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# Lung Cancer

## *Prevention, Management, and Emerging Therapies*

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# Vaccine Therapy for Lung Cancer

**John Nemunaitis and Jack Roth**

**Abstract** Evidence of lung cancer sensitivity to immune reactivity continues to accumulate. However, consistent therapeutic opportunities remain limited. Recently, as a result of increased awareness of immunoreactive components and new technological development, a new crop of therapeutic vaccines are being explored for the purpose of modulating immunity against lung cancer. This review summarizes the key investigative opportunities.

**Keywords** Vaccine • Gene • Cancer • Lung

## Introduction

Evidence of an endogenous immune modulating effect in NSCLC is suggested based on heterogeneity of clinical progression, which is observed among patients with the same histological type of malignancy (1, 2). Rarely, improved survival of lung cancer patients who develop empyema has been observed (3). Furthermore, biopsy of responsive disease has occasionally demonstrated the isolation of tumor-infiltrating lymphocytes within the cancer parenchyma, suggestive of endogenous immune affect (4). There is also evidence for shared antigens in lung cancers (5–12), as is seen in other tumor types (13, 14). Dendritic cells, which are responsible for the induction of antitumor immunity in tumor-bearing hosts by a process of antigenic cross-presentation (15, 16),

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have been shown to be activated in NSCLC. A great deal has been learned over the past 30 years regarding the potential application of immune-directed therapeutics in lung cancer. Furthermore, recent advances in molecular biology have allowed us to identify new antigens, cytokines, and mechanisms that enhance our understanding toward the development of immunotherapeutic approaches.

The role of Dendritic Cells (DCs) in cell-mediated immunity has been extensively investigated (17–21). DCs have been found to play a central role in the induction of antitumor immunity in tumor-bearing hosts by a process of antigenic cross-presentation and have displayed activity in NSCLC (22). They efficiently display antigens on major histocompatability complexes (MHC II) ultimately stimulating proliferation and activation of $CD4^+$ and $CD8^+$ T cells. $CD4^+$ cells further augment the activity of natural killer cells and macrophages, in addition to amplifying antigen-specific immunity by local secretion of cytokines (23–27). These attributes make DCs a pivotal component in the therapeutic strategies of many current immune based therapies in NSCLC.

However, previous approaches to immunotherapy in lung cancer have failed to realize the potential of this promising strategy. There are several hypotheses to explain potential lack of activity, including 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 mechanism. For example, in NSCLC, a high proportion of the tumor-infiltrating lymphocytes are immunosuppressive T regulatory cells ($CD4^+$ $CD25^+$) that secrete transforming growth factor-β (TFG-β) and express a high level of cytotoxic T lymphocyte (CTL) antigen-4 (28, 29). These cells have been shown to impede immune activation by facilitating T cell tolerance to tumor associated antigens rather than cross-priming $CD8^+$ T cells. This results in the nonproliferation of killer T cells that recognize the tumor and will not attack it (28–34). Elevated levels of IL-10 and TFG-β are found in patients with NSCLC. Animal models have shown immune suppression that is mediated by these cytokines, serving as a defense for malignant cells against the body's immune system (35–44).

These factors are dealt with in different ways with more recent vaccine therapeutics described in this review.

## Non-Small Cell Lung Cancer Vaccine Development

### *Belagenpumatucel*

Belagenpumatucel (45) is a nonviral gene-based allogeneic vaccine that incorporates the TGF-β2 antisense gene into a cocktail of four different NSCLC cell lines. Elevated levels of TGF-β2 are linked to immunosuppression in cancer patients (46–51), and the level of TGF-β2 is inversely correlated with prognosis in patients with NSCLC (52). TGF-β2 has antagonistic effects on natural killer cells, lymphokine-activated killer cells, and dendritic cells (35, 40, 41, 53–55).



