EXPERT REVIEWS

Current vaccine updates for lung cancer

Expert Rev. Vaccines 9(3), 323–335 (2010)

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Current treatments for lung cancer are far from optimal. Several immunotherapeutic strategies involving vaccines incorporating different tumor-associated antigens to induce immune responses against tumors are being tested in clinical trials internationally. Although small, benefits have indeed been observed from the early studies of these vaccines, and the future is looking brighter for lung cancer patients as a handful of these immunotherapies reach Phase III trials. In addition, optimizing the induced immune response by these vaccines has become a priority, and a number of techniques are being considered, including addition of adjuvants and combining vaccines, which affect synergy based on their mechanism of action. This review is an update on the current vaccines in production, the benefits observed from their most recent studies, and the upcoming plans for improvements in these immunotherapies.

KEYWORDS: immunotherapy • lung cancer • tumor-associated antigen • vaccine

Lung cancer is the most prevalent form of cancer, accounting for 1.2 million cases annually, and is the leading cause of cancer-related deaths worldwide with 160,000 deaths per year [1,2]. Prognosis and treatment options for both small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC) depend on the stage of the tumor at diagnosis, and with approximately 75% of patients with lung cancer initially diagnosed as having advanced-stage disease, treatments are very limited [3,4]. Currently, no advanced-stage patients are cured of this disease and the median survival for frontline treatment success remains less than 1 year, emphasizing the dire need for new approaches to combat this disease [5].

The current treatments for lung cancer include surgery for early-stage disease, and chemotherapy and radiation for advanced-stage lung cancer [6]. Patients with advanced-stage disease undergoing chemotherapy often have a limited response duration and suffer toxic effects related to chemotherapy, particularly with prolonged administration of combination chemotherapy. Vaccines may provide a therapeutic opportunity when added to treatment regimens early after initial chemotherapy (i.e., after four cycles in a responsive or stable disease [SD] setting). In addition, certain monoclonal antibodies and angiogenesis inhibitors, which target epidermal and endothelial growth factor receptors (cetuximab and bevacizumab), have recently been approved for use as therapy in advanced NSCLC with small survival benefits [7]. Although these methods can somewhat improve the outcome of lung cancer, it is obvious that more effective treatment means are becoming increasingly necessary.

Historically, lung cancer has not been considered an immunogenic disease. However, it was observed that lung cancer cells could potentially be converted to convey immunogenic properties by cytokine stimulation or genetic modification, and this observation would eventually lead to experimentation with certain immunotherapies in this disease [8,9]. In the 1990s, it was observed that patients with immunodeficient diseases, such as AIDS, had a markedly increased risk of developing NSCLC and other solid tumors [10,11]. At this time, tumor-infiltrating lymphocytes were detected in lung tumor tissue, and alhough they seemed to be directed against the tumor, their function had been blocked by cancer-derived elements [12]. The first studies investigating immunotherapy in lung cancer involved nonspecific immune-inducing agents, and vaccines came soon afterwards, with a somewhat notable survival advantage in lung cancer patients [13,14]. Currently, a number of ongoing trials are examining different vaccine therapeutic strategies. Because NSCLC makes up 80% of lung cancer cases and is associated with poor outcomes in survival, a special emphasis has been put on investigation of this particular histology.

 10.1586/ERV.10.12 ISSN 1476-0584

In order to induce an immune response in lung cancer, not only must specific anti-tumor cells be present, but also immune cells must be plentiful and active at the tumor site, and they must be able to locate and access the tumor [15]. Antigens, which the immune system uses to recognize tumors, are often lacking in lung cancers, making them target molecules for tumor vaccines. They are often found in autologous or allogeneic tumor cells, proteins or peptide epitopes [16]. Many of the tumor-associated antigens (TAAs) in lung cancer have been identified, opening numerous doors for expanded antigen-specific vaccine immunotherapy [17]. As a result, immunotherapy in NSCLC has expanded to also include focused targeting of anti-tumor elements. Vaccines require an immune system that can correctly distinguish cancer cells from normal cells through TAA recognition [18,19]. Currently, many vaccine strategies also incorporate an adjuvant or mixture of adjuvants, dendritic cells (DCs), recombinant cytokines or gene-transferred cytokines, as will be reviewed [20,21].

With new technology and a better understanding of the immune system, new tumor-related antigens and adjuvants can be identified, and more efficient vaccines can be produced to combat lung cancer. Suggestive patient survival benefits have been observed through early clinical trials with certain vaccines (see Table 1 for a complete list of current vaccines), and further investigation is underway to optimize the immune response and hopefully further impact survival in lung cancer.

Nonspecific immune modulators for NSCLC

Early studies of immunotherapy in lung cancer began with immunomodulator adjuvants that activated the immune system in a general fashion. Nonspecific adjuvants, such as killed bacteria and bacterial lipopolysaccharides, have been shown to induce immune responses in many types of tumors, including NSCLC. Although major clinical benefits have not been observed [22], one vaccine in particular, SRL172, may warrant further investigation based on a recent study.

