	Unresectable stage III NSCLC
Number of patients	17	171	Goal: 1322
Number treated with L-BLP25 vaccine	16	88	Estimated: 881
Vaccine treatments			
Cyclophosphamide dosage (mg/m^2; 3 days prior to vaccine injection)	300	300	300; saline in placebo arm
L-BLP25 dosage (µg)	20 or 200	1000	1000
Vaccination schedule (weeks)	0, 2, 5, and 9	0, 1, 2, 3, 4, 5, 6 and 7	0, 1, 2, 3, 4, 5, 6 and 7
Primary treatment evaluation	Week 11	Week 8	Week 8
Maintenance vaccine dosage and schedule	None	1000 µg, every 6 weeks until progression	1000 µg, every 6 weeks until progression
Results			
Median OS in all patients	5.4 months in 20 µg group, 14.6 months in 200 µg group ($p = 0.484$)	Control arm: 13.0 months[†] Treatment arm: 17.4 months	To be determined[†]
OS in stage IIIB locoregional NSCLC	Not defined	Control arm: 13.3 months Treatment arm: not reached (>54% alive)	To be determined
OS in stage IIIB with malignant pleural effusion or stage IV NSCLC	Not defined	Control arm: 12.9 months Treatment arm: 15.1 months	Not applicable

[†]Median OS in all patients was the primary end point of the trial.

BSC: Best supportive care; L-BLP25: BLP25 liposomal vaccine; NSCLC: Non-small-cell lung cancer; OS: Overall survival; START: Stimulating Targeted Antigenic Responses to NSCLC.

chemotherapy regimen and ECOG performance status of 0, 1 or 2. In total, 171 patients enrolled and were randomly assigned in an unblinded one-to-one fashion to either L-BLP25 plus best supportive care (BSC) or to BSC alone. Patients in the treatment arm received a single dose of 300 mg/m^2 intravenous cyclophosphamide, followed by eight weekly subcutaneous doses of 1000 µg L-BLP25 as primary treatment. Maintenance immunizations given every 6 weeks thereafter were at the discretion of the investigator. BSC was provided to all patients and could include pain medication, nutritional and psychosocial support, and second-line chemotherapy and/or palliative radiation for treatment of disease progression.

Patients were followed and assessed for safety, survival, QoL and immune response. In total, 87 of 88 patients (98.9%) in the vaccine arm and 79 of 83 patients (95.2%) in the BSC arm reported adverse events (AEs). Most AEs were related to underlying disease rather than the vaccine. Grade 1 flu-like symptoms were the most common study-drug related AE. Other events included injection-site reactions (all grade 1) and nausea secondary to cyclophosphamide. One severe AE in the L-BLP25 arm was pneumonia, possibly related to the vaccine. Overall median survival in the L-BLP-25 arm was 17.4 months compared with 13 months in the BSC arm ($p = 0.066$) and the 2-year survival rate was 43.2 and 28.9%, respectively. Patients with

locoregional stage IIIB NSCLC had the greatest difference in survival with a 2-year survival rate of 60% for the vaccine arm versus 36.7% for the BSC arm. These 2-year survival rates are significantly better than most previously published data [15]. QoL analysis in this trial was based on the Functional Assessment of Cancer Therapy – Lung questionnaire and the Trial Outcome Index change scores. Overall, patients in the L-BLP25 arm demonstrated a QoL advantage over the BSC patients. Immune response was assessed by T-cell proliferation assays in 78 of 88 (88.6%) patients in the vaccine arm. In total, 16 patients had a positive MUC1-specific T-cell proliferative response induced by the vaccine. Patients who demonstrated such a response had a median survival of 27.6 months, as compared with 16.7 months in those without the response. However, only two of the 16 had stage IIIB disease. Therefore, immune response and survival cannot be correlated based on these data.

Results from this study suggest a survival benefit for L-BLP25 when administered to patients with locoregional stage IIIB NSCLC, but not those with malignant pleural effusions or stage IV disease. These promising results have led to the currently enrolling Phase III Stimulating Targeted Antigenic Responses to NSCLC (START) trial of L-BLP25 in unresectable stage III NSCLC.

The START trial is a multicenter Phase III, randomized, double-blind, placebo-controlled study of the cancer vaccine L-BLP25 or BLP25 liposomal (Stimuvax®), targeting the MUC1 antigen in NSCLC subjects with unresectable stage III disease [103]. This trial is sponsored by EMD Serono in collaboration with Merck KGaA and began enrollment in December 2006. This is an international trial with a total of 295 participating sites. The estimated enrollment number is 1322 patients, with an estimated study completion date of December 2011.

