24 *Immunological Approaches to the Treatment of Lung Cancer*

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■ INTRODUCTION

Evidence of an endogenous immune-modulating effect in non–small cell lung cancer (NSCLC) is suggested based on heterogeneity of clinical progression observed in patients with the same histologic type of malignancy (1,2). There is also evidence for shared antigens in lung cancers (3–10) as seen in other tumor types (11,12). Dendritic cells (DCs), responsible for antigen presentation and induction of antitumor immunity in tumor-bearing hosts (13,14), have been shown to be activated in NSCLC, and biopsies of responsive disease have occasionally demonstrated tumor-infiltrating lymphocytes within the cancer, suggestive of endogenous immune effect (15). Lastly, improved survival of lung cancer patients who develop empyema has been rarely observed (16), further suggesting a potential positive role of the modulated host immune system against cancer.

Recent advances in molecular biology have identified antigens, cytokines, and mechanisms that have furthered our understanding of immunotherapeutic approaches.

The role of DCs in cell-mediated immunity has been extensively investigated (17–21). DCs play a central role in the induction of antitumor immunity through tumor antigen cross-presentation and the efficient display of these antigens in the context of major histocompatability complexes (MHC). This ultimately results in stimulation, proliferation, and activation of CD4+ and CD8+ T cells. CD4+ cells further augment the activity of natural killer (NK) cells and macrophages, in addition to amplifying antigen-specific immunity by local secretion of cytokines (22–26). These attributes make DCs a pivotal component in 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 (27,28). These cells have been shown to impede immune activation by facilitating T-cell tolerance to tumor-associated antigens (TAAs) rather than cross-priming CD8+ T cells, resulting in the nonproliferation of killer T cells that recognize the tumor (27–33). Additionally, elevated levels of interleukin-10 (IL-10) and TFG-β found in patients with NSCLC have been shown in animal models to mediate immunosuppression, which may in turn alter host defense against malignant cells (34–43). These mechanisms are manipulated in different ways in the design of recent vaccine therapeutics described in this review.

■ NSCLC VACCINE DEVELOPMENT

Belagenpumatucel

Belagenpumatucel-L (Lucanix) (44) 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 (45–50), and the level of TGF-β2 is inversely correlated with prognosis in patients with NSCLC (51). TGF-β2 has antagonistic effects on NK cells, lymphokine-activated killer cells, and DCs (34,39,40,52–54). 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 (10–14,55–58). 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 (44). Patients were randomized to one of the three dose

cohorts. Grade 3 arm swelling existed in one patient with no other serious side effects. Of all 75 patients, the median survival was 441 days with a 1-year survival of 54%. 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%. This compared favorably with the historical 2-year survival rate of <20% of stage IIIB/IV NSCLC patients (3–6,59,60). Furthermore, induction of an enhanced immune response to tumor antigen correlated with a more favorable outcome. Immune function was explored in the 61 advanced-stage (IIIB/IV) patients. Cytokine production (interferon [IFN]-γ, $P = 0.006$; IL-6, $P = 0.004$; and IL-4, $P = 0.007$) was induced, an antibody-mediated response to vaccine human leukocyte antigen (HLA) antigen was observed ($P = 0.014$), and there was a trend toward a correlation between a cell-mediated response and achievement of stable disease or better ($P = 0.086$).

In a recent open-label phase II trial of belagenpumatucel involving 21 confirmed stage IV NSCLC patients, safety and efficacy as well as the correlation between circulating tumor cells (CTCs) in blood and overall survival of advanced NSCLC patients were investigated. Patients were given intradermal (61) immunization of 2.5×10^7 TGF-β2 antisense gene–transfected allogeneic tumor cells (belagenpumatucel) one time per month for a 16-month period. The trial took place from September 2005 to January 2008.

There were no significant grade 3 or 4 toxicities related to therapy. There was grade 2 transient injection-site erythema in three patients and grade 1 and 2 injection-site induration in five patients.

