# Immunotherapy for Lung Cancer

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**Abstract:** Reports of tumor regression after infection date back as far as 1550 BC. In the twentieth century, Dr. William Coley, witnessing regression of a malignant tumor in one of his patients after a bacterial infection, developed the first cancer treatment vaccine derived from killed bacteria, with some reported success. However, despite decades of research, no specific, active tumor vaccine has been approved for the treatment of cancer. In lung cancer, initial attempts to modulate the immune system with nonspecific therapies were unsuccessful. However, more sophisticated specific vaccines have now been developed, and an increasing number are being evaluated in randomized phase 3 trials, raising hopes that vaccines may be an additional novel therapy for patients with lung cancer. This article reviews the following seven vaccines, which have entered randomized trials: L-BLP25 (Stimuvax), BEC-2, 1E10, PF-3512676 (Promune), melanoma-associated antigen A3 immunotherapeutic, granulocyte-macrophage colony-stimulating factor-transduced allogeneic cancer cellular immunotherapy, and belagenpumatucel-L (Lucanix).

**Key Words:** Immunotherapy, Lung cancer, Cellular immune system, Vaccines.

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Lung cancer is the leading cause of cancer-related death.[1] Early micrometastatic disease is the predominant reason for treatment failure, even in apparently early-stage disease.[2,3] Identifying new adjuvant therapies beyond chemotherapy and radiation will be critical if we hope to improve cure rates for patients with surgically resected disease. However, novel treatments must also have a favorable toxicity profile because lung cancer is predominantly a disease of elderly populations and comorbidities are common.[4] Although not without risk,[5,6] vaccine therapy has the potential to meet these requirements. In lung cancer, initial attempts to modulate the immune system with nonspecific approaches were unsuccessful. However, with a greater understanding of the immune system and improved technology to allow for the identification of new antigenic targets and to enable production of more sophisticated vaccines, an increasing number of lung cancer vaccines have shown early promise and now are being evaluated in randomized phase 3 trials.

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## CELLULAR IMMUNE SYSTEM OVERVIEW

The cellular immune system involves a complex interplay of receptor-mediated cellular events that are regulated by cytokines and result in target cell death by activated cytotoxic T-lymphocytes.[7,8] Cellular immunity is initiated by uptake of antigens by antigen-presenting cells (APCs). The most important APC is the dendritic cell, which monitors the environment for potential antigens (Figure 1). Antigens are internalized and short peptide sequences are displayed on the extracellular surface of the APC in conjunction with the major histocompatibility complex (MHC) class II molecule. The dendritic cell, displaying the antigenic peptide, circulates from the periphery to the draining lymph nodes, where it matures and comes into contact with naive T lymphocytes.[9]

APCs require contact with the appropriate $CD4^+$ T-helper lymphocyte before they can activate specific effector $CD8^+$ cytotoxic T lymphocytes. Two signals are required for T-cell activation. Interaction must take place between the specific T-cell receptor and the APC MHC-peptide molecule, and this must be followed by activation of the costimulatory molecules B7.1 and B7.2. Failure to activate the second signal results in immune tolerance and is one mechanism by which tumors can evade the immune system.[10–12]

Activated cytotoxic T-lymphocytes circulate to the periphery and recognize affected cells that display the complementary peptide-MHC class 1 molecule on the cell surface. Target cell death is effected by granule exocytosis or expression of FAS ligand, with both mechanisms activating apoptotic cell death.[13] Memory lymphocytes are produced that enable rapid expansion of T lymphocytes in the event of rechallenge with the same antigen.[14] Cytokines are crucial for the regulation of the immune system, with the balance of cytokines directing the response to one of activation or down-regulation.[15]

Tumors have mechanisms to evade the immune system[7,9,10] (Figure 1). First, tumors arise from self and may, therefore, be poorly immunogenic. Second, tumor cells down-regulate antigens, decrease expression of MHC class I molecules, interfere with APCs, and secrete cytokines that promotes immune tolerance and immunosuppression. Furthermore, tumor cells can be resistant to the effect of cytotoxic T lymphocytes by failing to activate apoptosis.

