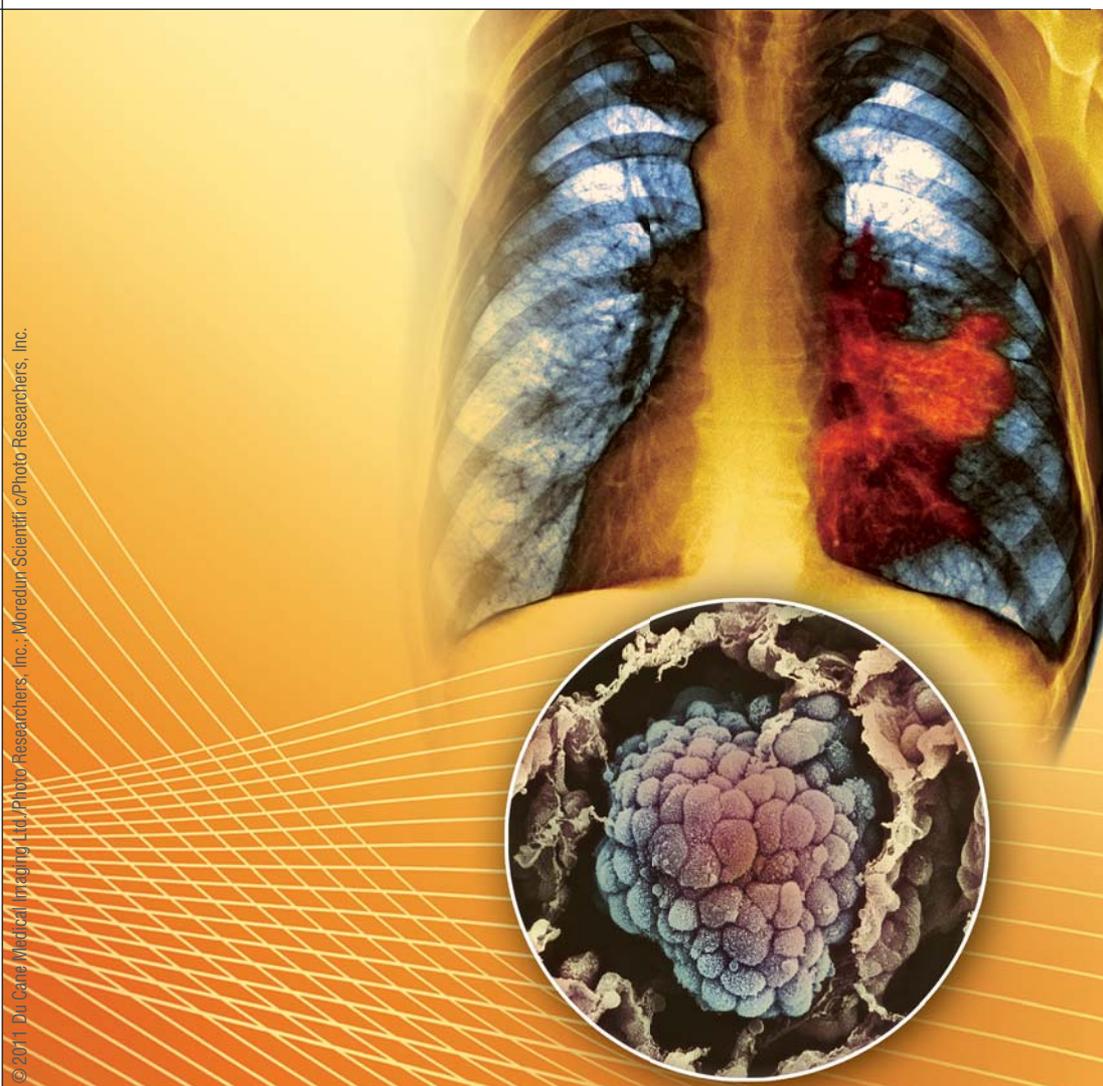


Advances in Immunotherapy for NSCLC

CME Learning Objectives

At the conclusion of this activity, participants should be able to:

- Explain current immunotherapy strategies being evaluated in non-small cell lung cancer (NSCLC).
- Evaluate key efficacy and safety data from clinical trials of protein-based, peptide-based, and cellular-based vaccines.
- Identify effective vaccine strategies for early-stage and advanced-stage NSCLC based on patient and disease characteristics.



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Introduction

Non-small cell lung cancer (NSCLC) remains one of the most prevalent neoplasms, and despite many years of intensive research, it responds poorly to currently available therapy in all but very localized stages of the disease. One of the most promising approaches to the treatment of this and other refractory solid tumors is the development of immunotherapy to manipulate the relationships between tumors and the immune system. Early data using several different mechanisms of action provide hope for new, effective monotherapies and combination treatments for lung cancer. Some of these therapies are already in use for other refractory diseases, including melanoma.

Vindico Medical Education, in conjunction with HEMONC TODAY, sponsored a symposium in June 2011 to review the current and future avenues of research in immunotherapy for NSCLC. The symposium presented the major approaches to tumor vaccination and reviewed biology of novel targets for immunotherapy in oncology. The most recently available data from clinical trials, many of which are ongoing, were evaluated. Questions concerning prognostic markers to individualize treatment were also discussed.

The symposium provided the material for this monograph, which will inform clinicians involved in the treatment of NSCLC of the most recent advances in immunological research, which will undoubtedly soon result in additions to the oncological armamentarium for lung cancer therapy. I thank the speakers for sharing their valuable knowledge and perspectives on these exciting new developments, and for participating in the preparation of this monograph.

Roy S. Herbst, MD, PhD
Course Chair

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CME Pretest

Advances in Immunotherapy for NSCLC

CME Instructions

Answer each pretest question by entering it in the space provided on the registration form on page 18. Responses to the pretest will not affect CME credit for this activity and will only be used to assess the efficacy of the activity.