Participants in the START trial are required to have unresectable stage III NSCLC, and will have had primary chemoradiotherapy, completed between 4 and 12 weeks prior to randomization. Concomitant or sequential chemoradiotherapy must consist of at least two cycles of a platinum-based chemotherapy and at least 50 Gy of radiation. An ECOG performance status must be 0 or 1, and patients must have adequate bone marrow stores.

For the Phase III trial of L-BLP25, patients will be randomized in a two-to-one fashion to the experimental or the placebo arm, respectively. The experimental arm involves a single intravenous infusion of cyclophosphamide (300 mg/m^2), to inhibit suppressor T-cell function and enhance the immune response to the vaccine [14]. After 3 days, they will receive the first of eight weekly L-BLP25 vaccinations (1000 µg), according to the primary-treatment phase. The injections will be administered to four anatomic sites to ideally stimulate a greater number of lymph nodes [16]. This is followed by a maintenance phase starting at week 13, with vaccinations at 6-week intervals until documented disease progression. Patients randomized to the placebo arm will receive saline instead of cyclophosphamide and placebo instead of L-BLP25. The primary outcome measure is overall survival, and secondary end points include time to symptom progression, time to progression, 1-, 2- and 3-year survival and safety.

Melanoma antigen gene-A3 antigen-specific cancer immunotherapeutic agent

The melanoma antigen gene (MAGE) families are found on the X-chromosome and encode for tumor-specific antigens that are recognized by T lymphocytes. They are frequently expressed in many tumor types, including lung cancer, sarcoma, esophagus (in 47% of tumors), head and neck (49%), metastatic melanoma (76%) and bladder cancer (36%) [17,18]. MAGE-A3 is expressed in 35–85% of cases of NSCLC, most often in the squamous histology, and is associated with a poor outcome [17,19–21]. MAGE-A3 belongs to the family of cancer/testis antigens, a category of immunogenic proteins that are highly restricted to tumors [22]. Cancer/testis antigens are not expressed in normal human tissues, except the placenta and testis, which are unable to act as antigen-presenting cells to stimulate the immune system because they lack an essential human leukocyte antigen protein [23]. There have been reported cases in NSCLC in which patients naturally develop antibodies to MAGE-A3, suggesting that this antigen may be able to spontaneously evoke immune responses [24].

The functions of the MAGE proteins are still largely unknown, but there is evidence to suggest their involvement in regulation of cell cycle and apoptosis, as well as during embryonic development [20]. In a murine model, MAGE-A3 has recently been shown to inhibit *in vitro* activation of procaspase-12 to caspase-12. Caspase-12 has the ability to induce apoptosis in response to cell insults, suggesting that MAGE-A3 protein expression may inhibit apoptosis and provide cancer cells with a survival advantage [25]. In addition, recently published data have shown that MAGE proteins bind to and activate really interesting new gene (RING) ubiquitin ligases [26]. There is evidence that such complexes are able to degrade p53, thus contributing to tumorigenesis.

The MAGE-A3 antigen-specific cancer immunotherapeutic (ASCI) has shown promise in melanoma, where expression of MAGE-A3 has been shown to successfully predict the worst outcomes [27]. Data presented at the 2008 Annual Meeting of the American Society of Clinical Oncology detailed a Phase II study of its

use as first-line metastatic treatment for MAGE-A3-positive patients with metastatic melanoma [28]. In total, 72 patients enrolled and were randomly treated with the MAGE-A3 recombinant protein combined with one of two different co-stimulatory adjuvants, AS02B and AS15. The AS02B adjuvant contains monophosphoryl lipid A and QS-21, a saponin from the bark of the South American *Quillaja saponaria* Molina tree [29,30]. The AS15 adjuvant contains monophosphoryl lipid A, QS-21 and cytosine-phosphate-guanine (CpG) oligonucleotides.

Objective responses to the MAGE-A3 ASCI were noted in three patients and stable disease in 11, both favoring the AS15 adjuvant. Anti-MAGE-A3 antibodies titers and $CD4^+$ T-cell responses were also better with AS15. A current Phase III trial of the MAGE-A3 ASCI in melanoma is currently recruiting and is known as DERMA (Adjuvant Immunotherapy with MAGE-A3 in Melanoma) [104]. This trial is enrolling MAGE-A3-positive patients with stage IIIB or IIIC cutaneous melanoma with macroscopic lymph node involvement. Approximately 1300 patients will be randomized in a two-to-one fashion to treatment with MAGE-A3 ASCI or placebo, with a primary end point of disease-free survival.