Using an antisense gene to inhibit TGF-β2, several groups have demonstrated an inhibition of cellular TGF-β2 expression resulting in an increased immunogenicity of gene-modified cancer cells (12–16, 56–59). In a recent Phase II study involving 75 early- ($n=14$) and late-stage ($n=61$) NSCLC patients, a dose-related effect of belagenpumatucel was defined (Table 1) (45). Patients were randomized to one of the three dose cohorts. In 41 advanced-stage (IIIB, IV) patients, the investigators found no adverse toxicity and an impressive survival advantage at dose levels $\geq 2.5 \times 10^7$ cells/injection, with an estimated 2-year survival of 47% in response to Lucanix™. This compared favorably with the historical 2-year survival rate of <20% of stage IIIB/IV NSCLC patients (5–8, 60, 61). Furthermore, there was a correlation of positive outcome with induction of immune enhancement of tumor antigen recognition. Immune function was explored in the 61 advanced stage (IIIB/IV) patients. Cytokine production (IFN-γ, $p=0.006$; IL-6, $p=0.004$; and IL-4, $p=0.007$) was induced, an antibody-mediated response to vaccine HLA antigen was observed ($p=0.014$), and there was a trend toward the correlation between a cell-mediated response and achievement of stable disease or better ($p=0.086$). Subsequent phase II investigation in stage IIIB/IV NSCLC patients further validated survival results observed in the initial phase I/II trial. Median survival in the subsequent trial was 19 months. There was a suggestion that circulating tumor cells may predict for a worse response (62).

### *GVAX*

Vaccines transduced with granulocyte–macrophage colony-stimulating factor (GM–CSF) gene were potent inducers of tumor immunity in animal models (63). Secretion of GM–CSF by genetically modified tumor cells induced local tumor antigen expression and stimulated cytokine release at the vaccine site, which activated and attracted antigen-presenting cells, thereby inducing a tumor-specific cellular immune response (64). Preclinical studies conducted with GVAX showed no significant local and systemic toxicities at clinically relevant doses (63, 65–67).

Several phase I/II human trials using GM–CSF-secreting autologous or allogeneic tumor cell vaccines have been performed (Table 1) (68–73). One multicenter phase I/II trial involving patients with early stage and advanced-stage NSCLC evaluated an autologous GVAX vaccine (10). For vaccine preparation, tumor tissue was obtained surgically or by thoracentesis in the case of malignant effusions. Cells were exposed overnight to an adenoviral vector supernatant (Ad-GM). GVAX was administered intradermally. A total of 43 NSCLC patients (10 early stage, 33 late-stage) were vaccinated. The most common vaccine-related adverse events were local vaccine injection site reactions (93%), followed by fatigue (16%) and nausea (12%). Three advanced-stage patients achieved durable, complete tumor regressions. Two remain without disease more than 5 years following vaccine. Both had failed prior frontline and second-line therapy prior to vaccination and had multisite disease. One complete responder showed an in vitro T cell response to autologous