- 1. Anticancer immunotherapy is based on the concept that:**
 - A. Healthy and cancerous cells generate identical surface antigens.
 - B. Mutated cancer cells express “non-self” surface antigens.
 - C. The human immune system is incapable of tumor cell cytolysis.
 - D. Cancer cells are incapable of generating surface antigens.
- 2. Which vaccine has both an activation and an anti-inhibitory effect on T cells?**
 - A. FANG
 - B. Lucanix
 - C. GVAX
 - D. Ipilimumab
- 3. Results of studies examining Lucanix demonstrated that:**
 - A. An immune response correlated with lack of disease progression.
 - B. TGFβ1 expression was significantly suppressed.
 - C. Survival was similar among all doses examined.
 - D. Efficacy of Lucanix did not compare favorably with existing second-line chemotherapy.
- 4. The phase 2 studies of talactoferrin demonstrated that:**
 - A. Talactoferrin significantly improves survival in patients with refractory NSCLC who had failed chemotherapy.
 - B. Talactoferrin was ineffective as a first-line agent.
 - C. The frequency of serious adverse events are a concern.
 - D. Talactoferrin was effective as a first-line and second-line agent, although the results were not statistically significant.

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Overview

Immunotherapy is emerging as a promising approach to the treatment of non–small cell lung cancer (NSCLC), which has had generally limited treatment options with poor survival rates. Stimulating

the patient's own immune system to eliminate tumor cells using vaccines may eventually offer improved outcomes with limited adverse events. In this program, leading researchers will discuss the immunotherapy strategies being developed and the efficacy and safety data from clinical trials to date. In addition, approaches to discussing and implementing vaccination programs with patients will be discussed.

Target Audience

The intended audience for the activities is oncologists, hematologists, and other health care professionals involved in the treatment of patients with non–small cell lung cancer.

Unlabeled and Investigational Usage

The audience is advised that this continuing medical education activity may contain references to unlabeled uses of FDA-approved products or to products not approved by the FDA for use in the United States. The faculty members have been made aware of their obligation to disclose such usage. All activity participants will be informed if any speakers/authors intend to discuss either non-FDA approved or investigational use of products/devices.

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Non–small Cell Lung Cancer: Novel Immunotherapy Strategies

Ronald B. Natale, MD

Cancer cells derive from normal cells by genetic mutations. The alterations and rearrangements in these mutations should be viewed by the immune system as “non-self.” The presence of cancer cells should therefore result in the activation of the immune system. The cytolytic power of the immune system can be harnessed to destroy cells that express “non-self” antigens on their surfaces; this effect is observed in the rejection of foreign tissue following organ transplantation. The goal of immunotherapy in non–small cell lung cancer (NSCLC) is to manipulate the immune system to recognize the “non-self” antigens that are expressed in tumor cells. In order to achieve this, the immunotherapeutic agent must overcome the defenses of the tumor cells, such as surface proteins that downregulate the host T cells, preventing the immune system from being activated by the tumor.

There are 2 approaches to manipulating the immune system in patients with NSCLC as well as other malignancies. One is the use of molecules that directly modulate the immune system. Such molecules include talactoferrin, BMS-936558 (an anti-PD1 IgG4 monoclonal antibody), and ipilimumab (Yervoy, Bristol-Myers Squibb), which has been approved for the treatment of melanoma. The second approach is to employ tumor-specific vaccines such as melanoma-associated antigen-A3 (MAGE-A3) vaccine, the granulocyte-macrophage colony-stimulating factor (GMCSF) vaccine (GVAX, Biosante Pharmaceuticals), belagenpumatucel-L (Lucanix, NovaRx) and the transforming growth factor β 2 (TGF β 2) antisense vaccine combined with human recombinant GMCSF (TAG). There is also a new derivation of the latter, with activity against a wider spectrum of TGF β proteins, a bi-shRNA-furin and GMCSF augmented autologous tumor cell vaccine (FANG, Gradalis, Inc).

Immunomodulation

There are a variety of immunomodulators that show potential to be used for immunotherapy in NSCLC. For example, the 80 kilodalton human recombinant lactoferrin protein talactoferrin is produced in *Aspergillus niger*. Lactoferrin is expressed in immune cells and throughout the body. This protein is found in high concentrations in milk and colostrum, and has a central role in establishing

the immune system, including gut-associated lymphoid tissue (GALT), in infants. Oral talactoferrin is transported by M cells into Peyer’s patches, structures in the GALT that act as antigenic sampling sites. They detect luminal antigens, induce an immunological response, and prevent antigen translocation across the mucosal epithelium. When talactoferrin is introduced into Peyer’s patches, it induces migration and maturation of dendritic cells.¹

BMS-936558 (MDX-1106) is a human IgG4 monoclonal antibody directed against PD1. The programmed death receptor PD1 has a central role in T-cell regulation. BMS-936558 binds with high affinity to PD1, blocking its interaction with the ligands PDL-1 and PDL-2, thereby influencing T-cell regulation.² BMS-936558 has no antibody-dependent or cell-dependent cytotoxicity (ADCC/CDCC).

Ipilimumab is a monoclonal antibody directed against cytotoxic T lymphocyte-associated antigen 4 (CTLA4).³ CTLA4 blocks T-cell activation. Therefore, by blocking CTLA4, ipilimumab promotes T-cell activation and augments the immune response to the endogenous tumor cell population.

Vaccine Therapy

Several conditions must be satisfied in order for a vaccine to be successful. First of all, the vaccine must take advantage of a tumor-associated antigen that is expressed almost, if not completely, exclusively in the tumor cell population rather than in healthy cells. Mutations in the cancer cells should confer this exclusivity of novel antigen expression. A successful vaccine should also induce an effective cellular or humoral immune response. Many modern vaccines use adjuvants, which enhance the response in several ways: they promote the recruitment of antigen-presenting cells; they modulate cytokines to augment the immune response; and they help to sustain antigen presentation so that memory can be developed, which results in a more vigorous immune response to the tumor vaccine.