SRL172

SRL172 is a suspension of heat-killed *Mycobaterium vaccae*, which has been shown to be immunogenic [23]. *M. vaccae* is nonpathogenic in humans and has been investigated for treating TB, asthma and other types of cancer [24–27]. The SRL172 vaccine approach relies on inducing a general immune response that promotes TAA recognition by activating antigen-presenting cells and natural killer (NK) cells. In a Phase II study, first-line treatment was given to 29 NSCLC patients followed by either three weekly intradermal SRL172 injections or best supportive care [28]. Local injection-site reactions were the only toxicities observed related to the vaccine. Median survival for the 28 evaluable patients was 9.4 months versus 7.5 months in patients who received only chemotherapy, and the 1-year survival rate was 42%. However, no significant immune response was observed.

A Phase III study was initiated in 2004 [29]. SRL172 was administered monthly for 3 months and every 3–6 months thereafter. Although quality of life was improved in all patients who received vaccine, no changes in overall survival (OS) or progression-free survival (PFS) were observed. However, subset analysis with longer follow-up observed that patients with adenocarcinoma who received SRL172 did, in fact, experience a survival advantage over those who received only chemotherapy (302 vs 177 days) [30].

Peptide/protein-based vaccines for NSCLC

Antigens play an important role in the activation of the immune system against tumor cells. As mentioned before, tumors are recognized by the presence of antigens, which are processed and presented to the immune system in order to elicit an immune response. These particular vaccines take advantage of the fact that certain antigens may be absent in lung cancer and target these specific antigens, usually with the help of an adjuvant and a delivery vehicle, such as DCs. In order for an antigen to be considered for a vaccine, it must be consistently present in the particular tumor, absent or somehow different in normal cells, and show immunogenicity (for enhancement) or tumorigenicity (for suppression) [31]. Antigen-based vaccines are usually presented as modified proteins or T-cell epitope peptides [9]. Furthermore, antigen-based vaccines are commonly incorporated with adjuvants to optimize the release of the antigen and further stimulate the immune system. Currently, the antigen-based vaccine method is well established in early clinical trials, and safety and feasibility have been demonstrated.

MAGE-A3

Melanoma-associated antigen (MAGE)-A3 is a tumor-specific antigen expressed almost exclusively in various types of cancer, including NSCLC. It is present in approximately 50% of advanced-stage lung cancer and 35% of early-stage, making it a desirable target [32]. In addition, MAGE-A3 contains epitopes that are recognized by cytotoxic T cells and its immunogenicity has been repeatedly proven in early clinical trials [22]. A recombinant MAGE-A3 fusion protein was used to create the vaccine, and when tested it was proven to activate $CD4^+$ and $CD8^+$ T cells in early-stage NSCLC patients. Initially, a pilot study was conducted for patients with resected stage I/II NSCLC who expressed MAGE-A3 [33]. Of the 17 who were eligible to enroll, nine patients received the MAGE-A3 vaccine and eight received the MAGE-A3 plus the AS02B adjuvant. Seven of the eight patients who received the adjuvant developed antibodies to MAGE compared with three of the nine MAGE-only patients.

Owing to the success of these results, a Phase II study of this vaccine was conducted [34]. Final results for this trial evaluating MAGE-A3 as a cancer immunotherapy were reported in 2007. A total of 182 patients who were MAGE-A3 positive and had completely resected stage IB/II NSCLC were eligible to receive vaccinations, which were administered every 3 weeks for a total of five vaccinations. There were no serious toxicities found to be attributed to the study. Those who received the vaccine experienced a 27% reduction in the relative risk of recurrence compared with those who received the placebo. A follow-up study was performed 3 years after vaccination, revealing 14 patients who were still disease free [35]. Because of these impressive

Table 1. Summary of recent lung cancer vaccines.