Twenty of 21 patients enrolled were evaluable, with a median survival of 562 days; however, those patients whose baseline CTC levels were 0 to 1 had a significantly improved median survival of 660 days ($P = 0.025$). This adds further support to the hypothesis that lower CTC count may be correlated with better survival (61).

A phase III trial is ongoing to test the effect of belagenpumatucel in patients with stage IIIB, IV NSCLC who demonstrate initial responsiveness to platinum-based therapy.

There have been several investigations involving immune stimulation through TGF-β "blockade." One technique involves a TGF-β type 1 receptor kinase inhibitor, SM16. Inhibition of this particular receptor was shown to increase immunostimulatory cytokines and ICAM-1. In addition, there was an increase in number and function of antitumor CD8+ cells in mice containing lung cancer tumors (62).

Another study was done to explore the effectiveness of silencing TGF-β1. Tumor cultures of SW1 melanoma and Ag104 sarcoma cells were transfected with short hairpin RNA (shRNA) that inhibited the production of TGF-β1 utilizing a lentivirus vector. The concentration of TGF-β1 decreased by 98% in the SW1 culture and by 94% in the Ag104 culture. To explore the efficacy of using these TGF-β1 inhibited tumor cultures (SW1-TGF-β1 or Ag104-TGF-β1) as vaccines, in vivo studies were performed in mice. In one study, four SW1 mice treated with the SW1-TGF-β1 culture had a significant delay in tumor growth and two had a complete regression. All four of the control mice had consistent tumor growth. Similar results were found in studies with the Ag104-TGF-β1 cells (63).

GVAX

Vaccines transduced with granulocyte-macrophage colony-stimulating factor (GM-CSF) gene were potent inducers of tumor immunity in animal models (64). 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 (65). Preclinical studies conducted with GVAX showed no significant local and systemic toxicities at clinically relevant doses (64,66–68).

Several phase I/II human trials using GM-CSF–secreting autologous or allogeneic tumor cell vaccines have been performed (69–74). One multicenter phase I/II trial involving patients with early-stage and advanced-stage NSCLC evaluated an autologous GVAX vaccine (8). 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 regression. 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 tumor-pulsed DCs after vaccination. Survival at 1 year was 44% for all advanced-stage–treated patients and median survival was 12 months. Medial survival among patients receiving vaccines secreting GM-CSF at a rate of ≥40 ng/24 hours/10^6 cells was 17 months, 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 (75). 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.

α-Galactosylceramide

αGalCer is a glycolipid-based vaccine that has demonstrated capacity to activate Vα24 NK T cells which have been shown to demonstrate antitumor activity via several mechanisms including the production of cytokines such as IFN-γ. Combination with peripheral blood mononuclear cells pulsed with low-dose IL-2 and GM-CSF appeared to enhance vaccine activity (76). A phase 1 study involving 11 patients with NSCLC demonstrated minimal toxicity (grade I or II toxicity) and predicted immune response. However, only two patients achieved stable disease.

In a more recent phase I–II trial of the same vaccine, 23 advanced-stage NSCLC patients received treatment and 17 patients completed the study which took place from February 2004 to August 2006 (77). In 10 of the 17 patients, there was a measurable increase in IFN-γ producing cells. More significantly, those 10 patients had a 2-year survival of 60% and also had an appreciably greater median survival of 31.9 months in comparison with the 9.7-month median survival of the unresponsive patients ($P = 0.0015$). The median survival of the unresponsive patients is consistent with historical survival of similar patients undergoing standard treatment.