**Table 1** Update of demographics and responses for recent vaccine trials in NSCLC

| Vaccine | # Patients | Stage | Side effects | Median survival | 1-Year survival | Response | Reference |
|---|---|---|---|---|---|---|---|
| Allogenic Ad B7.1, HLA-A modified cell vaccine | 19 | IIIB, IV | Minor skin erythema | 18 months | 52% | 1 PR/5SD | (117) |
| Dendritic cells pulsed with Allo NSCLC line 1650 | 16 | IA–IIIB | Minor skin erythema and fatigue | N/A | N/A | 6/16 ag specific responses | (22) |
| Dexosome (auto derived DC-exosomes loaded with MAGE from NSCLC) | 13 | IIIB, IV | Grade 1–2; inj site reaction, flu-like symptoms, edema and pain | ND | ND | 3/9 induced response to MAGE | (159) |
| Recombinant EGF protein | 40 | III, IV | Grade 2; chills, fever, vomiting, nausea, hypertension, cephalgea, dizziness, flushing, pain at inj site | 8.2 mo | ND | 12/40 SD | (124) |
| Recombinant EGF protein | 43 | IIIB, IV | Grade 1–2; fever, chills, nausea, vomiting, tremors, anorexia, pain | Low dose: 6.43 mo High dose: 8.4 mo | ND | ND | (122) |
| GVAX (Ad-GMCSF gene transfected auto NSCLC vaccine) | 35 | IV | Grade 1–2; erythema and indurations, fatigue, flu-like symptoms | ND | ND | 5SD 1MR | (72) |
| GVAX (Ad-GMCSF gene transfected auto NSCLC vaccine) | 33 | IIIB/IV | Grade 1–2 reaction at inj site, fatigue, nausea, dyspnea | 12 mo | 44% | 3 CR 6 SD | (10) |
| Belagenpumatucel (TGFβ$^2$ AS gene transfected Allo NSCLC vaccine) | 75 | II, III, IV | Grade 3; arm swelling ($n=1$) | 441 days (IIIB/IV only) | 54% (IIIB/IV only) | 6 PR | (194) |
| Telomerase peptide (GY 1001 and HR 2822) vaccine | 26 | IIIB, IV (I, III A) | Mild induration and erythema at inj site, chills, fever | 8.5 mo | 36% | ND | (152) |
| Allogenic NSCLC murine (1,3) galactosyltransferase transfected cell vaccine | 7 | IV | Grade 1–2: inj pain, erythema, fatigue, hypertension, bradycardia cough, diarrhea, dyspnea, headache, nausea, vomiting, pleural effusion | ND | ND | 4SD | (161) |
| MAGE 3 protein vaccine | 17 | I/II | ND | ND | ND | ND | (138) |
| L523S protein vaccine | 13 | IB/II | Grade 1,2: erythema, site pain, nausea, flu-like, hypertension | ND | 100% | 2 PD≤1 yr | (121) |
| GVAX Bystander vaccine | 49 | IIIB/IV | Grade 1,2: inj pain, fatigue, dyspnea, nausea, fever | 7 mo | 31% | 7SD≥12 wks | (74) |
| GVAX | 10 | IB/II | Grade 1,2 inj site reaction, fatigue, nausea, dysprea | ND | 100% | 4 SD≥20 mo | (10) |
| L BLP25 | 88 | IIIB/IV | Grade 1,2 inj site reaction, fatigue, nausea | 17.4 mo | ND | ND | (81) |

*Ad* Adenovirus; *Allo* allogenic; *NSCLC* Non small cell lung cancer; *Auto* autologous; *inj* injection; *N/A* not applicable; *ND* not done; *mo* months; *PR* partial response; *SD* stable disease; *ag* antigen; *MR* minor response; *CR* complete response; *PD* progressive disease; *yr* year; *wks* weeks

tumor-pulsed dendritic cells after vaccination. Survival at 1 year was 44% for all advanced stage treated patients and median survival was 12 months. Median survival among patients receiving vaccines secreting GM–CSF at a rate of ≥40 ng/24 h/$10^6$ cells was 17 months, when compared with 7 months for those receiving vaccines secreting less GM–CSF.

A subsequent trial in advanced NSCLC using a vaccine composed of autologous tumor cells mixed with an allogeneic GM–CSF-secreting cell line (K562 cells) failed to demonstrate evidence of clinical efficacy (74). Evidence of vaccine-induced immune activation was demonstrated; however, objective tumor responses were not seen despite a 25-fold higher GM-CSF secretion concentration with the bystander GVAX vaccine.

## *L-BLP-25*

Mucin (MUC)-1 is a high molecular weight protein containing large amounts of *O*-linked sugars and is expressed on the apical borders of most normal secretory epithelial cells (75). It is expressed in many cancers, including NSCLC (76). Tumor-associated MUC1 is antigenically distinct from normal MUC1 (77). Recent studies have identified that MUC1 is associated with cellular transformation, as demonstrated by tumorigenicity (78), and can confer resistance to genotoxic agents (79). Both the oligosaccharide portion and the tandem repeat of the MUC extracellular domain have potential for immunotherapeutic activity.

L-BLP-25 vaccine has been tested in three NSCLC trials (Table 1) (80). Three doses and two regimens were tested, including one regime using liposomal IL-2 as an adjuvant. Recently, results of a phase III study (81) of L-BLP-25 in 171 advanced stage NSCLC patients were reported (74). Patients with stable or responding stage IIIB or IV NSCLC following standard first-line chemotherapy were randomized to either L-BLP-25 (88 patients) or best supportive care (83 patients). There was a 4.4 month longer median survival for patients on the L-BLP-25 arm (17.4 vs. 13 months), although this did not reach statistical significance. The median survival for a subset of 35 stage IIIB patients who received vaccine was 30 months versus 13.3 months for the 30 who received best supportive care ($p=0.09$). There were no major toxicities.