Many of these vaccines are now engineered to interfere with immune tolerance to tumor antigens, as in the use of “knockdown” TGF β 2. TGF β 2 inhibits processes such as activation of antigen-presenting cells, natural killer (NK) cell function, and T-cell and B-cell functions. Thus, knocking down expression of TGF β 2 would augment the

activation of these components.⁴ Another example of this approach is the inhibition of CTLA4, which is the mechanism of action for ipilimumab.

A lot of research is dedicated to the identification of antigen sources that could be used for immunotherapy in NSCLC in order to present these antigens to the dendritic cell population in the human body. These include naked DNA, mRNA, viral-like particles, viral vectors, bacterial vectors, recombinant proteins, novel peptides and proteins, and tumor cells (Figure).⁵ This article will focus on recombinant proteins, peptides, and tumor cells.

Protein-based vaccines

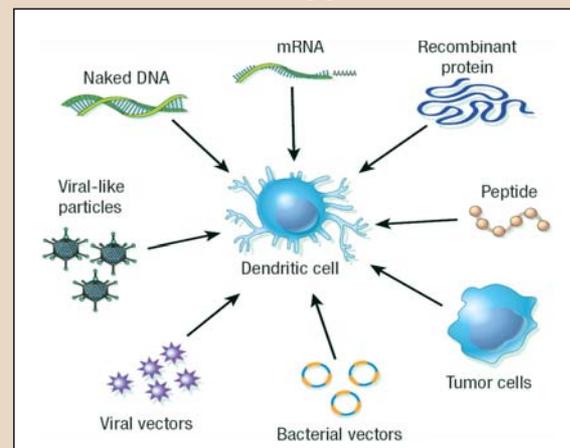
The MAGE-A3 vaccine is a protein vaccine that is currently in phase 3 trials. MAGE-A3 is an almost exclusively tumor-specific antigen. MAGE-A3 has increased expression with increasing stage of disease and is associated with a poor prognosis. In lung cancer, approximately 30% of tumors express the MAGE-A3 antigen. The MAGE-A3 vaccine is composed of purified recombinant MAGE-A3 protein fused to protein D. The vaccine incorporates a second-generation adjuvant system.⁶⁻⁹

Peptide-based vaccines

The BLP25 liposome vaccine L-BLP25 (Stimuvax, Oncothyreon) simulates the mucin MUC1.¹⁰ The normal form of MUC1 is expressed on a number of epithelial surfaces throughout the human body, particularly the oral and gastrointestinal mucosa. In most epithelial cancers, an aberrant form of MUC1 is highly expressed, rendering it an attractive therapeutic target. Stimuvax mimics an exposed core peptide that forms a part of the oncogenic protein. MUC1 is involved in cell-cell adhesion. The aberrant form of MUC1 functions as an oncogene, promoting invasion and metastasis of tumor cells and conferring resistance to genotoxic agents. These characteristics contribute to the poor prognosis associated with the aberrant form of MUC1. MUC1 can affect antitumor immune response, as it endogenously suppresses T-cell function, impairs dendritic cell function, and prevents NK cell binding.¹¹ One of the key characteristics of MUC1 that enable its role in promoting tumor growth is its strongly glycosylated extracellular region, which is known to bind growth factors. By accumulating growth factors near the tumor cell surface, MUC1 promotes cancer cell growth. Furthermore, the hydrophilic nature of the glycosylated region of MUC1 acts as a barrier to hydrophobic anticancer drugs.

MUC1 is observed in a variety of epithelial malignancies, expressed in approximately 90% of breast cancer; almost all NSCLC and nasopharyngeal cancer; about 80% of renal cell, colorectal, ovarian, and head and neck cancer;

Figure. Antigen Sources for Immunotherapy in NSCLC



A lot of research is dedicated to the identification of antigen sources that could be used for immunotherapy in NSCLC.

Source: Romero P. *Clin Lung Cancer*. 2008;9(suppl 1):S28-S36.

and in 70% to 80% of mesothelioma, gastric, prostate, and pancreatic cancers (Table, page 6). MUC1 also occurs in more than one-half of multiple myeloma and in approximately one-third of esophageal cancers.

Stimuvax contains the BLP25 lipopeptide as well as the adjuvant monophosphoryl lipid A to enhance the immune response. Liposomal components include cholesterol, dimyristoyl phosphatidylglycerol (DMPG), and dipalmitoyl phosphatidylcholine (DPPC), which have a direct effect on the immune system and also enhance targeted uptake by dendritic cells. The proposed mechanism is that Stimuvax is taken up by antigen-presenting cells, then processed and presented to both major histocompatibility complex (MHC) class I and class II cells.¹⁰ The MHC class I cells are cytotoxic T cells that are then activated and will directly target cancer cells that express the MUC1 antigen. The class II cells are helper T cells, which in turn stimulate the B-cell system and the production of endogenous antibodies. These antibodies result in antigen-dependent, cell-mediated cytotoxicity against the tumor cell population. Both of these mechanisms result in cancer cell death.