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 (78). It is expressed in many cancers, including NSCLC (79). Tumor-associated MUC1 is antigenically distinct from normal MUC1 (80). Recent studies have identified that MUC1 is associated with cellular transformation, as demonstrated by tumorigenicity (81), and can confer resistance to genotoxic agents (82). 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 (83). Three doses and two regimens were tested, including one regimen using liposomal IL-2 as an adjuvant. Recently, results of a phase III study (84) of L-BLP-25 in 171 advanced-stage NSCLC patients were reported (75). 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 are 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 TAAs frequently overexpressed in NSCLC (i.e., carcinoembryonic antigen [CEA] (85), p53 (86,87), HER-2/*neu* (88,89), and melanoma antigens [MAGE] 2 and 3) (90). These TAAs have been used in previous vaccine studies involving patients with NSCLC (91–110) and have been extensively characterized in the literature. IDM-2101 is composed of 10 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 MHC class I molecules, providing coverage of approximately 45% of the general population. The 10th synthetic peptide is the pan-DR epitope (PADRE), a rationally designed helper T-lymphocyte epitope included to augment the magnitude and duration of CTL responses (111).

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 (112). 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 with 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 (113). 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 (96,114–116). 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 (117–120). In a Phase I trial, Raez et al. (121) used an allogeneic NSCLC tumor cell line (AD100) transfected with B7.1 (CD80) and HLA-A1 or -A2 to generate CD8 CTL responses. 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 due to 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 (121,122). The Kaplan-Meyer 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 (123,124). L523S is expressed in approximately 80% of NSCLC cells (123,124). 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. 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 (125). No significant toxic effect was identified. All but one 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. (126) 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 (126–128). Results from these studies have demonstrated that vaccination with EGF is immunogenic and appears to be well-tolerated.

In one study, 43 patients with stage IIIB/IV NSCLC randomly received either a single dosage or a double dose (126). 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 (127,128). 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.

Previous results described justified a randomized phase II trial of 80 late-stage (IIIB/IV) NSCLC patients that was recently completed (129). Patients were randomized to either vaccine or standard therapy. Mild, grade 1 and 2 toxic events were associated with the vaccine. The investigators classified patients whose anti-EGF antibody titers were at least 1:4,000 or four times their preimmunization values to have GAR. Of the vaccinated patients, 51.4% of them achieved GAR while no patients achieved GAR in the control group. The vaccine did decrease EGF concentration in 64.3% of vaccinated patients and those who achieved GAR survived significantly longer, with an 11.7-month median survival as opposed to 3.6 months in those with poor antibody response. Overall, there was a slight advantage for vaccinated patients with a 6.47-month median survival versus the 5.33-month median survival for the patients on the control arm. One-year survival was nonsignificantly higher ($P = 0.096$) in vaccinated patients at 67% in comparison with 33% for the controls.

A subsequent study investigated the same EGF-based vaccine in combination with chemotherapy in 20 advanced NSCLC patients (130). No serious side effects related to the combination therapy were observed. Also, median survival and 1-year survival were both encouraging at 9.3 months and 70%, respectively, suggesting support of further testing in combination with chemotherapy.

Melanoma-Associated Antigen E-3 Vaccine

MAGE-3 is the most commonly expressed testicular cancer antigen and is expressed in testicular germ cells, but no other normal tissue (131). It is aberrantly expressed in a wide variety of tumors, including NSCLC (131). Several $CD8^+$ T-cell epitopes of MAGE-3 have been identified in vitro (132–140), including HLA-A1–restricted epitope 168–176 (141), and HLA-A2–restricted epitope 271–279 (142). 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 (143). Clinical vaccination studies using full-length recombinant proteins may offer potential advantages in that this antigen includes the full range of epitopes for CD4+ and CD8+ T cells. In addition, it is likely that protein vaccination leads to 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 (144).

Atanackovic et al. (144) used a MAGE-3 protein as a vaccine to induce $CD4^+$ T cells in patients with stage I or II NSCLC. 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 the MAGE-3 protein alone, 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 completed and recently reported (145), involving 182 (122 vaccine and 60 placebo) early-stage (IB, II) NSCLC MAGE-A3 positive patients. No significant toxicity issues were identified, and preliminary analysis revealed a 33% disease-free survival improvement in the vaccinated arm compared with the placebo arm. Results trended toward significance in the stage II patients.