The induction of an immune response in NSCLC patients vaccinated with a MAGE-A3 recombinant protein was demonstrated and published in 2004 (Table 2) [31]. This Phase II study included 17 patients with stage I or II NSCLC who had undergone surgical resection of their MAGE-A3 gene-expressing tumor (assessed by reverse transcription-PCR). The trial's vaccine was a DNA-recombinant fusion protein of MAGE-A3, injected with or without the AS02B adjuvant.

Participants in this study received vaccine preparations as follows: the first nine were given 300 µg of MAGE-A3 protein alone, the second eight were given 300 µg of MAGE-A3 protein plus the adjuvant. Vaccines were dose every 3 weeks for a total of four doses. The resultant production of anti-MAGE-A3 antibodies was measured by ELISA. Modest levels of such antibodies were found in three of the patients treated with vaccine alone, and marked levels were seen in seven of the patients treated with vaccine plus adjuvant. A $CD4^+$ T-cell response against MAGE-A3 was seen in only one of the patients treated with vaccine alone, but in four of the patients treated with vaccine plus adjuvant.

The MAGE-A3 ASCI was studied in a randomized Phase II study and data was presented at the 2006 and 2007 American Society of Clinical Oncology Annual Meetings [21]. This trial included 182 patients from 59 centers in 14 European countries with completely resected, pathologic stage IB or II NSCLC. The vaccine in this study was a MAGE-A3 recombinant protein combined with the AS02B adjuvant. Patients were randomly assigned in a double-blind, two-to-one fashion to the MAGE-A3 ASCI or placebo, both given intramuscularly. Patients received five injections at 3-week intervals, followed by eight injections at 3-month intervals. Stratification was based on stage, histology (squamous vs other) and lymph-node procedure (minimal sampling vs radical mediastinal lymphadenectomy). Disease-free interval was the primary end point; safety, disease-free survival and overall survival were the secondary end points.

A total of 122 patients with stage IB NSCLC and 60 patients with stage II NSCLC were randomized and followed for a median of 28 months. During that time, 67 recurrences of NSCLC were noted. The MAGE-A3 treatment group had a more favorable disease-free interval with a hazard ratio of 0.74 (95% CI: 0.44–1.20%; p = 0.107). Disease-free survival and overall survival also favored the MAGE-A3 treatment group with a hazard ratio of 0.73 (95% CI: 0.45–1.16%; p = 0.093) and 0.66 (95% CI: 0.36–1.20%; p = 0.088), respectively. With a mean follow-up of 28 months, 30.6% of patients in the vaccine arm had disease recurrence, compared with 43.3% in the placebo arm [29]. In addition, treatment was relatively well tolerated. None of the trial's end points met statistical significance, but enough survival benefit was appreciated to support Phase III evaluation.

MAGE-A3 as Adjuvant Non-Small-Cell Lung Cancer Immunotherapy (MAGRIT) is a randomized, double-blind, placebo-controlled Phase III study to assess the efficacy of an ASCI as adjuvant therapy in patients with resectable MAGE-A3-positive NSCLC [105]. This trial is sponsored by GlaxoSmithKline and opened to enrollment in October 2007. There are 556 participating locations in the USA, Canada, South America, Europe, Asia, and Australia, with an estimated enrollment of 2270 and an estimated completion date of March 2022.

For the Phase III trial, enrolled patients are expected to have stage IB, II or IIIA NSCLC after complete surgical resection and tumors must show expression of the MAGE-A3 gene. This vaccine is the first to be tested in the postoperative adjuvant setting, where lung cancer vaccines may have the most benefit, because tumor burden is low and immunotherapy will have more time to induce an antitumor effect [18,29]. In this trial, prior neoadjuvant chemotherapy or radiotherapy is not permitted, but up to four cycles of adjuvant platinum-based chemotherapy can be given between surgery and randomization. Approximately half of the participants will have received adjuvant chemotherapy prior to randomization. ECOG performance status can be 0, 1 or 2; and patients must have adequate bone-marrow reserve

Table 2. Summary of clinical trials of melanoma antigen gene-A3 antigen-specific cancer immunotherapeutic agent targeting the melanoma antigen gene-A3 antigen in non-small-cell lung cancer.