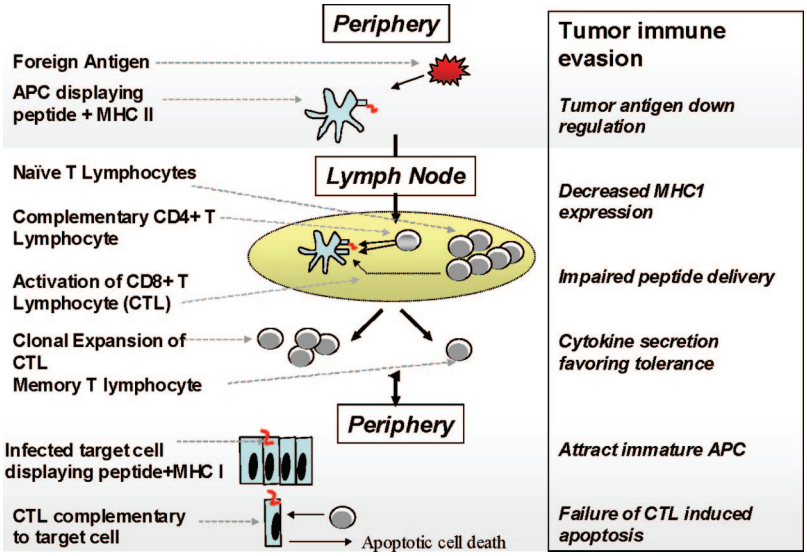


**FIGURE 1.** Schematic diagram of cell specific immune system and mechanism of tumor cell evasion. APC indicates antigen-presenting cell; TL, T lymphocyte; CTL, cytotoxic T lymphocyte; MHC, major histocompatibility complex. Foreign antigen is phagocytosed by APCs (e.g., dendritic cells). Antigen is degraded into small peptide sequences that are displayed in combination with MHC II. The APC travels to draining lymph nodes and is in contact with naive T lymphocytes. The APC is activated by interacting with appropriate $CD4^+$ T-helper cells, after which complementary CTLs are activated by the APC by interaction with MHC and costimulatory molecules. Activated CTLs circulate to the periphery searching for specific target. The CTLs induce apoptosis of target cells displaying complementary peptide in combination with MHC class I molecules.

## VACCINE TRIAL METHODS

Standard phase 1 to 2 trial methods used in the assessment of cytotoxic chemotherapy may not be appropriate for the assessment of vaccines.[16] Defining the maximum tolerated dose may not be relevant for vaccines, which tend to have a favorable side effect profile. Conversely, determining a dose that has biologic activity is crucial. Immune assays have the potential to act as surrogate markers of response; however, to date, immune response has shown poor correlation with clinical response. Furthermore, standard response criteria may not be meaningful in vaccine therapy because delayed responses may occur and the patients under study may not have bulky or measurable disease. Although new therapies are frequently assessed in heavily pretreated patients with advanced disease, this may not be appropriate for vaccines that require repeated vaccine administration and time for an immune response to be mounted. In fact, vaccine therapy may be most effective in patient populations with minimal residual (microscopic) disease. Many vaccines are specific in nature, and so a general patient population may not be optimal for phase 1 and 2 studies. Finally, for many vaccines, assays are not available for pharmacokinetic studies.

## ANTIGEN-SPECIFIC VACCINES

Requirements for a tumor antigen to be a suitable target for vaccine therapy are summarized in Table 1.[17] Essentially, an antigen should be expressed uniformly in the tumor type of interest, differ from normal cells, and preferably should be tumorigenic and immunogenic.