Currently, a randomized, double-blind, placebo-controlled phase III trial with a target accrual of over 2,200 stage IB, II, and IIIA NSCLC patients is ongoing. The trial began in June 2007 and explores the vaccine both following adjuvant chemotherapy and without chemotherapy. The primary end-point for the trial is disease-free survival (146).

Transcriptase Catalytic Subunit Antigen Vaccine

It is well established that T cells of the human immune system can recognize telomerase (147–155). Although telomerase is also expressed in some normal cells, such as bone marrow stem cells (156) and epithelial cells in gastrointestinal tract crypts (157), 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 (158). HR2822 is a second peptide corresponding to sequences 540–548 of hTERT. Brunsvig et al. (159) initiated a phase I/II trial 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 (160–162). Vesicles released from DCs (dexosomes) have been demonstrated to play a role in the activation of the immune response (163,164). In vitro, dexosomes have the capacity to present antigen to naïve $CD8^+$ cytolytic T cells and CD4+ T cells (161,165). Purified dexosomes were shown to be effective in both suppressing tumor growth and eradicating an established tumor in murine models (160). Morse et al. developed a vaccine using DC–derived exosomes loaded with MAGE tumor antigens (166). The phase I trial enrolled 13 patients with stage IIIB or IV NSCLC demonstrating MAGE-A3 or -A4 expression. Autologous DCs were harvested to produce dexosomes. They were loaded with MAGE-A3, -A4, -A10, and -3DPG4 peptides. Dexosome therapy was administered to nine patients. Patients experienced grade 1 to 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 nonhuman mammalian cells and

are the primary antigen source responsible for hyperacute xenograft rejection. Expression of agal epitopes after gene transfer (using a retroviral vector) in human A375 melanoma cells prevented tumor formation in nude mice (167).

Preliminary results by Morris et al. (168), 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. Toxicity involved grade 1 to 2 pain at the injection site, local skin reaction, fatigue, and hypertension. Four patients had stable disease for >16 months.

NSCLC Dendritic Cell Vaccines

DCs are potent antigen-presenting cells. As part of a phase II study, Hirshowitz et al. 169 (17–21) recently generated DC vaccines from CD14+ precursors, which were pulsed with apoptotic 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. There were 10 patients who experienced skin erythema at the injection site and 4 patients experienced minor fatigue. No patients experienced a serious adverse event. Five patients showed a tumor antigen-independent response, and 6 patients showed an antigen-specific response. The study concluded that the vaccine was safe and demonstrated biological activity.

Another phase I trial utilizing peripheral blood mononuclear cells from 15 patients with several different metastatic tumor types (melanoma, lung, renal cell carcinoma, sarcoma, and breast cancer) was also recently described (170). The DCs were stimulated with autologous tumor lysates and infused intravenously every 21 days for four total treatments. Toxicity was mild and included fever on the day of injection as well as well as asthenia. Seven of the 15 patients experienced stable disease for at least 3 months and 7 progressed while on treatment. The median time to progression was 3 months indicating that this DC approach should be pursued in further clinical testing.

Others have looked at use of postsurgical chemotherapy in combination with immunotherapy utilizing DCs and activated T-killer cells in late-stage lung cancer patients (171). The T-killer cells and DCs were harvested from tumor-draining lymph nodes and supplemented with peripheral blood lymphocytes. Thirty-one patients received four courses of chemotherapy in combination with immunotherapy every 2 months over the course of 2 years. These 31 patients were divided into two groups—those with N2 disease (group A) and those with N0 or N1 (group B). Group A received chemotherapy (calboplatin and paclitaqxel) and then underwent surgery. Group B went straight to surgery, and then both groups received a combination of chemotherapy and DC therapy or chemotherapy only. Those eligible for combination therapy received two to four courses and then continued DC therapy every 2 months for 2 more years. Group A was treated with docetaxel while group B received calboplatin and paclitaxel. In both cases, DC therapy was administered 5 to 7 days after chemotherapy. Twenty-eight patients in total received the DC therapy.