Vaccine/antigen	Mechanism	Adverse effects	Immune response	Survival	Ongoing study phase	Ref.
L-BLP25	MUC1	Mild injection-site reactions	MUC1-specific T cells	MS: 17.4 months; OS: 30.6 months for stage IIIB NSCLC	III	[41]
EGF + p64K	EGF	Flu-like symptoms; injection-site reactions	GARs and anti-EGF antibodies	MS: 8.2 months	II/III	[46]
MAGE-A3	MAGE-A3 epitopes	No serious toxicities	MAGE-A3-specific antibodies	14 complete regression	III	[35]
GV1001	hTERT	No serious adverse events	GV1001-specific responses and CTLs	7 SD, 18 PD; MS: 8.5 months; 1 CR	II	[53]
1E10	NeuGCGM3	Grade 1/2; injection-site reaction	IgM/IgG antibodies to NeuGCGM3	MS for SD/PR: 11.5 months; OS: 9.9 months	II	[59]
aGal	Activate anti-agal antibodies	No serious toxicities	ND	4 SD for more than 16 weeks	I/II	[118]
Lucanix™	TGF-β2	One grade 3 (arm swelling)	HLA antibody responses to vaccine and cytokine production	2-year survival of 47%	III	[99]
GVAX (autologous)	Increasing GM-CSF secretion to activate APCs	Local injection-site reaction	Infliltration of $CD4^+$ and $CD8^+$ T cells, and $CD1a^+$ DCs	5 SD, 1 MR; OS: 12 months	II	[112]
GVAX bystander	Optimizing GM-CSF secretion	Greater injection-site reaction than GVAX	Vaccine-induced immune activation	7 SD >12 weeks, MS: 7 months	NP	[113]
B7.1	Upregulation of B7.1	Minimal skin erythema	Tumor-specific $CD8^+$ T cells	5 SD, 1 PR; OS: 18 months	I/II	[116]
DC vaccine – Her2/neu, CEA, WT1, MAGE-2, survivin	Various overexpressed antigens	Injection-site reaction, minor fatigue	Antigen-specific responses	ND	NP	[121]
DC vaccine – CEA	CEA	No serious adverse events	Decreased serum CEA levels; CEA-specific immune response	SD in numerous patients, depending on study	I	[122–124]
SRL172	General antigen recognition	Local injection-site reaction	ND	In patients with adenocarcinoma, OS: 10 months	NP	[29]
TG4010	MUC1 and upregulation of IL-2	Injection-site reaction; minor fatigue; flu-like symptoms	MUC1-specific cellular response in all evaluable patients	OS: 14.9 months; 1-year survival 60%	II/III	[42]
Fuc-GM1	Overexpressed fucosyl GM1 in SCLC	Local injection-site reaction	Increased levels of anti-Fuc GM1 IgM	3 regression-free for ≥18 months	II	[130]
PolySA	Polysialic acid	Local injection-site reaction; flu-like symptoms	IgM antibodies to polySA	5 PF; 6 survived ≥30 months	II	[134]
IDM-2101	CEA, Her2/neu, p53, MAGE2,3, peptides	Injection-site erythema	CTL induction to vaccine epitopes	Epitope-related survival advantage; MS: 17.3 months	NP	[91]

APC: Antigen-presenting cell; CEA: Carcinoembryonic antigen; CR: Complete response; CTL: Cytotoxic T lymphocyte; DC: Dendritic cell; GAR: Good antibody response; GM-CSF: Granulocyte–macrophage colony-stimulating factor; GVAX: Granulocyte–macrophage colony-stimulating factor gene-based vaccine; hTERT: Human telomerase reverse transcriptase; MAGE: Melanoma-associated antigen; MS: Median survival; MUC: Mucin; ND: Not determined; NP: No progress; NSCLC: Non-small-cell lung cancer; OS: Overall survival; PD: Progressive disease; PR: Partial response; SCLC: Small-cell lung cancer; SD: Stable disease.

results, an international Phase III study (MAGE-3 as Adjuvant Non-Small Cell Lung Cancer Immunotherapy [MAGRIT]) is now enrolling over 2000 patients with resected stage IB, II or IIIA MAGE-A3-positive NSCLC [201].

L-BLP25

Mucin 1 (MUC1) is a glycoprotein normally expressed on epithelial cells and is overexpressed in many different malignancies, including NSCLC [36]. In addition, the glycosylation pattern for MUC1 in cancer cells is immunologically distinct from that of MUC1 found in healthy human cells. In fact, it has been shown to play a role in tumorigenesis and is highly immunogenic in humans, making it an attractive target [37].

The L-BLP25 vaccine, which consists of the immunogenic peptide associated with monophosphoryl lipid A as an immunoadjuvant, is essentially a liposomal delivery system. This liposomal formation has been proven to improve accessibility to antigen-presenting cells, aiding the immune system. This vaccine, administered subcutaneously weekly for 8 weeks along with a single dose of cyclophosphamide, was applied in stage IIIB and IV patients who had responded to first-line lung cancer therapy in a Phase II study [38]. Cyclophosphasmide is an immunoadjuvant that has been shown to regulate the activity of suppressor T cells [39,40]. A total of 171 patients were enrolled in the study, randomized to receive either the vaccine or best supportive care. There was no significant toxicity related to the L-BLP25 vaccine, only mild injection-site reactions. Of the 78 patients who were eligible for immune-response evaluation, 16 had an antigen-specific T-cell response to MUC1. After analysis, it was shown that there was no significant difference in survival as a whole (the median survival for patients who received the vaccine was 17.4 months versus 13 months for those who received only best supportive care).

However, in a 2-year follow-up, a survival benefit was shown in a subset of patients, those with stage IIIB locoregional disease who received L-BLP25 (OS of 30.6 months, compared with an OS of 13.3 months for the control arm [41]). Based on these promising results, an international, multicenter Phase III is now ongoing, involving 1300 patients, specifically for those with stage III NSCLC who have previously received chemotherapy and radiation therapy [201].