Tumor cell-based vaccines

There are a variety of tumor cell-based vaccines in development. Lucanix was derived from 4 human NSCLC cell lines, which were transfected with pCHEK/human TGFβ2 antisense (pCHEK/HBA-2), a vector containing the TGFβ2 antisense transgene, and then irradiated.¹²

The tumor cell-based TGFβ2 Antisense + rhGMCSF (TAG) vaccine was developed using a more sophisticated

Table. Overview of MUC1 Expression in Human Tumors

Tumor Type	MUC1 Expression (%)	Number of Tissues
Breast ¹	91	1,447
NSCLC ²	99	231
RCCa ³	84	133
Colorectal ⁴	81	243
Ovarian ⁵	83	63
H&N SCCa ⁶	82	29
Nasopharyngeal ⁷	100	38
Gastric ⁸	77	136
Prostate ⁹	79	89
Pancreatic ¹⁰	81	53
Mesothelioma ¹¹	75	20
Multiple myeloma ^{12,13}	59	125
Esophageal ¹⁴	32	53

Key: H&N SCC — head and neck squamous cell carcinoma; NSCLC — non-small cell lung cancer; RCC — renal cell carcinoma

Sources: 1. Rakha EA, et al. *Mod Pathol*. 2005;18:1295-1304; 2. Data on file, Merck Serono; 3. Langner C, et al. *Mod Pathol*. 2004;17:180-188; 4. Baldus SE, et al. *Histopathology*. 2002;40:440-449; 5. Chauhan SC, et al. *Mod Pathol*. 2006;19:1386-1394; 6. Croce MV, et al. *Pathol Oncol Res*. 2001;7:284-291; 7. Zhong XY, et al. *Zentralbl Pathol*. 1993;139:281-286; 8. Utsunomiya T, et al. *Clin Cancer Res*. 1998;4:2605-2614; 9. DeNardo SJ, et al. *Clin Cancer Res*. 2005;11:7187S-7194S; 10. Qu CF, et al. *Br J Cancer*. 2004;91:2086-2093; 11. Saad RS, et al. *Diagn Cytopathol*. 2005;32:156-159; 12. Pileri S, et al. *Eur J Haematol Suppl*. 1989;51:52-59; 13. Cloosen S, et al. *Br J Haematol*. 2006;135:513-516; 14. Kijima H, et al. *Anticancer Res*. 2001;21:1285-1289.

approach. The patient's autologous tumor cells are transfected with TGFβ2 antisense and recombinant human GMCSF, irradiated, and then used in the vaccination process.¹³

Much of the immune inhibitory activity of the TGFβ proteins is known to be associated with the isoform TGFβ1, which is unaffected by TGFβ2 antisense. By combining a furin RNA inhibitor with the immunostimulation provided by GMCSF, researchers were able to produce the FANG vaccine, with broad TGFβ-blocking activity as well as the ability to activate T cells.¹⁴

In summary, there are reasons for optimism in the future of NSCLC therapy. There has been recognition and

characterization of tumor-associated antigens that have a relatively high level of specificity. Antigen processing and presentation is now better understood, particularly the role of antigen-presenting cells such as dendritic cells. The role of immunomodulatory cytokines, including TGFβ2, interleukin (IL)-2, and IL-10, that are capable of augmenting the immune response has been clarified. Furthermore, the role of homeostatic controls such as suppressor T cells, CD4⁺ T cells, and CD25⁺ T cells, has been recognized. Tools have been developed during the past decade that allow the manipulation and monitoring of the immune response with greater specificity. Finally, the recent positive results of clinical trials of immunotherapeutic agents in prostate cancer and melanoma lead to an expectation of positive results in other highly refractory malignancies such as NSCLC.

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Full references are available at www.HemOncToday.com. Click on the Education Lab.

Protein Vaccines, Peptide Vaccines, and Non-vaccine Immunotherapies: Clinical Trials Update

Charles A. Butts, MD

Protein-based and peptide-based antigen vaccines are among the most promising forms of cancer vaccine therapy. There are a number of different agents in late-stage clinical trials for the treatment of non-small cell lung cancer (NSCLC) (Table 1). For example, a vaccine based on the protein melanoma-associated antigen-A3 (MAGE-A3) is now in phase 3 trials. Epidermal growth factor (EGF) is expressed in up to 85% of NSCLC tumors, and a vaccine based on this protein is in phase 2/3 clinical trials. The BLP25 peptide vaccine (Stimuvax, Oncothyreon), which is based on the MUC1 tumor antigen, is also being investigated in a phase 3 trial, while several other peptide vaccines are in phase 2, including the telomerase reverse transcriptase (TERT) subunit vaccine, which is expressed in more than 85% of NSCLC tumors.¹⁻⁵

Protein-based Vaccines

MAGE-A3

MAGE-A3 is almost exclusively a tumor-specific antigen; other than in the testes and the placenta, it is not expressed in normal cells. MAGE-A3 is associated with a poor prognosis.⁶ MAGE-A3 is associated with increasing expression with increased stage in NSCLC, and data suggest that it is overexpressed more often in squamous cell carcinoma than in adenocarcinoma.³ Importantly, because MAGE-A3 is expressed in only 30% to 50% of NSCLC,² patients must be tested for tumoral expression of the protein before inclusion in clinical trials, which is not the case for a number of other vaccines. MAGE-A3 can be detected in tumor tissue by the use of reverse transcription polymerase chain reaction (RT-PCR).

In a randomized phase 2 trial of MAGE-A3, 182 patients were randomized 2-to-1 to the vaccine or placebo.⁷ Treatment consisted of an induction phase of 5 administrations at 3-week intervals followed by a 2-year maintenance phase of 8 administrations every 3 months. The trial was conducted before the introduction of chemotherapy as standard adjuvant therapy for NSCLC, so the population consisted of MAGE-A3-positive patients with completely resected stage IB/II disease without adjuvant chemotherapy. The primary endpoint was disease-free

interval; secondary endpoints included safety, disease-free survival, and overall survival.