The clinically meaningful survival advantages seen for stage IIIB patients is encouraging. A phase III randomized trial of L-BLP-25 for unresectable stage III NSCLC patients with response or stable disease after chemoradiation is now ongoing.

## *IDM-2101*

IDM-2101 is a peptide-based vaccine designed to induce CTLs against five tumor associated antigens (TAAs) frequently overexpressed in NSCLC [i.e., carcinoembryonic antigen (CEA) (82), p53 (83, 84), HER-2/*neu* (85, 86), and melanoma antigens (MAGE) 2 and 3 (87)]. These TAAs have been used in previous vaccine studies involving patients with NSCLC (88–107) and have been extensively characterized



in the literature. IDM-2101 is composed of ten synthetic peptides from these TAAs. Nine of the peptides represent CTL epitopes and each CTL epitope is restricted by HLA-A2.1 and at least one other member of the HLA-A2 superfamily of major histocompatibility complex class I molecules, providing coverage of approximately 45% of the general population. The tenth synthetic peptide is the pan-DR epitope (PADRE), a rationally designed helper T-lymphocyte (HTL) epitope included to augment the magnitude and duration of CTL responses (108).

IDM-2101 was tested in an open label phase II study involving 63 HLA-A2-positive stage IIIB/IV NSCLC patients who had failed prior chemotherapy. No significant adverse events were noted. Low-grade erythema and pain at the injection site were the most common side effects. One-year survival in the treated patients was 60%, and median survival was 17.3 months. One complete and one partial response were identified. Survival was longer in patients demonstrating an immune response to epitope peptides ($P<0.001$). Overall, treated patients appeared to do well when compared to historical controls.

Immune responses in 33 patients collectively showed induction of CTLs to all of the vaccine epitopes. Although patient to patient variability was observed with respect to the frequency and magnitude of the CTL responses, 85% of tested patients responded to at least two epitopes. These data are consistent with results from an earlier phase I trial (109). Moreover, longer survival was shown in patients achieving responses to two or more epitopes ($P<0.001$).

## *B7.1 Vaccine*

B7.1 ($CD80^+$) is a costimulating molecule associated with induction of a T and NK cell response (93, 110–112). Tumor cells transfected with B7.1 and HLA molecules have been shown to stimulate an avid immune response by direct antigen presentation and direct activation of T cells, in addition to allowing cross-presentation (113–116). In a Phase I trial, Raez et al. (117) used an allogeneic NSCLC tumor cell line (AD100) transfected with B7.1 (CD80) and HLA-A1 or -A2 to generate CD8 CTL responses (Table 1). Patients who were HLA-A1 or -A2 allotype received the corresponding HLA-matched vaccine. A total of 19 patients with stage IIIB/IV NSCLC were treated, and most had received prior chemotherapy. Patients who were neither HLA-A1 nor -A2 received the HLA-A1-transfected vaccine.

A total of 18 patients received at least one full course of treatment. One patient was removed before the completion of the first course because of a serious adverse event not associated with the vaccine. Three more patients experienced serious adverse events, which were also not associated with the vaccine. Side effects included minimal skin erythema for four patients.

One patient showed a partial response for 13 months and five patients had stable disease ranging from 1.6 to >52 months (117, 118). The Kaplan–Meier estimate for the survival for the 19 patients was 18 months. One-year survival was estimated at 52%. The low toxicity and good survival in this study suggested benefit from clinical vaccination.