TG4010

TG4010 is a gene vaccine that expresses MUC1 antigen in combination with human IL-2 using a modified vaccinia virus (MVA-MUC1-IL-2). A recent Phase II study was conducted to evaluate the immune response induced by this vaccine in advanced-stage NSCLC patients [42]. A total of 65 patients were randomized into two arms and treated until disease progression. Arm one involved 44 patients who received TG4010 combined with chemotherapy upfront, and TG4010 monotherapy was administered to 21 patients in arm two. No significant toxic events were observed. All of the 37 evaluable patients experienced a MUC-1-specific cellular response. The OS for arm one was 12.7 months and it was 14.9 months for arm two. The 1-year survival rate was 53%. In the follow-up study for this trial, 148 patients were randomized to TG4010 weekly for 6 weeks plus chemotherapy or chemotherapy alone. Of the evaluable patients at the end of the study, 45% experienced PFS for more than 6 months, reported at the American Society of Clinical Oncology in June 2008. No Phase IIB results have yet been published, although the study was completed at the end of 2007. However, because the primary end point of 40% PFS was met, a Phase II/III study is currently ongoing, further investigating TG4010 in advanced-stage NSCLC patients [101].

EGF vaccine

The EGF receptor (EGFR), or HER1, is a transmembrane receptor represented in a number of solid tumors, including NSCLC, making it a viable immunotherapeutic target. Its overexpression has been known to lead to cell proliferation and differentiation [43]. Erlotinib and gefitinib, which are known to target the EGFR tyrosine kinase, have already been clinically approved to treat NSCLC, and many other anti-EGFR antibodies are currently under investigation [44]. In addition, immunotherapies involving EGFR are being studied extensively. A vaccine that prevents ligand binding and the subsequent signaling cascade by inducing an immune response against self-produced EGF has been involved in several pilot trials. It was first administered to patients in 1998, along with a low dose of cyclophosphamide. In 2003, the same group conducted two additional studies with the EGF vaccine and different adjuvants, which were pooled together [45]. A total of 20 patients who were previously treated for advanced-stage NSCLC were randomly immunized with EGF plus p64K, a recombinant protein known to act as an immunologic carrier protein, or EGF plus p64K emulsified in montanide ISA 51 in the first trial. In the second trial, the same vaccine randomizations occurred, but all patients received the low dose of cyclophosphamide 3 days before vaccination. No significant toxicity was observed. The pooled results revealed that the vaccine was more effective with higher anti-EGF antibody responses when emulsified in montanide ISA 51, or when the patient received the cyclophosphamide. Those who had good antibody responses (GARs) had a better median survival than those who did not (9.1 vs 4.5 months), and GARs were seen in those who received the vaccine with the montanide ISA 51. The median survival time for all patients was 8.2 months.

In a Phase II trial, 80 patients received either the EGF-p64K vaccine or best supportive care alone after first-line lung cancer treatment [46]. Patients were immunized weekly for 4 weeks and then monthly. They also received the cyclophosphamide adjuvant 3 days before the first vaccination. Again, toxicity was minimal, limited to local skin reactions at the injection site and flu-like symptoms. GARs were achieved in approximately half of the vaccinated patients, and serum EGF concentrations were greatly reduced. Survival was somewhat significant in immunized patients under 60 years of age and was in correlation with the robust immune response. Recently, a clinical trial was performed in order to test the safety and feasibility of combining chemotherapy with an even higher dose of the EGF vaccine.

This successful trial has led to an ongoing Phase II/III study to determine the correlation between survival and the antibody titers observed [47]. In addition, the EGF-p64K vaccine has been approved for clinical use in Cuba [48].

GV1001

Human telomerase reverse transcriptase (hTERT) subunit is known to be upregulated in over 85% of NSCLC, and is highly overexpressed in a number of tumors, so much so that T cells recognize it as a TAA [49,50]. GV1001 is a peptide-based vaccine that corresponds to sequences 611–626 of hTERT with strong human leukocyte antigen (HLA) class II binding properties. Studies have also shown that GV1001 epitopes have immunogenic tendencies, with the ability to recruit $CD4^+$ and $CD8^+$ T cells [51,52].

In a Phase I/II study with hTERT peptides, 26 patients with advanced-stage NSCLC received either GV1001 or I540 (HR2822), another telomerase peptide representing an HLA class I restricted epitope with granulocyte–macrophage colony-stimulating factor (GM-CSF) as an immunoadjuvant [53]. A total of 4–21 vaccinations were given intradermally at three different dose levels over 10 weeks. There were no serious adverse events observed, even in the bone marrow and gastrointestinal epithelium, where telomerase is also commonly found. Of the 24 evaluable patients, 13 had GV1001-specific responses and only two had responses against I540. Overall, seven patients demonstrated SD and 18 were found to have progressive disease (PD). One patient had a complete regression and developed cytotoxic T lymphocytes (CTLs) specifically for GV1001. Median survival for all patients was 8.5 months. Obviously, GV1001 induced a more significant immune response than the I540 peptide. This promising information led to a Phase II trial being conducted in Norway [54]. Patients with stage III NSCLC will receive either vaccination with GV1001 or placebo after chemoradiotherapy.