Although the improvement in disease-free interval of the vaccine was not statistically significant compared to placebo (hazard ratio [HR], 0.73; $P=.107$), there was a strong signal in favor of the vaccine. Improvement in disease-free survival was also not statistically significant (HR, 0.73; $P=.093$). A 27% decrease in the relative risk for lung cancer recurrence compared to surgery alone was observed. This result led to a confirmatory phase 3 trial, the MAGE-A3 as Adjuvant, Non-small Cell Lung Cancer Immunotherapy (MAGRIT) study, which is examining the

Table 1. Protein and Peptide Antigen-based Targets in Immunotherapy for NSCLC

Antigen	Expression in NSCLC	Phase of Clinical Development
Protein		
MAGE-A3	30% to 50%	3*
EGF	75% to 85%†	2/3*
Peptide		
MUC1	>60%	3*
IDM-2101‡	NA	2
p53/K-ras	NA	2
TERT subunit	>85%	2*

*Ongoing trial

† Overexpression of EGFR and its ligands

‡ Mix of carcinoembryonic antigen (CEA), p53, human embryonic growth factor receptor (HER)-2, MAGE-2, and MAGE-3 peptides

Key: EGF — epidermal growth factor; MAGE — melanoma-associated antigen; TERT — telomerase reverse transcriptase; NA — not applicable

Sources: Kakimi K, et al. *Lung Cancer*. 2009;65:1-8; Siemel W, et al. *Eur J Cardiothorac Surg*. 2004;25:131-134; Gure AD, et al. *Clin Cancer Res*. 2005;11:8055-8062; Romero P. *Clin Lung Cancer*. 2009;9(suppl 1):S28-S36; Ramos TC, et al. *Cancer Biol Ther*. 2006;5:145-149.

MAGE-A3 vaccine as adjuvant therapy.⁸ The cohort of patients eligible for the trial has been expanded to include those with resected stage IB to IIIA NSCLC, whose tumors express the MAGE-A3 protein.

The goal of this trial is to recruit more than 2,200 patients in the adjuvant setting. Platinum-based adjuvant chemotherapy is now the standard of care, so patients will receive up to 4 cycles of platinum-based chemotherapy before being randomized 2-to-1 to vaccine or placebo. There is a cohort of patients who are not receiving chemotherapy because of either disease stage or contraindications. This cohort will also be randomized to vaccine or placebo. The primary endpoint of the MAGRIT study is disease-free survival. Secondary endpoints are overall survival; lung cancer-specific survival; 2-, 3-, 4-, and 5-year disease-free survival; anti-MAGE-A3 and anti-protein D seropositivity; and safety profile and serious adverse events.

The trial has been successful in screening and accruing patients to date. MAGRIT opened in October 2007 at 580 sites globally. As of March 2011, almost 11,000 patients had been screened and nearly 10,000 tumor samples have been tested, of which approximately one-third (3,235) were MAGE-A3-positive. Almost 1,700 patients had been randomized at that time. Expectations are that the trial will complete accrual by the end of 2011, and at that time approximately 12,000 patients in the adjuvant setting will have been screened.

Peptide-based Vaccines

Stimuvax

Stimuvax is a liposomal peptide vaccine that uses as its tumor antigen the peptide backbone of the MUC1 glycoprotein. This vaccine includes the adjuvant monophosphoryl lipid A, which stimulates a non-specific immune response. The liposomal components of the vaccine facilitate immune recognition.

A randomized phase 2 trial of Stimuvax was conducted in patients with advanced NSCLC (stage IIIB/IV) who had undergone chemotherapy or chemoradiation and demonstrated a response or stable disease to their initial therapy.^{9,10} Those patients were then randomized to receive vaccine or supportive care, with the primary endpoints of survival and safety. The vaccine produced a non-significant increase in overall survival in the intent-to-treat (ITT) population (HR, 0.74; $P=.112$), although there was a stronger trend toward significance in survival in patients with localized stage IIIB disease

(HR, 0.524; $P=.069$). Many of the latter had chemoradiation as their initial therapy. In this stage IIIB patient subgroup, after nearly 5 years of follow-up, survival in the Stimuvax arm was 30.6 months vs. 13.3 months in the control arm, with a 2-year survival of 49% vs. 27%.

To further investigate the promising results observed in this study with Stimuvax in the stage III setting, the phase 3 START trial was designed to focus on this patient population.¹¹ Eligibility criteria for START include patients with unresectable stage III NSCLC who have undergone chemoradiation and are stable or responding, along with Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1. The original study design called for the recruitment of more than 1,300 patients, making this the largest stage III NSCLC trial to date. Patients are randomized 2-to-1 to vaccine or placebo. Treatment consists of 8 cycles of primary treatment followed by maintenance until disease progression. The primary endpoint is survival. Secondary endpoints are time to progression; time to symptom progression; 1-, 2-, and 3-year survival; and safety.

The START trial was interrupted in March 2010 when the FDA placed a clinical hold on Stimuvax. This was the result of a serious adverse reaction in a phase 2 trial of

Stimuvax in multiple myeloma. A patient in this study died of encephalitis. This trial was investigating the use of an intensive schedule of low-dose cyclophosphamide administered with the Stimuvax vaccine. The hold was subsequently removed in June 2010, and additional monitoring for serious adverse events was put in place in the START trial.

As a result of the delay, it was felt that patients who were in the middle of their induction phase of the vaccine, or had been within 6 months of starting in the trial, would probably not have developed a sufficient immune response to the vaccine by the time they were placed on hold. Because the delay was 3 months in duration, those patients were replaced when the trial reopened. The accrual goal was therefore increased to 1,476 patients. As of May 2011, 1,822 patients had been screened and 1,444 randomized. Screening has now been completed.

Non-vaccine Immunotherapy

Talactoferrin

Talactoferrin, a dendritic cell activator, is a recombinant form of lactoferrin, the active immune agent in human breast milk and colostrum. Oral talactoferrin is transported by M cells into the Peyer's patches of the gut-associated

In the stage IIIB patient subgroup, survival in the Stimuvax arm was 30.6 months vs. 13.3 months in the control arm.