There were no significant toxicities other than low-grade fever, chills, fatigue, and nausea on the day of immunotherapy. Two- and 5-year survival of 88.9% and 52.9%, respectively, are encouraging and support an evaluation of efficacy in a phase III trial. There was also a correlation between the number of cells transferred and the rate of patient survival. Patients receiving more than 5×10^{10} cells had a 5-year survival rate of 80.8% compared with 38.5% in those who received less.

Cyclophilin B

Cyclophilin-B (CypB) is a ubiquitous protein playing an important role in protein folding (172,173), 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 (174).

Gohara et al. (175) investigated the immune response in advanced-stage lung cancer patients treated with CypB vaccine. Sixteen HLA-A24 positive patients, 15 with NSCLC and 1 with small cell lung cancer (SCLC), were treated with CypB or modified CypB peptide vaccine following completion of chemotherapy. All patients had stable disease at 5-week follow-up. Following vaccination, IFN-γ production by peripheral blood mononuclear cells isolated from patient sera was elevated in 3 of 12 patients. Overall survival for NSCLC patients receiving CypB or modified CypB vaccine was 67+ and 28+ weeks, respectively. One patient with SCLC was not evaluable for response.

1E10 Vaccine

The 1E10 vaccine is a murine anti-idiotypic antibody that was primarily created by the immunization of BALB/c mice containing P3, an idiotypic antibody which recognizes gangliosides containing NeuGc. Such gangliosides are reasonable targets for immunotherapeutic techniques as they have been detected in a number of different tumor types, including lung cancer. In fact, there has been recent data to suggest that NeuGcGM3 is correlated closely with tumor progression (176). It was hypothesized that the 1E10 idiotypic vaccination could produce an idiotypic cascade specific to the NeuGcGM3 antigen.

In a recent study aimed to investigate efficacy of the 1E10 vaccine, 20 advanced-stage NSCLC patients were administered 15 doses of the vaccine over an 18-month period (177). Those patients who received at least five doses of the vaccine were considered immunologically evaluable.

The study investigated via serum analysis whether antibodies against both the 1E10 vaccine itself and against the NeuGcGM3 ganglioside were produced in vaccinated patients. Of the 20 patients, 18 elicited an immune response against the vaccine and 16 produced an immune response against the ganglioside. The 1E10 antibodies, however, showed no success in inducing cell death and it was development of the anti-NeuGcGM2 antibodies that showed a distinct significance in patient survival. The median survival time of all patients on study was 10.6 months, but a dramatic improvement in survival was observed in patients who developed antibodies against NeuGcGM3 (median survival of 14.26 months) compared with those who did not (median survival 6.35 months). There were no significant advanced-grade side effects observed in the study.

■ SMALL CELL LUNG CANCER VACCINE DEVELOPMENT

Fucosyl GM1

The ganglioside fucosyl-GM1 is a carbohydrate molecule present in most cases of SCLC (178,179), 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 (180). 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 (180). The specificity of fucosyl-GM1 to SCLC makes it a potential target for immunotherapy.

Dickler et al. (181) treated 13 patients with Fuc-GM1 isolated from bovine thyroid tissue; 10 patients completed the study and were evaluable. All 10 patients demonstrated high titers of IgM and IgG antibodies to Fuc-GM1. The most common toxicity was local skin reaction, lasting 2 to 5 days. Three of 6 patients who completed the entire course of vaccinations remained relapse free at 18, 24, and 30 months from diagnosis. Subsequently, Krug et al. (182) administered synthetic fucosyl-GM1 after conventional chemotherapy to 17 patients. Five of 6 patients at the high dose demonstrated increased levels of antifucosyl GM1 IgM. Three of 6 patients receiving the middle dose showed antifucosyl GM1 IgM production, and none of 5 patients at the low dose showed elevated IgM levels. Toxicities were minimal.