1E10

Components of malignant tissues that are not present in normal tissues are potentially beneficial immunotherapeutic focuses. This is why Neu-glycosylated (NeuGc)-containing gangliosides have been targeted in a number of different studies [55–57]. In order to generate immune responses to these glycolipids, anti-idiotype antibodies (Ab2) have been used to essentially mimic the tumor-associated gangliosides. In particular, a murine Ab2 vaccine called 1E10 is related to and can successfully recognize NeuGcGM3 [58]. 1E10 Phase I trials for patients with melanoma, breast cancer and SCLC have divulged a notable immune response against tumor cells in correlation with prolonged survival [59–61]. In 2007, a study was executed with this anti-idiotype vaccine in stage IIIb/IV NSCLC patients who had already completed first-line treatment [62]. Immunizations were given intradermally every 2 weeks for the first five doses and then monthly for the remaining ten doses. The only toxicities of this trial were classified as grade 1 or 2, with the main side effect being an injection-site reaction. Out of the 71 patients who were enrolled in the study, 18 remained alive at the time of analysis. The OS for all patients enrolled was 9.9 months and the median survival for the 51 patients who achieved a partial response (PR) or SD was 11.5 months (compared with 6.5 months for PD patients).

After this initial study, a Phase II study with this vaccine was conducted to investigate the correlation between immune responses and survival times in patients [63]. A total of 20 patients who were eligible for enrollment were injected in the same manner as stated for the previous clinical trial [62]. There were no unexpected or serious adverse events observed in this study. Of the 20 patients who participated, 18 developed antibodies against 1E10 and 16 had IgM and/or IgG antibodies to NeuGcGM3. After analysis, it was shown that nine of the patients generated antibodies with the capability of recognizing and killing tumor cells expressing NeuGcGM3. The median survival (months) for all patients was 10.6 months, and there was a significant difference in survival time for patients who developed IgM and/or IgG antibodies compared with those who did not (17.3 vs 6.35 months). Based on the impressive immune response induced by this vaccine, along with the noteworthy increase in survival time, a Phase II clinical trial has been set up and is currently ongoing for 1E10 to further investigate its' effectiveness in NSCLC patients [63].

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] [64], p53 [65,66], HER-2/*neu* [67,68], and MAGE 2 and 3 [69]). These TAAs have been used in previous vaccine studies involving patients with NSCLC [70–88,201] 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 MHC 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 [89].

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 treatment [90]. No significant adverse events were noted. Low-grade erythema and pain at the injection site were the most common side effects. The 1-year survival in the treated patients was 60%, and median survival was 17.3 months. One CR and one PR were identified. Survival was longer in patients demonstrating an immune response to epitope peptides ($p < 0.001$). Overall, treated patients appeared to do well 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 [91]. Moreover, longer survival was seen in patients achieving responses to two or more epitopes ($p < 0.001$).

Tumor cell-based vaccines for NSCLC

Whole-cell vaccines contain an entire array of antigens, both known and unknown to researchers, which can induce an immune response to many TAAs. Both allogeneic and autologous whole-cell vaccines have been clinically studied in lung cancer patients. Allogeneic vaccines do not require extensive preparation with patient-specific tissue because lung cancer cell lines are used instead. However, antigens in these cell lines may have limited specificity compared with the host tumor. Autologous vaccines are patient specific, but they require tumor resection. Newer allogeneic and autologous tumor cell-based vaccines are constructed to contain immune-suppressing proteins or immune-activating cytokines in order to induce an immune response.

Lucanix™

TGF-β2 has been shown to have suppressive effects on the immune system in cancer patients, specifically on natural killer cells and DCs due to overexpression [92–96]. It is commonly found in elevated quantities in patients who have glioblastoma and NSCLC, with increasing levels in patients with a poor prognosis [97]. Preclinical studies have proven that inhibition of TGF-β2 can increase the immunogenicity of tumor vaccines by providing a source of multiple TAAs. Lucanix™ (NovaRx, San Diego, CA, USA) is a gene-modified, allogeneic tumor cell vaccine composed of four different NSCLC cancer cell lines modified to secrete a TGF-β2 antisense gene in order to inhibit TGF-β2 expression and, in turn, increase immunogenicity [98–102].

Limitations of this vaccine do arise, however, in that the immune effect is limited to TGF-β2. TGF-β1 appears to have a greater inhibiting effect in non-glioblastoma/NSCLC tumors [103–106].