—Charles A. Butts, MD

Table 2. Progression-free Survival and Overall Survival with Talactoferrin as First-line Chemotherapy

	Intent to Treat			Evaluable*		
	TLF/C/P	Placebo/C/P	Improvement	TLF/C/P	Placebo/C/P	Improvement
Median PFS (months)	7.0	4.2	67%	7.0	4.2	67%
Median OS (months)	10.4	8.5	22%	11.3	8.5	33%
Duration of Response† (weeks)	32.7	23.6	39%			

* Prospectively defined as patients who received at least 1 dose of study drug as well as 1 dose of C/P, and who had at least 1 CT scan after start of treatment (excludes patients who died [5] or dropped out [5] prior to the first post-treatment CT).

† Duration of response was measured from date of first occurrence of a confirmed response to date of tumor progression or death. N=38, with 23 and 15 patients in the TLF and placebo arm, respectively.

Key: C — carboplatin; P — paclitaxel; OS — overall survival; PFS — progression-free survival; TLF — talactoferrin

Source: Wang Y, et al. *J Clin Oncol*. 2006;24(18S):7095.

lymphoid tissue (GALT) — the largest immune organ in the body — where it induces migration of immature dendritic cells to the GALT and promotes their maturation.

A randomized phase 2 trial of talactoferrin (n=47) vs. placebo (n=53) evaluated overall survival in patients with refractory NSCLC.¹² Patients had failed either 1 or 2 lines of chemotherapy, at least 1 of which was platinum-based. This trial demonstrated a statistically significant improvement in survival in favor of the talactoferrin arm, both in the ITT population (HR, 0.68; $P=.0404$) and in the evaluable population (n=81; HR, 0.59; $P=.0171$).

A phase 3 trial (FORTIS-M) examining oral talactoferrin as an adjunct in prior nonresponders to treatment is now underway. The study population consists of 720 patients with stage IIIB/IV NSCLC, who have failed 2 or more prior regimens.¹³ Patients were randomized 2:1 to talactoferrin or placebo. This trial completed its accrual in March 2011.

Talactoferrin is also being investigated as first-line therapy in combination with chemotherapy.¹⁴ A phase 2 trial randomized 110 patients 1:1 to carboplatin/paclitaxel plus either talactoferrin or placebo. The primary endpoint in this trial was confirmed response rate by CT scan using Response Evaluation Criteria In Solid Tumors (RECIST). The trial met its primary endpoint; in the evaluable patient group, there was a significant benefit with treatment, with response rates of 47% vs. 29%, respectively ($P=.05$). Response rate in the ITT patients (n=110) showed a trend towards improvement in the talactoferrin arm compared to placebo, at 42% vs. 27%, respectively ($P=.08$). Patients treated with talactoferrin also had improved median progression-free survival and overall survival rates (Table 2). Patients

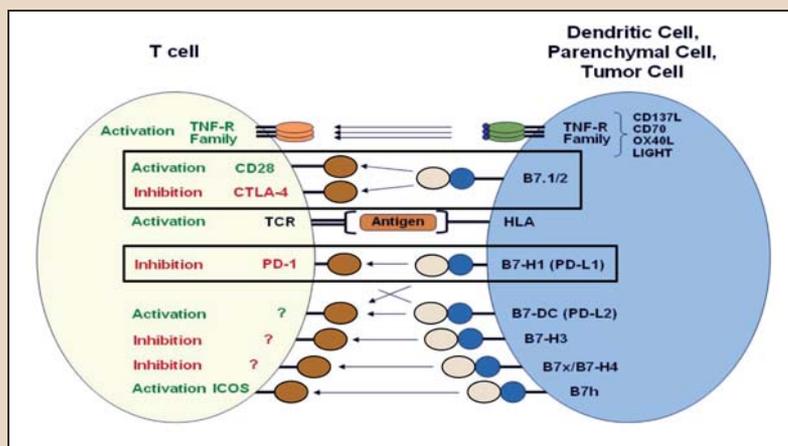
with a partial response experienced significantly improved survival rates vs. those who did not respond to treatment ($P<.01$). Talactoferrin appeared to be well-tolerated, with no drug-related serious adverse events.

To further evaluate talactoferrin in this setting, FORTIS-C, another large phase 3 trial, is currently recruiting. This will be a randomized, double-blind, placebo-controlled study of oral talactoferrin in combination with carboplatin and paclitaxel as first-line therapy in patients with locally advanced or metastatic NSCLC. The study will include 1,100 patients with previously untreated stage IIIB/IV NSCLC.

T-cell Activation/Inhibition

The vaccines that have been discussed function primarily by increasing the T-cell response to antigens. However, the interface between T cells and other cells, such as dendritic cells, is complex. In addition to molecules that promote activation when presented to the T-cell receptors, there are a number of other molecules that have an inhibitory effect on T cells (Figure, page 10). One of these molecules is cytotoxic T lymphocyte-associated antigen 4 (CTLA4). In the normal activation of T cells, the antigen is presented to the T-cell receptors by antigen-presenting cells via the major histocompatibility complex present on their surface. This process also requires co-stimulation with B7 presenting to CD28 to activate the T cells. Normally, when the T cells are activated, CTLA4 becomes expressed on the surface, and because it binds with greater affinity to B7 than does CD28, it decreases the co-stimulation and inhibits the T-cell activation, acting as a negative feedback mechanism to prevent a hyperactive immune response.

Figure. T Cell Activation/Inhibition



There are a variety of molecules that regulate T-cell function, rendering them attractive targets for immunotherapy.

Key: CTLA4 — cytotoxic T lymphocyte-associated antigen; ICOS — inducible T-cell co-stimulator; TCR — T-cell receptor; TNF-R — tumor necrosis factor receptor

Sources: Walunas TL, et al. *J Exp Med.* 1996;183(6):2541-2550; Carter L, et al. *Eur J Immunol.* 2002;32(3):634-643.