	Atanackovic *et al.* (2004) [31]	Vansteenkiste *et al.* (2007) [21]	MAGRIT, currently enrolling [103]
Study designs			
Trial design	Phase II, open-label, experimental vaccination study to measure $CD4^+$ T-cell response	Phase II, double-blind, placebo-controlled study, evaluating disease-free survival in patients, randomized (2:1) to post-operation MAGE-A3 ASCI vs placebo	Phase III, double-blind, placebo-controlled study, evaluating disease-free survival in patients, randomized (2:1) to post-operation MAGE-A3 ASCI vs placebo
Patient population	Completely resected MAGE-A3(+), stage I or II NSCLC	Completely resected MAGE-A3(+), stage IB or II NSCLC	Completely resected MAGE-A3(+), stage IB, II or IIIA NSCLC
Number of patients	17	182	Goal: 2270
Number treated with vaccine	17	~121 (not published)	Estimated: 1513
Vaccine treatments			
Vaccine	300 µg MAGE-A3 protein alone (n = 9, cohort 1) or MAGE-A3 with AS02B adjuvant (n = 8, cohort 2)	MAGE-A3 ASCI with AS02B adjuvant (monophosphoryl lipid A + QS21, a saponin)	MAGE-A3 ASCI with AS15 adjuvant (monophosphoryl lipid A + QS21 + CpG oligonucleotides)
Vaccination schedule	Every 3 weeks for four doses	Every 3 weeks for five doses	Every 3 weeks for five doses
Maintenance vaccine dosage and schedule	None	Every 3 months for eight doses	Every 3 months for eight doses
Results			
Disease-free survival	Trial not designed to assess efficacy	Favored MAGE-A3 treatment group HR 0.73 (95% CI: 0.45–1.16%, p = 0.093)†	To be determined†
Overall survival	Trial not designed to assess efficacy	Favored MAGE-A3 treatment group HR 0.66 (95% CI: 0.36–1.20%, p = 0.088)	To be determined
Immunogenicity	Three of nine patients in cohort 1 had a modest increase in antibodies against MAGE-A3 protein; seven of eight in cohort 2 had a marked increase in anti-MAGE-A3 antibodies; five total patients had a $CD4^+$ T-cell response (one in cohort 1, four in cohort 2)	Not evaluated	Anti-MAGE-A3 and Anti-protein D seropositivity rate will be a secondary end point

†Disease-free survival was the primary end point of the trial.

ASCI: Antigen-specific cancer immunotherapeutic; CpG: Cytosine-phosphate-guanine; MAGRIT: MAGE-A3 as Adjuvant Non-Small-Cell Lung Cancer Immunotherapy; NSCLC: Non-small-cell lung cancer.

and adequate hepatic and renal function. Eligible participants will be randomized in a two-to-one fashion to ASCI or placebo, respectively. The ASCI in this trial includes recombinant MAGE-A3 protein along with the AS15 adjuvant, which was studied in melanoma as described above. Treatment (ASCI or placebo) will involve 13 intramuscular injections over 27 months. The first five injections will be given every 3 weeks, the remaining eight injections will be given every 3 months. The primary end point is disease-free survival, and secondary end points include efficacy, immunogenicity, and safety.

Belagenpumatucel-L vaccine

Belagenpumatucel-L (Lucanix™) is a TGF-β2 antisense gene modified allogeneic tumor-cell vaccine. TGF-β is a regulatory protein, secreted from both healthy cells and cancer cells, and is involved in cell growth and cell function. In healthy cells, the TGF-β pathway restricts cell growth, differentiation and cell death, in part by inhibiting progression from the G1 phase to the S phase of the cell cycle [32]. However, in cancer cells, mutations in the signaling pathway lead to TGF-β resistance and cells grow without regulation [106]. When secreted by cancer cells, TGF-β2 has immunosuppressive properties that lead to the promotion of tumor-cell growth. These include antagonistic effects on natural killer cells and lymphokine-activated killer cells, and blocking of the maturation and chemotaxis of dendritic cells. In general, a higher level of mutated TGF-β has been associated with poorer outcomes [33].

The belagenpumatucel-L vaccine is made from four irradiated cell lines of NSCLC (two adenocarcinomas, one squamous carcinoma and one large-cell carcinoma). Using a plasmid vector, the NSCLC cells are transfected with an antisense gene to inhibit TGF-β2 [34,35]. This antisense gene was created by nucleic-acid sequencing of the TGF-β2 gene and designing a molecule to bind the gene's mRNA, thus preventing the translation of mRNA to the TGF-β2 protein [36]. The inhibition of TGF-β2 by the gene-modified cancer cells will ideally decrease local immunosuppression and result in enhanced antigen processing and presentation. Such a milieu will aid in the immune response against shared lung-cancer antigens by improving T-cell priming and increasing the production of cytokines and antibodies.