This vaccine was studied in a Phase II clinical trial, in which 75 patients (14 early stage and 61 late stage) randomly received one of the three doses (1.25, 2.5 or 5×10^7 cells/injection) intradermally monthly or every other month until PD criteria were fulfilled [98]. There was no significant adverse toxicity observed, and only one grade 3 event (arm swelling) was attributed to the vaccine. Those who received the lowest dose of Lucanix experienced shorter survival compared with the other two doses combined. A significant 2-year survival advantage of 47% was seen in 41 advanced-stage patients who received dose levels of 2.5×10^7 cells/injection or greater. In addition, 15% of the vaccinated patients achieved PRs. A total of 59% of the vaccinated patients had no progression after 16 weeks on treatment. Increased production of cytokines (IFN-γ, IL-6 and IL-4) was observed in all 20 patients with SD or better, and 11 of these 20 patients had HLA antibody responses to the vaccine, compared with two of the 16 PD patients. A second Phase II study was recently completed at the 2.5×10^7 cells/injection dose, and similar survival and safety was demonstrated [107]. A Phase III trial is currently ongoing to further investigate this vaccine.

GVAX

Granulocyte–macrophage colony-stimulating factor gene-based vaccine (GVAX), has been shown to induce a tumor-specific immune response by increasing antigen expression and attracting antigen-presenting cells to the vaccination site [108,109]. The GVAX vaccine contains a viral-based vector of the recombinant *GM-CSF* gene, which is transfected into surgically resected autologous tumor cells. GVAX was studied in a Phase I trial for patients with stage IIB–IV NSCLC [110]. A successful vaccine was created for 34 of the patients enrolled in the study and was administered intradermally weekly for 3 weeks and then every 2 weeks. Local injection-site reactions were the most common adverse events. Anti-tumor immunity was observed in 82% of vaccinated patients. Overall, five patients had SD, one patient had a mixed response and two patients who underwent surgical resection showed no evidence of cancer progression for more than 40 months. A Phase I/II clinical trial using a modified manufacturing process more suited to commercialization of autologous vaccines was conducted in early- and advanced-stage NSCLC patients. Results revealed a dose-related survival advantage [111]. A total of 43 patients initiated vaccine treatment (33 with advanced disease), with injections given biweekly for a total of three to six vaccinations. No significant toxicity was observed, with injection site reactions being the most common vaccine-related event. Complete tumor regressions were seen in three advanced-stage patients, two of which have experienced complete remission for longer than 5 years. Both are still in complete remission at the time of writing, even now, more than 8 years after initial treatment. The median OS for all patients was 12 months.

In an attempt to increase GM-CSF expression, a new vaccine called 'bystander' GVAX was designed and constructed [112]. Untransfected autologous tumor cell lines were mixed with an allogeneic GM-CSF-secreting cell line to create this vaccine. Despite a 25-fold higher GM-CSF secretion concentration, significant tumor regression was not seen. Toxicity and survival were less favorable compared with the first Phase I/II trial with GVAX. Owing to this, autologous tumor cell-transfected tissue is considered optimal over the bystander approach. Currently, a Phase II study is underway investigating the effects of GVAX in patients with advanced-stage bronchoalveolar lung cancer [101].

B7.1

B7.1, or CD80, is a protein expressed on antigen-presenting cells. It functions by binding to CD28 on T lymphocytes, another costimulatory molecule, in order to upregulate T-cell activity and cytokine production [75,113]. NSCLC tumor cells have the capability of downregulating B7.1, but tumor cells transfected with both HLA and B7.1 have been shown to induce immune responses in animal models. In a Phase I study, 19 advanced-stage NSCLC patients were vaccinated with an allogeneic, adenocarcinoma cell line modified with HLA-A and B7.1 transgenes [114]. Intradermal injections of 5×10^7 cells were given biweekly. Minimal toxicity was seen, with four serious adverse events unrelated to the vaccine. A total of 17 out of 18

patients experienced a tumor-specific $CD8^+$ immune response. Five patients achieved SD and one developed PR for 13 months. The 1-year survival for all patients who participated was 52%, with an OS of 18 months. Evaluation 1 year after this study revealed SD lasting from 1.6 weeks to more than 52 weeks [115]. There is a Phase I/II clinical trial currently underway at the University of Miami (FL, USA) investigating the feasibility and safety of the B7.1 vaccine in early-stage NSCLC patients [101].

α(1,3)-galactosyltransferase

α(1,3)-galactosyltransferase (agal) is an immunogenic protein that is normally only found on the surface of nonhuman mammalian cells. It is possible to induce an immune response against tumors using modified tumor cell lines [116]. One such vaccine containing three irradiated, genetically altered, human allogeneic lung cancer cell lines expressing murine agal was tested in a Phase I clinical trial [117]. Seven patients with stage IV NSCLC received injections intradermally every 4 weeks at four different doses (3×10^6, 10×10^6, 30×10^6 or 100×10^6 cells/vaccine). No significant toxicity was observed. SD was seen in four of the seven patients for more than 16 weeks. A Phase I/II is currently ongoing [101].