Although lung cancer is considered to be a poorly immunogenic malignancy, cytotoxic T lymphocytes have been identified in some studies. However, this evidence of an immune response in lung cancer patients has not been associated with significant improvement in outcome, and it is thought that greater and/or more specific stimulation of the immune system is needed. This has led to the development of numerous vaccines for the treatment of this malignancy. To optimize the immune response to vaccination, an adjuvant frequently is incorporated as part of the vaccine (Table 1).[18]

**TABLE 1.** Requirements for an Antigenic Target and Examples of Adjuvants

| | |
|---|---|
| Antigen requirements | Tumor specific/over or aberrantly expressed |
| | Common expression within tumor type |
| | Tumor metastases express antigen |
| | Tumorigenic |
| | Immunogenic |
| Adjuvant | Biological |
| | BCG |
| | Diphtheria toxoid |
| | Tetanus toxoid |
| | Monophosphoryl lipid |
| | Chemical |
| | Aluminum hydroxide |
| | Calcium salts |
| | Montanide ISA 51 |
| | Incomplete freund adjuvant |
| | Cytokines |
| | GM-CSF |

BCG, Bacille Calmette-Guérin; GM-CSF, granulocyte-macrophage colony-stimulating factor.

An adjuvant is a nonspecific immune stimulant administered to promote delivery of crucial APCs to the site of vaccination, with the potential to increase uptake of the specific vaccine antigen by the APC (Table 1).

### Mucin 1: A Cell Surface-Associated Antigen and L-BLP25 (BLP25)

Mucin 1 (MUC1) is a type 1 transmembrane protein expressed on epithelial cells. The function of MUC1 is uncertain, but in tumors, it is associated with reduced apoptosis, immunosuppression, chemoresistance, and poorer outcome. MUC1 overexpression, or aberrant glycosylation in tumors compared with normal tissue, makes it a potential target for vaccine therapy.[19–22]

 

L-BLP25 (Stimuvax; Biomira, Alberta, CA) is a liposome vaccine targeted to the extracellular core peptide of MUC1. The vaccine incorporates an adjuvant (monophosphoryl lipid) and three lipids to enhance delivery of the vaccine to the immune cells. Preclinical studies confirmed that the vaccine could elicit antigen-specific T-cell proliferation and interferon-γ secretion, and initial phase 1 and 2 trials showed that L-BLP25 had a favorable toxicity profile.[23–25]

A randomized phase 2B trial of L-BLP25 in patients with stage III/IV non-small cell lung cancer (NSCLC) after stable disease or response to primary chemotherapy has been completed, with updated survival data presented recently.[26] L-BLP25 was given weekly for 8 weeks (administered at four sites of the body to improve vaccine uptake in draining lymph nodes) with the option (at the investigator's discretion) to proceed to maintenance therapy, consisting of vaccination every 6 weeks starting in week 13. All patients received a single infusion of cyclophosphamide 3 days before vaccine administration, which has been shown to reduce activity of suppressor T cells.[26]

The study was powered to detect a 5-month prolongation of survival. There were 83 patients in the vaccination arm and 88 in the best supportive care (BSC) arm. Treatment was tolerable, with 96.6% of patients in the vaccine arm completing the planned eight injections and 69.3% proceeding to the maintenance phase. The most common adverse effects 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. From 78 samples that were evaluated, 16 demonstrated an antigen-specific T-cell response.

The median overall survival was 17.4 months for vaccination versus 13.0 months with BSC ($p = 0.66$; Figure 2*A*). In a post hoc analysis, patients with locoregional stage IIIB disease (38% of the total population) randomized to the vaccination arm had improved survival compared with the patients receiving BSC, although the difference did not reach statistical significance (hazard ratio, 0.52; 95% confidence interval, 0.261–1.052; $p = 0.69$). At the time of publication, the median survival of this subgroup had not been reached (Figure 2*B*). Updated survival data recently presented reported a median survival of 30.6 months compared with 13.3 months for patients receiving BSC, with a median follow-up of 53 months.[27] Only two of the samples that evaluated a specific T-cell response were from patients with IIIB disease, and so it is not possible to draw any conclusions regarding the utility of this as a surrogate marker of response from this study. Caution is required when interpreting data from an unplanned analysis of just 65 patients. However, the results are intriguing and a large international multicenter phase 3 trial of patients with inoperable stage III NSCLC after treatment with definitive chemoradiation is now under way.