The safety and efficacy of belagenpumatucel-L in NSCLC was investigated in a Phase II study and published in 2006 (Table 3) [34]. This trial included patients with stage II, IIIA, IIIB and IV NSCLC and an estimated overall tumor volume of less than or equal to 125 ml (excluding bony or lymph-node metastases). Smaller tumor volumes optimize vaccine effect, as demonstrated in preclinical data. Participants were required to have an ECOG performance status of 0, 1 or 2; and must have completed, or refused, conventional therapy. Adequate bone marrow and hepatic function were also required. Patients were assigned at random to one of three dose cohorts (1.25, 2.5 or 5 × 10^7 cells/injection) and intradermal vaccinations were dosed once a month or once every other month. End points included response, overall survival, progression-free survival, immune response and safety.

In total, 75 patients were enrolled onto the study and a total of 550 vaccinations were dosed. At week 16, a partial response rate of 15% was noted, with median tumor shrinkage of 63% and 59% of patients with stable disease. Of 40 patients with measurable disease, responses were seen in one out of 16 patients in cohort one (1.25 × 10^7 cells/injection), three out of 11 patients in cohort two (2.5 × 10^7 cells/injection) and two out of 13 patients in cohort three (5 × 10^7 cells/injection). All five responding participants were female; two had stage IIIB NSCLC and three had stage IV NSCLC; two cases were adenocarcinoma, two were squamous carcinoma and one was large-cell carcinoma. Patients receiving the higher-dosed vaccines (cohorts two and three combined) had improved 1- and 2-year survival over those in the low-dose group: 68% (95% CI: 55–80%) and 52% (95% CI: 35–68%), respectively, for the higher-dosed cohorts, and 39% (95% CI: 22–56%) and 20% (95% CI: 4–36%) for the low-dose cohort. The median survival for advanced-stage patients in cohorts two and three combined was estimated at 581 days, as compared with 252 days for cohort one ($p = 0.0186$).

Study participants were also followed for immune response using flow analysis to measure cytokine-producing cells. This was done prior to vaccination and again at weeks four and eight, and used cytokine-specific antibodies and an intracellular cytokine detection assay [34]. At week eight, vaccinated patients were found to have a greater frequency of cells producing TNF-α ($p = 0.01$). When compared with patients with progressive disease at 12 weeks, patients with stable disease or a response to treatment had higher levels of cells producing INF-γ, IL-6 and IL-4. In addition, a correlation was seen between positive clinical outcomes and the formation of novel antibodies to human leukocyte antigen molecules expressed by the vaccination cell lines [34]. Of the 20 patients with stable disease or better, 11 developed novel antibodies, while only two of the 16 patients with progressive disease developed antibodies.

Belagenpumatucel-L was well tolerated in this Phase II study. Two grade 3 events occured, possibly related to treatment; one grade 3 arm swelling and one case of chronic myelogenous leukemia, which was investigated thoroughly and thought unlikely secondary to the vaccine [34]. All other grade 3 and 4 events were attributed to underlying disease. Grade 1 and 2 AEs that may be attributed to the vaccine include flu-like symptoms and pain at the injection site.

The survival, tumor-free survival, overall survival and progression-free survival (STOP) trial is a randomized, double-blind, placebo-controlled Phase III trial to evaluate the efficacy of belagenpumatucel-L in advanced NSCLC [107]. STOP is an acronym for the expected end points of survival, tumor-free survival, overall survival and progression-free survival [36]. This

Table 3. Summary of clinical trials of belagenpumatucel-L in non-small-cell lung cancer.

	Nemunaitis *et al.* (2006) [34]	STOP, currently enrolling [107]
Study designs		
Trial design	Phase II, open-label, three-arm, dose-variable study to evaluate efficacy	Phase III, double-blind, placebo-controlled, randomized study evaluating efficacy of belagenpumatucel-L, and BSC vs placebo and BSC
Patient population	Stages II, IIIA, IIIB and IV NSCLC, after having completed or refused conventional therapy	Stage III or IV NSCLC; patients who have responded to or have stable disease following first-line chemotherapy
Number of patients	75	Goal: 700
Vaccine treatments		
Vaccine	Belagenpumatucel-L; given at one of three doses; 1.25, 2.5 or 5.0 × 10^7 cells injection	Belagenpumatucel-L; 2.5 x 10^7 cells/injection; or placebo injection
Vaccination schedule	Once a month or once every other month, to a maximum of 16 injections	Once a month for 18 months
Maintenance vaccine schedule	None	Once at 21 and 24 months
Results		
Response rate	15% partial response in late-stage (IIIB and IV) assessable patients; 59% had stable disease	To be determined
Overall survival (primary end point)	Median survival of 252 days for lowest-dose cohort vs 581 days in two higher-dose cohorts combined (p = 0.0186)	To be determined
Immunogenicity	Patients with stable disease or a treatment response had higher levels of INF-γ, IL-6 and IL-4, and more responding patients developed novel antibodies to the vaccination cell lines	Blood samples will be evaluated for cytokines, chemokines, IFN-γ, regulatory T-cell phenotype and function

BSC: Best supportive care; NSCLC: Non-small-cell lung cancer; STOP: Survival, tumor-free survival, overall survival and progression-free survival.

study is sponsored by NovaRx Corporation and opened to enrollment in July 2008. There are 74 participating locations in the USA, Canada, Europe and India. Enrollment is estimated at 700 participants, with completion anticipated in October 2011.