Recently, a new vaccine was synthesized which utilizes the fucosyl-GM1 molecule but has been altered to enhance immunogenicity. The investigators have incorporated an MHC-II binding site into the existing carbohydrate which should aid in its activation of T cells. In particular, the chosen sequence has the capacity to bind up to nine variants of the human HLA-DR. Clinical testing will be underway in the near future (183).

BEC2

Ganglioside GD3 is a cell surface glycosphingolipid with differential expression limited to cells of neuroectodermal origin and a subset of T lymphocytes (184–186). High levels of expression have been demonstrated in SCLC tumors and cell lines (187). 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 (188).

Grant et al. (189) treated 15 SCLC patients, 8 with extensive-stage and 7 with limited-stage disease, with BEC2 vaccination. Thirteen patients were evaluable for response; all developed IgM antibodies to BEC2, and 3 developed IgG antibodies. Duration of antibody production was variable, with at least 1 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. When compared with 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.

In a randomized, phase III study of BEC3 vaccine in combination with standard chemotherapy, 515 SCLC patients either received standard therapy plus vaccine or were randomized to standard treatment (190). Those randomized to the vaccination arm received five vaccinations of Bec2 (2.5 mg)/BCG cavvine over a 10-week period. The primary side effects were mild including transient skin ulcerations and mild flu-like symptoms.

The results did not show a clinical benefit, however. In fact, it was concluded that there was no improvement in survival, progression-free survival, or quality of life when receiving vaccine. Median survival was 14.3 months in vaccinated patients and 16.4 months in standard treatment patients.

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 (191,192). 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 (193). PolySA has been shown to be expressed abundantly by SCLC tissues (194–197), making it a potentially viable target for SCLC vaccine therapy.

Krug et al. (198) investigated the immunogenicity of polySA vaccination in 11 SCLC patients following conventional therapy. Two forms of polySA were administered to patients. Five patients received vaccination with polySA, and 6 patients received polySA manipulated by N-propionylation (NP-polySA), which has been shown to boost the IgG response in mice (199). One of 5 patients treated with unmodified polySA demonstrated an IgM response. Of the 6 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. The median survival of all patients receiving PolySA treatment was 22 months after the first vaccination. Common adverse effects were minimal and included injection-site reaction and flu-like symptoms lasting 2 to 4 days. Four patients reported sensory neuropathy.

Wilm's Tumor Gene

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 (200–202). WT1 is overexpressed in several hematologic malignancies as well as various solid tumors, including lung, breast, thyroid, and colorectal cancers (203,204). WT1-specific cytotoxic lymphocytes (CTL) lyse WT1 expressing tumor cells in vitro without damaging normal tissues that express WT1 physiologically (205,206).

Oka et al. (207) treated 26 patients, including 10 lung cancer patients (histologic type not specified), with WT1 vaccine following completion of conventional therapy. Three NSCLC patients showed decreased serum levels of tumor markers (CEA or SLX) following vaccination; 1 patient also showed a radiographic decrease in tumor size. One NSCLC patient had stable disease at follow-up; 4 patients developed progressive disease, and 2 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 approaches to vaccine therapy in lung cancer demonstrate promise of clinical efficacy. All appear remarkably safe. Limitations include identification of sensitive subset patient populations and surrogate measures of 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-β expression). Any one of these approaches has demonstrated evidence of activity in subsets of patients. However, phase III trials are required to determine conclusive relevance to lung cancer therapy. Data appear encouraging particularly in a setting of minimal disease early in the therapeutic course and at earlier stages of disease. It is also enticing to consider combinations of vaccines, particularly those with varied mechanisms of action. Future trials will undoubtedly explore combined vaccine approaches, or products with multiple immune-component modulation.

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