Ipilimumab

The anti-CTLA4 monoclonal antibody ipilimumab binds CTLA4 so it can no longer interact with B7, allowing B7 to continue to bind to CD28, thus preventing T-cell inactivation. Ipilimumab is recognized as a major advance in the treatment of malignant melanoma. A randomized phase 3 trial of ipilimumab with or without a vaccine in patients with advanced disease showed a significant improvement in survival, including durable responses in patients to 5 years, with no evidence of disease.¹⁵ Immune-related adverse events related to skin, gastrointestinal tract, liver, and endocrine system were observed in 60% of patients.

Ipilimumab has also shown activity against NSCLC. A randomized phase 2 trial with 3 arms compared 2 different ipilimumab regimens: ipilimumab given concurrently with carboplatin/paclitaxel chemotherapy, and a phased approach in which patients received 2 cycles of chemotherapy alone first, after which ipilimumab was added to the chemotherapy.¹⁶ The control arm consisted of chemotherapy alone. In the 2 ipilimumab arms, patients were permitted to continue on maintenance ipilimumab afterwards. The primary endpoint for the trial was immune-related progression-free survival. Both schedules showed an improvement in progression-free survival with ipilimumab; results for the phased schedule were statistically significant (HR, 0.686; $P=.026$), although the magnitude of the effect was small until about 3 months.

There was also a treatment benefit for the secondary endpoint of overall survival. In the phased ipilimumab arm, the

median survival was 12 months vs. 8 months in the control group. A phase 3 trial is planned to further explore the potential of ipilimumab in the treatment of NSCLC. This trial will recruit 800 patients with advanced or recurrent squamous NSCLC, comparing carboplatin/paclitaxel alone with carboplatin/paclitaxel plus ipilimumab in the phased approach.¹⁷

PD-1

Like CTLA4, the PD-1 receptor inhibits T-cell activity through interaction with B7 ligands, in this case PD-L1 and PD-L2. PD-1 ligands are expressed on dendritic cells, and also on some human epithelial tumor cells. Antibodies that have been developed to block PD-1 prevent the inhibitory signal from being delivered to the T cell. One

such molecule under investigation is BMS-936558, an IgG4 monoclonal antibody with no antibody-dependent or cell-dependent cytotoxicity (ADCC/CDCC) and a high affinity for PD-1. BMS-936558 blocks binding to both PD-L1 and PD-L2. BMS-936558 is in early development, but data from a phase 1 study were encouraging.¹⁸ Responses, which appeared to be durable, were detected in patients with melanoma, renal, and colorectal cancer. A mixed response was observed in a patient with NSCLC. Receptor occupancy lasted approximately 3 months at all tested doses. The serum half-life was 12 days to 20 days. Importantly, BMS-936558 was well-tolerated, unlike the CTLA4 blockers, including ipilimumab, which cause numerous significant immune-related adverse events. Common adverse events associated with BMS-936558 included rash, fatigue, lymphopenia, and arthralgia/myalgia.

A second phase 1 trial, again including patients with NSCLC, confirmed the durability and tolerability of this agent.¹⁹ Multiple doses (1, 3, and 10 mg/kg) administered at a schedule of once every 2 weeks were examined. In this trial, the maximum tolerated dose was not reached. Activity at the multiple dose levels was detected. The adverse event profile was consistent with an immunomodulatory mechanism of action. Responses again appeared to be durable. One patient with NSCLC showed a partial response, and has survived for more than 14 months at the 3 mg/kg dose in this study. Overall, 5 of 10 patients had durable stable disease at the higher doses. Some of those with stable disease had reduction in tumor burden, although they did not

meet the criteria for partial response. The stable disease in a number of these patients was very durable.

Pseudoprogession

There is a phenomenon known as pseudoprogession, or flare, which seems to be unique to immunotherapies, particularly with the CTLA4 blockers. Pseudoprogession was initially reported with ipilimumab, but was subsequently observed with other agents. In a number of studies, some patients experienced either significant increase in size of the target lesions, or development of new lesions, but eventually responded, some in a durable manner. Evidence of this was seen in response patterns in patients with melanoma treated with ipilimumab.²⁰ Some patients responded well initially, while others experienced an initial small increase in tumor, followed by a response. In another group, the target lesions increased to the point where they met RECIST criteria for progession, but subsequently showed significant improvement with time. There were also patients who developed new lesions that subsequently shrank with no further intervention.

A case of new lesions that resolved spontaneously was also observed in a phase 3 trial of belagenpumatucel-L (Bazhenova L, personal communication). A 47-year-old woman who had a complete response to initial chemotherapy with cisplatin/pemetrexed plus bevacizumab was randomized in the trial. A new lesion was detected at the first CT scan. This was a new lymph node in the para-aortic space that met the criteria for progession. A biopsy showed lymphocytes only, no malignancy. A repeat biopsy was planned, but the lesion resolved before this could be performed.

Because of the possibility of pseudoprogession, a longer time than expected may be required before an immune response is observed. Discontinuation of immunotherapy after apparent “early” disease progession may not be appropriate unless confirmed. For patients undergoing immunotherapy, it is recommended that suspected progession should be confirmed by further imaging or biopsy after 4 weeks in individuals who are not showing clinical deterioration.

Conclusions

Randomized phase 2 trials of a number of protein, peptide, and non-vaccine immunotherapies suggest that there is potential to improve survival. Furthermore, the initiation of some large randomized phase 3 trials —particularly START and MAGRIT — suggest that phase 3 clinical trials of immunotherapies on a global scale are feasible, and are supported by the oncology community. The MAGRIT trial will be the largest adjuvant trial ever to have been completed, and START is the largest trial in stage III disease.

These are significant achievements and demonstrate the interest that these agents have generated in patients and in the oncology community. Ultimately, data from the phase 3 trials over the next 1 to 2 years will determine whether these agents will enter the armamentarium of treatment for patients with NSCLC.