## GD3 and Anti-idiotype Antibody Bec2 Plus BCG Vaccine

GD3 is a cell surface ganglioside antigen. Gangliosides are involved in cell-cell recognition, cell matrix adhesion, and cell differentiation.[28–30] Bec2 is an anti-idiotype antibody that mimics GD3. Bec2 has been evaluated in patients with small cell lung cancer (SCLC) administered with BCG vaccine as an adjuvant to optimize the host immunologic response. Results of a small pilot study,[31] in which patients had prolonged survival after vaccination, led to a large 515-patient international phase 3 study in responding patients with limited SCLC after chemotherapy and radiotherapy.[32] Toxicity was minimal, but unfortunately there was no improvement in overall survival or progression-free survival (Figure 3*A*) or in quality of life in the vaccination arm compared with the observation arm ($p = 0.28$). Survival in patients who displayed a humoral response (71 of 213 cases assessed) was longer than nonhumoral responders; however, this finding was not statistically significant when correcting for differences between the two groups (Figure 3*B*).

GD3 is expressed in SCLC, but not in NSCLC, and in approximately two-thirds of cases, GD3 expression is up-regulated.[33,34] Assessment of GD3 expression was not mandated as part of this trial, and tumor samples were not collected, perhaps reflecting the difficulty obtaining tissue in a malignancy that is not treated surgically. It is therefore not be possible to determine if a subgroup of patients with GD3-overexpressing tumors might have gained greater benefit.

## Neu-Glycosylated Gangliosides and 1E10 Anti-idiotype Vaccine

1E10 is another anti-idiotype vaccine that mimics Neu-glycosylated gangliosides.[35] Neu-glycosylated sialic acid-containing ganglioside (NeuGc-GM3) is a variant of the normal Neu-acetylated sialic acid ganglioside, identified almost exclusively in transformed cells, making NeuGc-GM3 a potentially important therapeutic target.[36] Anti-anti-idiotype

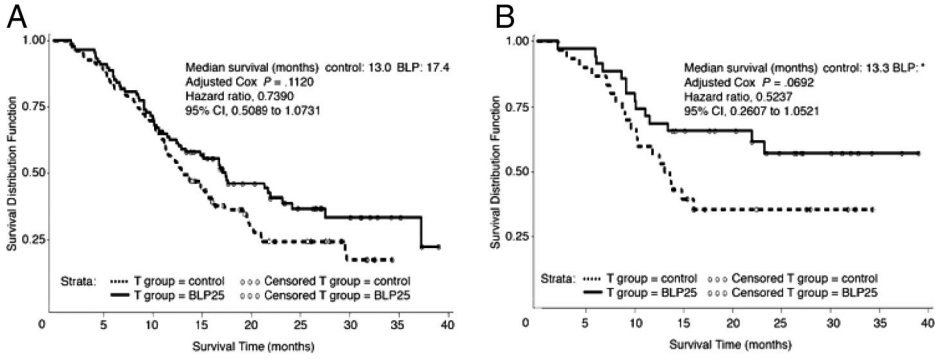


**FIGURE 2.** Kaplan Meier curves of survival analysis for the L-BLP25 study. *A,* All patients. *B,* Patients with locoregional stage 3B disease. BLP indicates BLP25 liposome vaccine. Reproduced with permission from Butts C, Murray N, Maksymiuk A, et al. *J Clin Oncol* 2005;23:6674–6681.