For the STOP trial, study participants need to have stage IIIA (T3 N2 only), IIIB or IV NSCLC that has responded to or remained stable after one regimen of platinum-based combination chemotherapy (up to six cycles), with or without concomitant radiotherapy. ECOG performance status can be 0, 1 or 2; and patients must have adequate bone-marrow reserve and adequate hepatic and renal function.

Enrolled subjects will be randomized to the treatment vaccine plus BSC or to BSC alone. Stratification is according to disease stage, response to front-line chemotherapy and prior treatment regimens (chemotherapy with or without radiotherapy, chemotherapy with or without bevacizumab). Treatment involves intradermal injections of belagenpumatucel-L or placebo, given once monthly for 18 months and then once at 21 and 24 months. The vaccine dose of 2.5 x 10^7 cells was chosen based on Phase II data showing better efficacy over the 1.25 × 10^7 cell dose. The primary end point is overall survival, and secondary end points are progression-free survival, QoL (based on the Lung Cancer Symptom Scale questionnaire), time-to-progression, best overall tumor response, response duration, rate of CNS metastases and AEs.

Future perspective

The use of vaccines and immunotherapy in NSCLC is a relatively young field. Based on preclinical studies and early-phase trials, there is data to suggest that such vaccines may alter the 'immunologic milieu' in some patients. It remains to be seen whether the current vaccine trials will meet their primary end points. It is, however, interesting that even in initial Phase I and II trials evidence for an immunologic response can be seen in some patients. There are still many questions that need to be explored in order to fully harness the power of immunotherapy. For example, why some patients can mount an immune reaction while others can not, remains an intriguing question that requires

further investigation. Can we identify a 'signature' that would identify patients who are more likely to benefit form this approach, and thus further personalize management of this difficult disease? Is targeting one protein sufficient to achieve a sustained immunologic response? Is it possible to show the activation of the host immune responses against a variety of tumor antigens by vaccinating against one target? The current trials will provide us with some answers but, as is the case with most clinical trials, many more questions will be raised. As we eagerly await the results of currently enrolling Phase III trials, investigators continue to look for novel antigen and better vaccine designs. As our knowledge in this field grows, we will be better able to select those patients and tumor characteristics that will benefit most from these therapies.

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Executive summary

- Cancer immunotherapy, specifically vaccines for non-small-cell lung cancer, is well tolerated and has shown modest benefit in early-phase studies.
- Lung cancer vaccines appear to confer more benefit in patients with early-stage disease and with smaller tumor burden.
- Antigen targets that have shown promise as non-small-cell lung cancer vaccines include mucin 1 and MAGE-A3. A vaccine based on TGF-β2 modified, allogeneic lung cancer cell lines has also shown promise of activity in early testing.
- Currently enrolling Phase III trials will determine whether or not such vaccines have a role in our future treatment algorithms.

Bibliography

Papers of special note have been highlighted as:

- ▪ of interest

1 Hirschowitz EA, Hiestand DM, Yannelli JR. Vaccines for lung cancer. *J. Thorac. Oncol.* 1(1), 93–104 (2006).

2 Singh R, Bandyopadhyay D. MUC1: a target molecule for cancer therapy. *Cancer Biol. Ther.* 6(4), 481–486 (2007).

3 Mok T, Wu YL, Zhnag L. A small step towards personalized medicine for non-small-cell lung cancer. *Discov. Med.* 8(43), 227–231 (2009).

4 Guan HH, Budzynski W, Koganty RR *et al.* Liposomal formulations of synthetic MUC1 peptides: effects of encapsulation versus surface display of peptides on immune responses. *Bioconjug. Chem.* 9(4), 451–458 (1998).

5 Ho SB, Niehans GA, Lyftogt C *et al.* Heterogeneity of mucin gene expression in normal and neoplastic tissues. *Cancer Res.* 53(3), 641–651 (1993).

6 Kam JL, Regimbald LH, Hilgers JH *et al.* MUC1 synthetic peptide inhibition of intercellular adhesion molecule-1 and MUC1 binding requires six tandem repeats. *Cancer Res.* 58(23), 5577–5581 (1998).