DC vaccines

One mechanism of immune system failure to eradicate tumor cells is because of inadequate antigen presence at the tumor site [6]. Pulsing DCs with antigens from allogeneic or autologous tumor cell lines is an efficient approach to induce an immune response. DCs are known to be efficient antigen-presenting cells that have the ability to activate CTLs [118,119]. Through the allogeneic method, uniformity is guaranteed, but the tumor must express the particular antigen used. If autologous cell lines are used, the tumor is guaranteed to express the antigen, but the antigens must be attained from the patient surgically.

In one Phase II study, autologous DCs were pulsed with allogeneic antigens from an NSCLC-irradiated cell line called 1650 that overexpressed Her2/neu, CEA, WT1, MAGE-2 and survivin [120]. Immunizations were given twice to 16 patients with various stages of NSCLC 1 month apart. Adverse events include local injection-site reaction and minor fatigue. Six of the vaccinated patients developed an antigen-specific response to the vaccine and five experienced PD. However, there was no clear correlation between the clinical outcome and the immune responses.

Another study was performed in 2004 to evaluate the efficacy of CEA in a DC vaccine [121]. Of the 18 patients enrolled, there were five with CEA-positive NSCLC. A total of five immunizations were given subcutaneously biweekly. Decreased levels of serum CEA were apparent in three NSCLC patients and four achieved SD. In a subsequent Phase I study, three NSCLC patients were immunized with an autologous DC vaccine transduced with CEA and costimulatory molecules [122]. Patients received three triweekly vaccinations in all. Of the 14 patients, five remained stable after vaccine was administered (it was not stated how many of these were NSCLC). CEA-specific T cells were apparent in ten patients, and one patient had decreased serum CEA levels. Recently, a Phase I study was conducted with DCs transduced with rF-CEA(6D)-TRICOM after administration of denileukin diftitox to deplete certain regulatory T cells [123]. CEA-specific immune responses were seen in patients but, as yet, no clinical outcomes have been reported.

SCLC vaccines

Less than a quarter of lung cancer cases are SCLC and immunotherapy for this cancer population has not been widely explored. In addition, SCLC is known to be an aggressive disease, so limits in time to immune induction with immunotherapy may be expected to have little potential to improve survival and prognosis. SCLC is often unresectable, so tissue is also often not readily available for vaccine production using whole-cell methods [124]. Despite these hindrances, there has been some investigation into using vaccines in SCLC, and this has even improved survival in a few cases.

One vaccine for SCLC has recently been evaluated in a Phase III trial using a nonspecific BEC2 plus Bacille Calmette–Guérin vaccine [125]. No survival benefit was seen. Other SCLC vaccine trials, however, are underway.

Fucosyl-GM1

Fucosyl-GM1 is a protein commonly found in SCLC but is absent in normal tissue or NSCLC, making it a specific target for vaccine therapy [126,127]. It was studied in 1999 in 13 SCLC patients after they had been treated with first-line therapy [128]. All of the ten evaluable patients showed high levels of IgM and IgG antibodies to Fuc-GM1, and three patients went into remission for 18 months or longer. In a Phase I study of synthetic Fuc-GM1 with a QS21 adjuvant, the vaccine was found to be feasible and safe [129]. Three different doses (30, 10 and 5 μg) were tested to optimize immune responses. Out of the 17 patients enrolled, eight experienced IgM anti-Fuc-GM1 responses, all of whom received dosages of 10 μg or more. There were no major toxicities observed. To further test this vaccine, a Phase II study using a 'tetravalent' vaccine in which Fuc-GM1 will be combined with antigens GM2, Globo H and polysialic acid (polySA) will soon be initiated in SCLC patients [130].

PolySA

Another target commonly found in SCLC is polysialic acid, which inhibits binding of cell adhesion molecules, thereby affecting metastatic spread of malignant cells [131,132]. A polySA vaccine was tested in a Phase I clinical trial that enrolled 11 patients [133]. Two forms of the vaccine were made, five received regular polySA and six received polySA manipulated with *N*-propionylation (NP-polySA). All patients with the manipulated vaccine developed IgM antibodies against polySA, and only one patient developed antibodies in the normal vaccine group. Toxicities included injection-site reactions and flu-like symptoms. Of all 11 patients, six experienced a survival time of 30 months or more, and five achieved progressive-free disease. Currently, polySA is being further investigated in a study using the tetravalent vaccine previously

mentioned, as well as in a Phase II clinical trial for SCLC. PolySA will be manipulated again with NP and the adjuvant QS21 will be added to the vaccine [101].

Key observations & conclusions

Although only small steps have been made in the process of finding an efficient and feasible vaccine for treating lung cancer, many promising discoveries have been made. Not only is the immune system finally being considered as a target for impacting lung cancer, several TAAs have been discovered, giving researchers a good basis for the development of various vaccines.

The most successful results have been seen in tumor cell-based vaccines, which incorporate a number of different antigens. Particularly encouraging results were seen with the vaccine Lucanix, evidenced by a 2-year survival rate of 47% in advanced-stage NSCLC patients.