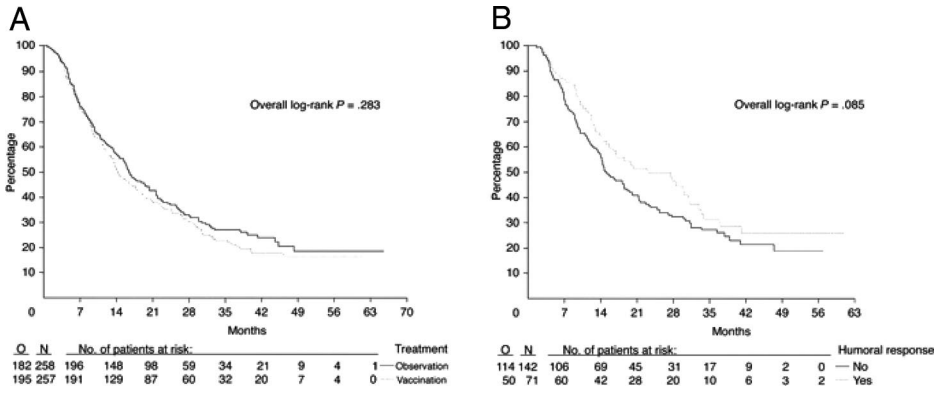


**FIGURE 3.** Kaplan-Meier curves of survival analysis for the Bec2/BCG Study. *A,* Overall survival analysis. *B,* Survival of patients with and without humoral response. Reproduced with permission from Giaccone G, Debruyne C, Felip E, et al. *J Clin Oncol* 2005;23:6854–6864.

antibody responses to 1E10 were identified in preclinical models, with antitumor activity.[37] Phase 1 trials, including a trial of 10 patients with SCLC, demonstrated a favorable toxicity profile, with the most common adverse effects being local injection site reaction and flu-like symptoms.[38] Efficacy as assessed by the development of antibodies against 1E10 and NeuGc-GM3 ganglioside was encouraging. A phase 2 trial of 1E10 is under way in patients with SCLC.

In addition, 1E10 has been evaluated in patients with NSCLC in a compassionate use study.[39] All patients had stage IIIB/IV disease and had completed standard therapy for the stage of their disease, achieving at least stable disease or better. Six intradermal vaccinations were administered biweekly, followed by a monthly maintenance phase. In a preliminary report of survival from 38 patients, the median survival had not been reached, with a median follow-up of 19 months and a mean survival of 12.94 months. In an unplanned exploratory analysis, a mean survival of 6 months for patients who received the same standard treatment but did not receive vaccination was reported. A phase 3 trial of 1E10 in patients with advanced NSCLC is planned.

## Toll-like Receptor 9 and PF-3512676

Toll-like receptors (TLRs) are a family of highly conserved receptors that regulate innate antigen-specific immunity via the recognition of pathogen-associated molecular patterns.[40] TLR9 is expressed on B and T lymphocytes, plasmacytoid cells, and dendritic cells. Activation of TLR9 may reduce immune tolerance and improve tumor antigen recognition and cell death via both innate and specific immune systems.[41] PF-3512676 (ProMune; Coley Pharmaceutical Group, Wellesley, MA), a TLR9 agonist, has been evaluated in a range of malignancies, including NSCLC. It has demonstrated some single-agent activity and has also been shown to be effective in combination with chemotherapy.

A randomized phase 2 trial of PF-3512676, in 112 chemonaive patients with stage IIIB/IV NSCLC, has recently been completed.[42] Patients received systemic therapy with carboplatin and paclitaxel and were randomized to receive no further treatment or subcutaneous vaccinations with PF-3512676 on days 8 and 15. A trend to improved survival was demonstrated favoring the vaccination arm. The combination of chemotherapy and vaccine was well tolerated, although there was an excess of myelosuppression. Other common adverse effects included mild injection site reactions and flu-like symptoms. After this, two phase 3 trials of PF-3512676 with platinum-based chemotherapy in patients with stage IIIB or IV NSCLC were commenced. Unfortunately, both of these trials have been discontinued recently after interim analysis by an independent data monitoring safety committee concluded there was no additional benefit from PF-3512676 over that of standard chemotherapy.[43]

## Melanoma-Associated Antigen A3 and Melanoma-Associated Antigen A3 Immunotherapeutic

Unlike the vaccines described above, melanoma-associated antigen A3 (MAGE-A3) is a tumor-specific antigen that is not expressed on normal cells. MAGE-A3 is expressed in 35% of NSCLC, has increasing expression rates with increasing stage, and may be associated with a poor prognosis.[44,45] A vaccine developed to target MAGE-A3 and evaluated initially in patients with metastatic melanoma demonstrated some evidence of activity, with five responses (including 4 mixed responses) reported from the 26 patients that received at least four vaccinations. CD4 T-lymphocyte response directed to the MAGE-A3 antigen was documented in one of the responders.[46]

The results of a randomized phase 2 trial of MAGE-A3 vaccine in patients with completely resected MAGE-A3-expressing NSCLC have been reported recently.[47] Expression of MAGE-A3 was an eligibility requirement, and the vaccine was evaluated in the postoperative adjuvant setting (the first to do so), where vaccines may ultimately have the greatest utility.