7 Ramanathan RK, Lee KM, McKolanis J *et al.* Phase I study of a MUC1 vaccine composed of different doses of MUC1 peptide with SB-AS2 adjuvant in resected and locally advanced pancreatic cancer. *Cancer Immunol. Immunother.* 54(3), 254–264 (2005).

8 Gilewski T, Adluri S, Ragupathi G *et al.* Vaccination of high-risk breast cancer patients with mucin-1 (MUC1) keyhole limpet hemocyanin conjugate plus QS-21. *Clin. Cancer Res.* 6(5), 1693–1701 (2000).

9 Sangha R, North S. L-BLP25: a MUC1-targeted peptide vaccine therapy in prostate cancer. *Expert Opin. Biol. Ther.* 7(11), 1723–1730 (2007).

10 Wang L, Chen H, Liu F *et al.* Monoclonal antibody targeting MUC1 and increasing sensitivity to docetaxel as a novel strategy in treating human epithelial ovarian cancer. *Cancer Lett.* 300(2), 122–133 (2011).

11 Butts C, Murray N, Maksymiuk A *et al.* Randomized Phase IIB trial of BLP25 liposome vaccine in stage IIIB and IV non-small-cell lung cancer. *J. Clin. Oncol.* 23(27), 6674–6681 (2005).

▪ **Contains the original data for the Phase I, II and III trials detailed in this review.**

12 Palmer M, Parker J, Modi S *et al.* Phase I study of the BLP25 (MUC1 peptide) liposomal vaccine for active specific immunotherapy in stage IIIB/IV non-small-cell lung cancer. *Clin. Lung Cancer* 3(1), 49–57 (2001).

▪ **Contains the original data for the Phase I, II and III trials detailed in this review.**

13 Adams MM, Damani P, Perl NR *et al.* Design and synthesis of potent quillaja saponin vaccine adjuvants. *J. Am. Chem. Soc.* 132(6), 1939–1945 (2010).

14 Bass KK, Mastrangelo MJ. Immunopotentiation with low-dose cyclophosphamide in the active specific immunotherapy of cancer. *Cancer Immunol. Immunother.* 47(1), 1–12 (1998).

15 Schiller JH, Harrington D, Belani CP *et al.* Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. *N. Engl. J. Med.* 346(2), 92–98 (2002).

16 Butts C, Murray RN, Smith CJ *et al.* A multicenter open-label study to assess the safety of a new formulation of BLP25 liposome vaccine in patients with unresectable stage III non-small-cell lung cancer. *Clin. Lung Cancer* 11(6), 391–395 (2010).

17 Schultz ES, Lethé B, Cambiaso CL *et al.* A MAGE-A3 peptide presented by HLA-DP4 is recognized on tumor cells by $CD4^+$ cytolytic T lymphocytes. *Cancer Res.* 60(22), 6272–6275 (2000).

18 Tyagi P, Mirakhur B. MAGRIT: the largest-ever Phase III lung cancer trial aims to establish a novel tumor-specific approach to therapy. *Clin. Lung Cancer* 10(5), 371–374 (2009).

19 Gure AO, Chua R, Williamson B *et al.* Cancer-testis genes are coordinately expressed and are markers of poor outcome in non-small cell lung cancer. *Clin. Cancer Res.* 11(22), 8055–8062 (2005).

20 Sang M, Wang L, Ding C *et al.* Melanoma-associated antigen genes – an update. *Cancer Lett.* 302(2), 85–90 (2010).

21 Vansteenkiste J, Zielinski M, Linder A *et al.* Final results of a multi-center, double-blind, randomized, placebo-controlled Phase II study to assess the efficacy of MAGE-A3 immunotherapeutic as adjuvant therapy in stage IB/II non-small cell lung cancer (NSCLC). *J. Clin. Oncol.* 25(Suppl. 18), 7554 (2007).

■ **Contains the original data for the Phase I, II and III trials detailed in this article.**

22 Scanlan MJ, Gure AO, Jungbluth AA, Old LJ, Chen YT. Cancer/testis antigens: an expanding family of targets for cancer immunotherapy. *Immunol. Rev.* 188, 22–32 (2002).

23 De Plaen E, Arden K, Traversari C *et al.* Structure, chromosomal localization, and expression of 12 genes of the MAGE family. *Immunogenetics* 40(5), 360–369 (1994).

24 Atanackovic D, Altorki NK, Cao Y *et al.* Booster vaccination of cancer patients with MAGE-A3 protein reveals long-term immunological memory or tolerance depending on priming. *Proc. Natl Acad. Sci. USA* 105(5), 1650–1655 (2008).