Two antigen-specific vaccines, MAGE-A3 and L-BLP25, are also involved in Phase III trials based on promising results. Stronger overall survival times have been seen in lung cancer using these immunotherapies. In addition, low levels of toxicity have been seen in all vaccines compared with other methods of cancer treatment, such as chemotherapy.

Despite strong hints of activity, immunotherapy in lung cancer remains unvalidated as a therapeutic opportunity. Dosing schedules for administering vaccines have not been optimized and surrogate assays of immune reactivity in coordination with response and survival have not been demonstrated. It has been suggested that treatment earlier in the patient's disease course, at a time of minimal disease (i.e., after surgical resection or radiation therapy/chemotherapy response) is more commonly associated with beneficial vaccine activity. However, such patients generally have longer survival and therefore require longer follow-up to confirm vaccine activity. Radiotherapy has been shown to diminish immune-regulating T cells, thereby facilitating immune-generated anti-tumor responses [134,135]. However, there is still a question of when the chemo- or radio-therapy should be administered before starting the vaccine. Many more clinical trials must be completed before an optimal strategy can be achieved.

There is also more to understanding the relationship between the immune system and cancer antigen modulation [15]. Not all TAAs of lung cancer have been investigated in immunotherapeutic studies, and there are understandably many more yet to be discovered. For example, the antigen survivin has recently been found to exist in large quantities in NSCLC [136]. Tumors, including those in lung cancer, have evolved ways of maneuvering around the immune system [15] and many vaccines that fail to induce an immune response do so as a result of these complex mechanisms.

Studies looking at reducing the inhibitory effects of T-regulatory cells (i.e., cyclophosphamide or CTLA-4 antibody) may also enhance the response to vaccine therapy [48].

Expert commentary

Our understanding of the immune system as it relates to cancer clearance and resistance has advanced over the last 5 years. Novel therapeutic approaches focusing on immune activation, reversal of immune inhibitors and enhancement of tumor antigens are now being actively investigated in clinical trials. The toxicity of such approaches is minimal, and observation of durable responses justifies Phase III testing. If successful, immune modulation will play an increasing role in the management of lung cancer, possibly in combination or sequence with standard therapy or perhaps as stand-alone therapy, particularly following 'high risk of recurrence' surgery or radiation therapy. However, several years of clinical validation testing and analysis are required before such approaches can be implemented into standard clinical routine. Patients achieving a response appear to benefit from durable activity. Perhaps one day, patients presenting for initial diagnostic surgery will have the opportunity to have tissue procured for the purpose of manufacturing a personalized vaccine that in turn, through immune-modulatory genes, may enhance immune activation, remove tumor-produced inhibition and focus attack on specific tumor antigens without toxicity. Unfortunately, most lung cancer patients present with symptomatic advanced or metastatic disease. As such, the 'slow' (3–4 months) response of immune induction with vaccines will probably justify therapeutic strategies utilizing upfront chemotherapy to induce initial response and stabilize disease to be followed by vaccine therapy.

Five-year view

It is anticipated that over the next 5 years, certain vaccine approaches will prove successful and warrant continued investigation while others will be abandoned. Completion of Phase III trials using Lucanix, MAGE-A3 and L-BLP25 are important milestones. Combined therapeutics to create synergy based on better understanding of mechanisms will enable new opportunities (e.g., immune activation combined with antigen education and inhibition of effector inhibition). Approaches exploring multiple methods of immune stimulation, in essence 'combination' immune therapy (i.e., utilizing immune activation [*GM-CSF* gene], combined with inhibition of cancer-related immune inhibitors [TGF-β inhibition via gene transfer] in whole-cell cancer vaccines [antigen education]) are likely scenarios currently undergoing initiation [137]. These may provide further understanding 5 years from now. Results of Phase I investigations are being generated.

Incorporation of adjuvants will also be seen in the coming years in the development of immunotherapies. Testing different adjuvants with vaccines may improve activity of antigen stimulation and could even prolong an immune response. Optimizing current vaccines with adjuvants, while optimizing dosage, delivery and schedules, will pose challenges, but should improve response.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Key issues

- Treatment for both non-small-cell lung cancer and small-cell lung cancer has reached a standstill, with no recent major improvements in first-line treatments.
- Lung cancer causes more deaths each year than breast, colorectal and prostate cancers combined.
- Although using the immune system to target lung cancer tumors has been thought of as unconventional, early clinical studies have shown clinical benefit from using immunotherapies.
- Antigen-based vaccines have the ability to target a specific tumor-associated antigen that is present in a host's tumor and not in normal tissue.
- Whole-cell vaccines can target multiple tumor-associated antigens at the same time.
- Dendritic cells are an efficient way to deliver antigen-presenting cells to the immune system to induce an immune response.
- Special attention should be given to vaccines that have entered Phase III studies, as they may make their way to the clinic in the near future if positive results ensue.
- In order to optimize an immune response, certain adjuvants or combinations of vaccines within disparate mechanisms are being explored.

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