## L523S Vaccine

L523S is a lung cancer antigen originally identified through screening of genes differentially expressed in cancer versus normal tissue (119, 120). L523S is expressed in ~80% of NSCLC cells (119, 120). The immunogenicity of L523S in humans was initially shown by detecting the presence of existent antibody and helper T cell responses to L523S in patients with lung cancer (BB-IND-#10833 public FDA database for this protocol IND). Subsequent studies further validated L532S immunogenicity by demonstrating that human CTLs could specifically recognize and kill cells that express L523S. In preclinical studies, the gene proved safe when injected intramuscularly as an expressive plasmid (pVAX/L523S) and when delivered following incorporation into an EIB-deleted adenovirus (Ad/L523S). In a phase I clinical trial in 13 stage IB, IIA, and IIB NSCLC patients, both delivery vehicles (pVAX/L523S and Ad/L523S) were used to administer the gene to three patients in each of three cohorts (121) (Table 1). No significant toxic effect was identified. All but 1 patient demonstrated at least twofold elevation in antiadenovirus antibodies; however, despite the positive preclinical studies, vaccination induced an immune response in only one patient in the phase I study. The reasons for a lack of significant detectable immune response are unknown. The use of alternative formulations and/or regimens and the assessment of other surrogate immune function parameters might be considered. Two patients developed disease recurrence and all remained alive after a median of 290 days follow up.

## Epidermal Growth Factor Vaccine

Overexpression of epidermal growth factor receptor (EGFR) and its ligand, epidermal growth factor (EGF), has been linked with the promotion of cell proliferation, survival and motility. EGF transduces signaling through EGFR following binding to this cell surface receptor, ultimately resulting in the stimulation of cell proliferation. The immunotherapy developed by Ramos et al. (122) induces an immune response against self-produced EGF. This vaccine is a human recombinant EGF linked to a P64K recombinant carrier protein from *Neisseria meningitides*. Several pilot trials have been completed (122–124). Results from these studies have demonstrated that vaccination with EGF is immunogenic and appears to be well-tolerated (Table 1).

In one study, 43 patients with stage IIIB/IV NSCLC randomly received either a single dosage or a double dose (122). Immune response against EGF was measured in 38 of the 43 patients, and 15 achieved a good antibody response (GAR) against EGF following vaccination. Kaplan–Meyer analysis separating patients by dose predicted a median estimated life expectancy of 6.4 months for patients who received the single dose, and 8.4 months for the patients who received the double dose. Based on immune response, however, patients classified as GARs had a life expectancy estimated at 12 months, whereas those who had a less favorable GAR had a life expectancy of 7 months.



Two other studies conducted by Gonzalez and colleagues compared the effect of different adjuvants on patients' antibody response (40). The patients were treated each time when antibody titers decreased to at least 50% of their induction phase peak titer. The pooled data of the two trials suggested that higher antibody responses were obtained when the vaccine was emulsified in adjuvant montanide ISA 51 or when low-dose cyclophosphamide was administered before the vaccination; however, the difference was not statistically significant. Median survival of GAR patients was 9.1 months, whereas poor antibody responding patients had a survival of 4.5 months.

## Melanoma-Associated Antigen E-3 Vaccine

MAGE-3 is the most commonly expressed cancer testis antigen and is expressed in testicular germ cells, but no other normal tissue (125). It is aberrantly expressed in a wide variety of tumors, including NSCLC (125). Several $CD8^+$ T cell epitopes of MAGE-3 have been identified in vitro (126–134), including HLA-A1-restricted epitope 168–176 (135), and HLA-A2-restricted epitope 271–279 (136). Based on these findings, synthetic peptides corresponding to these epitopes have been introduced into clinical vaccination studies, in which they were associated with regression of melanoma in individual cases (137). Clinical vaccination studies using full-length recombinant proteins have the advantage that this antigen potentially includes the full range of epitopes for $CD4^+$ and $CD8^+$ T cells. In addition, it is likely that protein vaccination leads to the presentation of epitopes in the context of various HLA alleles, and therefore, this type of vaccine should be applicable to any patient regardless of HLA restriction (138).

Atanackovic et al. (138) used a MAGE-3 protein as a vaccine to induce $CD4^+$ T cells in patients with stage I or II NSCLC (Table 1). All patients had undergone surgical resection of the primary lung tumor and had no evidence of disease at the onset of the study. Of the nine patients who received only the MAGE-3 protein, three developed an increase in antibodies against MAGE-3 protein and one had a $CD8^+$ T cell response. By comparison, of the eight patients who received MAGE 3 antigen combined with the adjuvant ASO2B, seven showed an increase in serum concentrations of anti-MAGE-3 and four had a $CD4^+$ response to HLA-DP4-restricted peptide. Based on these results, further testing in a larger randomized phase II trial was recently reported (139), involving 182 (122 vaccine and 60 placebo) early stage (IB, II) NSCLC MAGE-A3+ patients. No significant toxicity issues were identified and preliminary analysis revealed a 33% disease free survival improvement in the vaccinated arm when compared with the placebo arm. Results trended toward significant in the stage II patients.