■ **Contains the original data for the Phase I, II and III trials detailed in this article.**

25 Morishima N, Nakanishi K, Takenouchi H, Shibata T, Yasuhiko Y. An endoplasmic reticulum stress-specific caspase cascade in apoptosis. Cytochrome c-independent activation of caspase-9 by caspase-12. *J. Biol. Chem.* 277(37), 34287–34294 (2002).

26 Doyle JM, Gao J, Wang J, Yang M, Potts PR. MAGE-RING protein complexes comprise a family of E3 ubiquitin ligases. *Mol. Cell* 39(6), 963–974 (2010).

27 Koyanagi K, Mori T, O'Day SJ, Martinez SR, Wang H-J, Hoon DSB. Association of circulating tumor cells with serum tumor-related methylated DNA in peripheral blood of melanoma patients. *Cancer Res.* 66(12), 6111–6117 (2006).

28 Kruit WH, Suciu S, Dreno B *et al.* Immunization with recombinant MAGE-A3 protein combined with adjuvant systems AS15 or AS02B in patients with unresectable and progressive metastatic cutaneous melanoma: a randomized open-label Phase II study of the EORTC Melanoma Group (16032–18031). *J. Clin. Oncol.* 26(Suppl. 15), 9065 (2008).

29 Bradbury PA, Shepherd FA. Immunotherapy for lung cancer. *J. Thorac. Oncol.* 3(Suppl. 2), S164–S170 (2008).

30 Cluff CW. Monophosphoryl lipid A (MPL) as an adjuvant for anti-cancer vaccines: clinical results. *Adv. Exp. Med. Biol.* 667, 111–123 (2010).

31 Atanackovic D, Altorki NK, Stockert E *et al.* Vaccine-induced $CD4^+$ T cell responses to MAGE-3 protein in lung cancer patients. *J. Immunol.* 172(5), 3289–3296 (2004).

32 Pietenpol JA, Holt JT, Stein RW, Moses HT. Transforming growth factor β1 suppression of c-myc gene transcription: role in inhibition of keratinocyte proliferation. *Proc. Natl Acad. Sci. USA* 87(10), 3758–3762 (1990).

33 Kong F, Jirtle RL, Huang DH, Clough RW, Anscher MS. Plasma transforming growth factor-β1 level before radiotherapy correlates with long term outcome of patients with lung carcinoma. *Cancer* 86(9), 1712–1719 (1999).

34 Nemunaitis J, Dillman RO, Schwarzenberger PO *et al.* Phase II study of belagenpumatucel-L, a transforming growth factor β-2 antisense gene-modified allogeneic tumor cell vaccine in non-small-cell lung cancer. *J. Clin. Oncol.* 24(29), 4721–4730 (2006).

■ **Contains the original data for the Phase I, II and III trials detailed in this article.**

35 Nemunaitis J, Nemunaitis M, Senzer N *et al.* Phase II trial of belagenpumatucel-L, a TGF-β2 antisense gene modified allogeneic tumor vaccine in advanced non small cell lung cancer (NSCLC) patients. *Cancer Gene Ther.* 16(8), 620–624 (2009).

36 Askari FK, McDonnell WM. Antisense-oligonucleotide therapy. *N. Engl. J. Med.* 334(5), 316–318 (1996).

■ Websites

101 SEER Stat Fact Sheets: lung and bronchus, 2010–2011
www.seer.cancer.gov/statfacts/html/lungb.html#incidence-mortality

102 Lung Cancer Statistics, 2010–2011
www.cdc.gov/cancer/lung/statistics/index.htm

103 Cancer vaccine study for unresectable stage III non-small cell lung cancer, NCT00409188
www.clinicaltrials.gov/ct2/show/study/NCT00409188?term=cancer+vaccine+study+for+unresectable&rank=1&show_locs=Y#locn

■ **Contains the original data for the Phase I, II and III trials detailed in this article.**

104 ASCI in melanoma
www.ascitrials.com/melanoma

105 GSK1572932A antigen-specific cancer immunotherapeutic as adjuvant therapy in patients with non-small cell lung cancer
www.clinicaltrial.gov/ct2/show/NCT00480025?term=NCT00480025&rank=1

■ **Contains the original data for the Phase I, II and III trials detailed in this article.**

106 AACR cancer concepts: TGF-β
www.aacr.org/home/public--media/patients--family/fact-sheets/cancer-concepts/tgf-beta.aspx

107 Phase III Lucanix™ vaccine therapy in advanced non-small-cell lung cancer (NSCLC) following front-line chemotherapy (STOP)
www.clinicaltrials.gov/ct2/show/NCT00676507?term=NCT+00676507&rank=1

■ **Contains the original data for the Phase I, II and III trials detailed in this article.**