A total of 1089 lung cancer resection specimens were evaluated for MAGE-A3 expression, of which 363 were positive. Of these, 182 patients entered the study and were randomized to receive either placebo or active vaccination after complete resection for stage 1B to II NSCLC. The vaccine was administered intramuscularly every 3 weeks for a total of 5 vaccinations, followed by eight maintenance injections every 3 months. Treatment was well tolerated. A total of 117 grade 3 or 4 events were recorded, but only three were considered by the investigators to be related to the vaccine.

At the time of reporting, 30.6% of patients had recurred in the vaccine arm versus 43.3% in the placebo arm, with a median follow-up of 28 months. However, none of the

 

outcome end points (disease-free interval, disease-free survival, or overall survival) reached statistical significance. Although statistical significance was not met, the signal with respect to survival benefit was strong enough from the phase 2 trial to move into phase 3 evaluation. The phase 2 trial commenced before 2004, when postoperative adjuvant chemotherapy became standard of care for patients with NSCLC. In the phase 3 trial, MAGE-A3 vaccine will again be given in the adjuvant setting, but after completion of standard adjuvant chemotherapy.

## TUMOR CELL VACCINES

Whole cell vaccines have the advantage of exposing the host immune system to a full repertoire of tumor cell antigens, both known and unknown. Autologous and allogeneic tumor cell vaccines have been evaluated in lung cancer. Autologous vaccines are patient specific; however, they require individual patient tissue for their development, and it may take weeks to months for the vaccine to be prepared. Allogeneic vaccines, using lung cancer cell lines, do not have these logistical concerns, although these tumor antigens may lack specificity compared with the host tumor. To enhance conditions for optimal immune stimulation, genetically manipulated tumor cell vaccines have been developed, which secrete immune-activating cytokines or immune-suppressing proteins at the sites of vaccination. Granulocyte-macrophage colony-stimulating factor (GM-CSF)-transduced allogeneic cancer cellular immunotherapy (GVAX; Cell Genesys Inc., South San Francisco, CA) and belagenpumatucel-L (Lucanix; NovaRx Corporation, San Diego, CA) are two such vaccines.

### Granulocyte-Macrophage Colony-Stimulating Factor-Transduced Allogeneic Cancer Cellular Immunotherapy

GM-CSF induces antigen expression and attracts APCs to the site of vaccination.[48] A phase 1 trial of an autologous NSCLC vaccine transfected with adenovirus containing GM-CSF DNA demonstrated that vaccine preparation was feasible with tissue obtained from resected metastases or pleural effusions.[49] Vaccine was produced in 37 of the 38 patients recruited to the study. Vaccination was well tolerated, with grade 1 or 2 skin reactions at the site of vaccination being the most common adverse effect. Furthermore, there appeared to be activity. Five patients had stable disease and two patients, who both had undergone surgical resection of all known metastatic sites before vaccination, had prolonged remissions of more than 40 months.

GVAX is also an autologous tumor cell vaccine transfected with an adenovirus containing the GM-CSF gene.[50] GVAX has been investigated in a phase 1/2 study of 43 patients with NSCLC (33 with advanced disease). GVAX was administered every 2 weeks for a total of 3 to 6 vaccinations. The toxicity profile was satisfactory, with grade 2 or less local reactions at the site of vaccination being the most common adverse event, reported in 93% of patients. Other adverse events included fatigue, nausea, pain, and arthralgia. Three patients with metastatic disease achieved complete remissions, which have been maintained for almost 5 years in two cases.