## *Transcriptase Catalytic Subunit Antigen Vaccine*

It is well established that T cells of the human immune system can recognize telomerase (140–148). Although telomerase is also expressed in some normal cells, such as bone marrow stem cells (149) and epithelial cells in gastrointestinal tract crypts (150), it is highly expressed in virtually all cancer cells. GV1001 is a unique peptide corresponding to a sequence derived from the active site of the catalytic subunit of human telomerase reverse transcriptase (hTERT). It contains the 611–626 sequence of hTERT and is capable of binding to molecules encoded by multiple alleles of all three loci of HLA class II (151). HR2822 is a second peptide corresponding to sequences 540–548 of hTERT. Brunsvig et al. (152) initiated a phase I/II trial (Table 1) involving 26 patients with late-stage NSCLC. No clinically significant toxic events related to the treatment were reported. Importantly, no bone marrow or severe gastrointestinal toxicities were observed. Side effects were mild and included flu-like symptoms, chills and fever.

Eleven patients demonstrated an immune response against GV1001, and only two patients demonstrated a response to HR2822. After receiving booster shots, two patients were converted to immune responders. One patient with stage IIIA NSCLC showed a complete tumor response and developed GV1001-specific CTLs that could be cloned from peripheral blood. The median survival time for all 26 patients was 8.5 months.

## *Dexosome Vaccine*

Exosomes are cell-derived lipid vesicles that express high levels of a narrow spectrum of cell proteins (153–155). Vesicles released from dendritic cells (dexosomes) have been demonstrated to play a role in the activation of the immune response (156, 157). In vitro, dexosomes have the capacity to present antigen to naïve $CD8^+$ cytolytic T cells and $CD4^+$ T cells (154, 158). Purified dexosomes were shown to be effective in both suppressing tumor growth and eradicating an established tumor in murine models (153). Morse et al. developed a vaccine using dendritic cell-derived exosomes loaded with MAGE tumor antigens (159). The phase I trial enrolled 13 patients with stage IIIB or IV NSCLC demonstrating MAGE-A3 or -A4 expression. Autologous dendritic cells were harvested to produce dexosomes. They were loaded with MAGE-A3, -A4, -A10 and -3DPG4 peptides. Dexosome therapy was administered to nine patients (Table 1).

Patients experienced grade 1–2 toxicities, including injection site reactions, flu-like symptoms, edema, and pain. Three patients exhibited delayed type hypersensitivity reactions against MAGE peptides. Survival ranged from 52 to 665 days.



## *α(1,3)-Galactosyltransferase*

α(1,3)-Galactosyltransferase (agal) epitopes are present on the surface of most non human mammalian cells and are the primary antigen source inductive of hyperactive xenograft rejection. Expression of agal epitopes after gene transfer (using a retroviral vector) in human A375 melanoma cells prevented tumor formation in nude mice (160).

Preliminary results by Morris et al. (161) using three irradiated lung cancer cell lines genetically altered to express xenotransplantation antigens by retroviral transfer of the murine *agal* gene, were recently described in seven patients with stage IV, recurrent or refractory NSCLC (Table 1). Toxicity involved grade 1–2 pain at the injection site, local skin reaction, fatigue and hypertension. Four patients had stable disease for >16 months.

## *Non-Small Cell Lung Cancer Dendritic Cell Vaccines*

Dendritic cells are potent antigen-presenting cells. As part of a phase II study (22), Hirshowitz et al. (17–21) recently generated dendritic cell vaccines from $CD14^+$ precursors, which were pulsed with apoptic bodies of an allogeneic NSCLC cell line that overexpressed Her2/neu, CEA, WT1, MAGE-2 and survivin. A total of 16 patients with stage IA-IIIB NSCLC were vaccinated (Table 1).

There were ten patients who experienced skin erythema at the injection site and four patients experienced minor fatigue. No patients experienced a serious adverse event. Five patients showed a tumor antigen-independent response, and six patients showed an antigen-specific response. The study concluded that the vaccine was safe and demonstrated biological activity.