It is notable that 83 patients underwent tumor harvest for GVAX preparation in this trial, but only 43 patients received vaccination. In 16 cases, vaccine could not be manufactured because of insufficient tumor tissue, particularly when pleural fluid was used as the source of tumor cells. In addition, the median number of days from tissue collection to vaccine administration was 49. This highlights the logistic problems that arise from the development of autologous vaccines, which if developed for patients with advanced disease could result in an unacceptable lag time to first vaccination.[50] In this trial, the secretion of GM-CSF after GVAX vaccination was shown to correlate with outcome. In view of the potential significance of GM-CSF, a subsequent autologous vaccine combined with a GM-CSF-secreting cell line was developed. Unfortunately, although GM-CSF secretion appeared to be significantly higher, toxicity and clinical benefit were less favorable than with GVAX.[51] GVAX has now entered into phase 2 clinical trials in NSCLC.

Interestingly, in both the trial of GVAX and that reported by Salgia et al., 3 of the 4 patients who achieved prolonged remissions had bronchoalveolar carcinoma. It is hypothesized that bronchoalveolar carcinoma may have a viral origin, and so immunotherapy may be particularly interesting for this histologic subtype.[50] GVAX is being evaluated in a phase 2 trial specifically in patients with stage IIIB/IV bronchoalveolar carcinoma to investigate this further.

### Transforming Growth Factor $\beta$2 Antisense Gene-Modified Allogeneic Tumor Cell Vaccine: Belagenpumatucel-L

Belagenpumatucel-L (Lucanix; NovaRx Corporation) is developed from allogeneic NSCLC cell lines genetically modified to secrete an antisense oligonucleotide to transforming growth factor-$\beta$2 (TGF-$\beta$2). TGF-$\beta$2 is immunosuppressive, suppressing natural killer cells, activated killer cells, and dendritic cell activity, and has been identified as a poor prognostic factor in NSCLC.[52] Preclinical and early-phase studies showed that inhibition of TGF-$\beta$2 increased the immunogenicity of tumor vaccines. In contrast to GVAX, because belagenpumatucel-L uses allogeneic tumor cells, there is no requirement for individual patient tumor tissue or long preparation time.

A randomized phase 2 trial of 75 patients with stages II to IV NSCLC, after completion or patient refusal of standard chemotherapy, has been completed.[53] Patients were randomized to 1 of 3 dose levels (1.25, 2.5, or $5 \times 10^7$ cells per injection). Toxicity was minor, with only 1 grade 3 event attributed to the vaccine. There was a 16% response rate. Patients who received the lowest dose level had inferior survival compared with the other two doses combined. The estimated median survival for patients receiving $2.5 \times 10^7$ and $5 \times 10^7$ cells per injection was 581 days, compared with 252 days for patients receiving $1.25 \times 10^7$ cells per injection ($p = 0.0186$).

Biologic markers of immune system stimulation, including mononuclear cell cytokine production and development of antibody response to vaccine, correlated with response or stable disease, and there was a nonstatistically significant increase in ELISPOT response in patients with

partial response or stable disease compared with progressive disease. Belagenpumatucel-L is now entering phase 3 trials.

## CONCLUSIONS

It has not been possible to review all clinical trials evaluating new vaccines in this short review. We have, therefore, limited this review to those vaccines that have demonstrated activity worthy of further investigation. Vaccine therapy is feasible, and toxicity is minimal. Furthermore, there is evidence of activity with some vaccines. However, significant challenges exist. Neither the most effective adjuvant nor the optimum vaccine platform has been defined. In addition, researchers may need to move away from the ingrained standard trial method used for the investigation of cytotoxic chemotherapy. As with other targeted agents, it is probable that vaccines may only be beneficial for certain subgroups of patients, and so the advancement and incorporation of correlative science assays within vaccine trials will be crucial to avoid discarding a vaccine that may have potential benefit for some patients. Despite this, there are encouraging signs that vaccine therapy may have a role in the future treatment of patients with lung cancer.

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