## *Cyclophilin B*

Cyclophilin-B (CypB) is a ubiquitous protein playing an important role in protein folding (162, 163), and is expressed in both normal and cancerous cells. CypB-derived peptides are recognized by HLA-A24 restricted cytotoxic lymphocytes (CTL) isolated from lung adenocarcinoma. CypB peptides induce CTLs from leukemic patients, but failed to induce an immune response in cells isolated from patients with epithelial cancer or normal donors. Modification of a single amino acid of the CypB gene increases its immunogenicity and results in CTL activation in both cancer patients and healthy donors (164).

Gohara et al. investigated the immune response in advanced-stage lung cancer patients treated with CypB vaccine. Sixteen HLA-A24+ patients, fifteen with NSCLC, and one with SCLC were treated with CypB or modified CypB peptide vaccine following the completion of chemotherapy (165). All patients had stable disease at 5-week follow-up. Following vaccination, IFN-γ production by peripheral blood mononuclear cells isolated from patient sera were elevated in 3 of 12 patients. Overall survival for NSCLC patients receiving CypB or modified CypB vaccine was 67+ and 28+ weeks, respectively (Table 1). One patient with SCLC was not evaluable for response.

## Small Cell Lung Cancer Vaccine Development

### *Fucosyl-GM1*

The ganglioside fucosyl-GM1 is a carbohydrate molecule present in most cases of SCLC (166, 167), but absent in normal lung tissue. Immunostaining has demonstrated the presence of fucosyl-GM1 in culture media from SCLC cell lines, in tumor extracts, and in serum of mouse xenografts (168). Fucosyl-GM1 was detected in the serum of 4 of 20 SCLC patients with extensive-stage disease, but was not present in the serum of 12 patients with non-SCLC or in 20 healthy volunteers (168). The specificity of fucosyl-GM1 to SCLC makes it a potential target for immunotherapy.

Dickler et al. treated 13 patients with Fuc-GM1 isolated from bovine thyroid tissue; ten patients completed the study and were evaluable (169). All ten patients demonstrated high titers of IgM and IgG antibodies to Fuc-GM1. The most common toxicity was local skin reaction, lasting 2–5 days. Three of six patients who completed the entire course of vaccinations remained relapse free at 18, 24, and 30 months from diagnosis. Subsequently, Krug et al. administered synthetic fucosyl-GM1 after conventional chemotherapy to 17 patients (170). Five of six patients at the high dose demonstrated increased levels of antifucosyl GM1 IgM. Three of six patients receiving the middle dose showed antifucosyl GM1 IgM production, and none of five patients at the low dose showed elevated IgM levels. Toxicities were minimal (Table 2).

### *BEC2*

Ganglioside GD3 is a cell surface glycosphingolipid with differential expression limited to cells of neuroectodermal origin and a subset of T lymphocytes (171–173). High levels of expression have been demonstrated in SCLC tumors and cell lines (174). Because GD3 is present at low levels in normal tissues, it is poorly immunogenic. BEC2, an anti-idiotypic IgG2b mouse antibody that is structurally similar to GD3, demonstrated strong immunogenic properties in patients with melanoma (175).



**Table 2** Demographics and results of recent vaccine trials in SCLC

| Vaccine | Number of patients | Stage | Side effects | Median survival | 1-Year survival | Response | Reference |
|---|---|---|---|---|---|---|---|
| Fucosyl GM1 | 13 | 9ES, 4LS | Grade 1–3: local skin reaction, flu-like symptoms, sensory neuropathy | NA | NA | NA | (169) |
| Fucosyl GM1 | 16 | 6ES, 10LS | Grade 1–2: local skin reaction, myalgia, sensory neuropathy | 17.5 months from 1st vaccination | 69% | NA | (170) |
| BEC2 | 15 | 8ES, 7LS | Grade 1–3: local skin reaction, fever | 20.5 months from diagnosis | NA | NA | (176) |
| PolySA | 13 | 8ES, 5LS | Grade 1–4: local skin reaction, peripheral neuropathy | 22 months from 1st vaccination | 61% | NA | (184) |
| p53 | 29 | ES | Grade 2: fatigue, arthralgia | 11.8 months from 1st vaccination | 11% | 1 PR, 7 SD | (196) |

*ES* extensive stage; *LS* limited stage; *NA* not available; *PR* partial response; *SD* stable disease

Grant et al. treated 15 SCLC patients, 8 with extensive-stage and 7 with limited stage disease, with BEC2 vaccination (176). Thirteen patients were evaluable for response; all developed IgM antibodies to BEC2, and three developed IgG antibodies. Duration of antibody production was variable, with at least one patient demonstrating measurable antibody production 1 year following treatment. Median survival was 20.5 months from diagnosis, and patients with measurable anti-GD3 antibodies showed the longest relapse-free intervals (Table 2). When compared to SCLC patients treated with conventional therapy alone, the authors found patients treated with BEC2 vaccine to have longer than expected survival time, though not statistically significant. Significant toxicity was minimized to local skin irritation.

### *PolySA*

Polysialic acid (polySA) is found on the surface of Gram-negative bacteria (such as group B meningococcus), embryonic neural crest cells, and some malignancies of neural crest origin (177, 178). The large size and negative charge of this molecule inhibit binding of cell adhesion molecules, and it is this property that is believed to contribute to its role in neural crest cell migration and early metastasis of malignant cells (179). PolySA has been shown to be expressed abundantly by SCLC tissues (180–183), making it a potentially viable target for SCLC vaccine therapy.

Krug et al. investigated the immunogenicity of polySA vaccination in 11 SCLC patients following conventional therapy (184). Two forms of polySA were administered to patients. Five patients received vaccination with polySA, and six patients received polySA manipulated by N-propionylation (NP-polySA), which has been shown to boost the IgG response in mice (185). One of five patients treated with unmodified polySA demonstrated an IgM response. Of the six patients vaccinated with NP-polySA, all produced measurable IgM antibody responses. In five of the six cases, these antibodies cross-reacted with unmodified polySA. Flow cytometry confirmed the presence of IgM antibodies reactive to SCLC cell lines. Despite the demonstrable production of IgM antibodies to polySA, complement-dependent lysis of polySA-positive tumor cells with human complement could not be demonstrated. Common adverse effects were minimal and included injection-site reaction and flu-like symptoms lasting 2–4 days (Table 2). Four patients reported sensory neuropathy.

### *WT1*

The Wilm's tumor gene (WT1) is responsible for Wilm's tumor, a pediatric renal cancer, and encodes a protein involved in cell proliferation and differentiation, apoptosis, and organ development (186–188). WT1 is overexpressed in several hematological malignancies as well as various solid tumors, including lung, breast, thyroid and colorectal cancers (189, 190). WT1-specific cytotoxic lymphocytes



(CTL) lyse WT1 expressing tumor cells in vitro without damaging normal tissues that express WT1 physiologically (191, 192).

Oka et al. treated 26 patients, including 10 lung cancer patients (histological type not specified), with WT1 vaccine following the completion of conventional therapy (193). Three NSCLC patients showed decreased serum levels of tumor markers (CEA or SLX) following vaccination; one patient also showed a decrease in tumor size radiographically. One NSCLC patient had stable disease at follow-up; four patients developed progressive disease, and two were unevaluable. Three patients demonstrated increased activity of WT1-specific CTL activity. A correlation ($p=0.0397$) between immunological and clinical response was observed for all study patients.

## Conclusion

In conclusion, several vaccine opportunities demonstrate evidence of activity (195). All appear remarkably safe. Limitations, however, involve identification of sensitive subset patient populations and surrogate measures to indicate relevant immune reactivity. Vaccines described in this review focus on different elements of immune reactivity (i.e., antigen exposure, dendritic activation, T cell activation, inhibition of T regulatory cells, inhibition of TGF beta expression). Each of these approaches has demonstrated evidence of activity in subsets of patients. However, phase III trials are required to conclusively determine relevance to lung cancer. Data appear encouraging, particularly in the setting of minimal disease early in the therapeutic course and at earlier stages of disease. It is also enticing to consider the combination of vaccines, particularly those with varied mechanisms of action. Future trials will undoubtedly explore combination vaccine approaches or products with multiple immune component stimulation.

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