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Personalized Vaccine Strategies in Early-stage and Advanced-stage Non-small Cell Lung Cancer

John Nemunaitis, MD

Unlike many approaches to cancer treatment, vaccines are closely tailored to the precise mechanisms by which specific types of tumor cells interact with the immune system. Each vaccine represents a unique approach to the problem of ensuring that a strong immunogenic response is elicited by the non-self proteins contained within the tumor. Because of the safety profile observed in these agents, there is a potential to use them efficiently in combination, so as to simultaneously enhance several different aspects of the interaction between the tumor and immune system.

Development of successful vaccines is based on 3 important principles: to educate the immune system against specific tumor antigens; to stimulate the immune system, and improve its ability to effectively target the cancer — in essence, to attempt to break the tolerance that the cancer has been able to evoke in the body; and to block the inhibitors that cancer cells produce to hinder the immune response.

A large number of vaccines, both gene-based and nongene-based, are currently under investigation for use in the treatment of non-small cell lung cancer (NSCLC). This article will examine several novel gene-based vaccines that illustrate the ways in which these principles can be successfully employed to improve the visibility and vulnerability of tumor cells to our immune systems.

Granulocyte-macrophage Colony-stimulating Factor Gene Vaccine (GMCSF)

The GMCSF vaccine (GVAX, Biosante Pharmaceuticals) induces immune activation and exposes tumor antigens. A trial of this approach in prostate cancer, using allogeneic cell lines, was unsuccessful. The technique was modified for use in patients with NSCLC.¹ Autologous lung cancer cells harvested from the patients were genetically modified with an adenoviral vector (Ad-GM) to secrete human GMCSF. After irradiation, they were administered intradermally into the patient.

A phase 1/2 trial of GVAX in NSCLC was encouraging.² Tumors were harvested from 83 patients, 20 with early-stage NSCLC and 63 with advanced-stage NSCLC. Vaccines were successfully manufactured for 67 patients, of whom 43 were vaccinated. Intradermal injections were given every 2 weeks for a total of 3 to 6 vaccinations. The vaccine was well-tolerated; the most common toxicity was a local injection-site reaction (93%). Three of 33 advanced-stage patients who had already failed standard first-line therapy, and 2 of whom had bronchiolo-alveolar carcinoma, had durable complete tumor responses (lasting 6, 18, and 22 months). Many patients treated with the vaccine survived for longer periods than would otherwise have been expected. There appeared to be a vaccine dose-related survival advantage:

longer survival was observed in patients receiving vaccines secreting GMCSF at more than 40 ng/24 hour per 10⁶ cells (median survival, 17 months; 95% confidence interval [CI], 6-23)

than in patients receiving vaccines secreting less GMCSF (median survival, 7 months; 95% CI, 4-10; $P=.028$). Assays on a subset of these patients appeared to show a correlation with immune function. However, the majority of patients did not experience durable responses.

Belagenpumatucel-L

Belagenpumatucel-L (Lucanix, NovaRx) blocks the action of transforming growth factor β 2 (TGF β 2), a potent immune response inhibitor produced by some lung cancer cells.³ The TGF β proteins are involved in the growth and regulation of normal cells. However, in cancer, they inhibit a variety of functions that the immune system uses to attack tumor cells, thus protecting the tumor. Elevated levels of TGF β 2 are inversely correlated with prognosis in NSCLC. The actions of TGF β 2 include:

- inhibition of activation of T cells and B cells;
- inhibition of the activation of antigen-presenting cells;
- inhibition of natural killer (NK) cell function;

Vaccines are closely tailored to the precise mechanisms by which specific types of tumor cells interact with the immune system.

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- inhibition of γ -interferon production by immune effector cells;
- induction of FoxP3 expression and the generation of T regulatory cells.

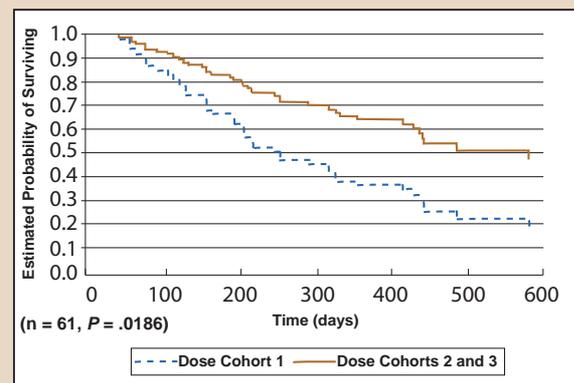
Lucanix is a nonviral gene-based vaccine. This vaccine is synthesized by incorporating a TGF β 2 antisense gene into a pool of allogeneic tumor cells.³ The use of allogeneic cell lines avoids some of the limitations and difficulties of autologous vaccine therapy, including the necessity of harvesting adequate quantities of tumor tissue, difficulties in producing reliable transfection, and long delays between harvesting and vaccine treatment.

A randomized phase 2 trial of Lucanix examined 3 different doses, 1.25×10^7 cells/injection, 2.5×10^7 cells/injection, and 5.0×10^7 cells/injection.⁴ The dose was administered as an intradermal injection once per month for 4 months, then once a month or every other month for a total of 12 months. Samples were studied for immune function analysis. The majority of the 75 patients in the study had non-resectable stage III or IV disease. An average of 7 vaccinations were administered to each patient. The vaccine was well-tolerated. Of 40 patients with measurable disease, 5 (13%) had a radiographic response. An immune response occurred in many patients, which correlated with lack of disease progression, and there was a dose-related effect on overall survival; patients in the 2 higher dose cohorts had a significantly longer survival time than those on the lower dose ($P = .0186$; Figure). There was also a correlation between immune response and survival in the study patients. Efforts are continuing to characterize patients who are likely to be more responsive to this vaccine, either initially or during the course of treatment.

Phase 2 data for both GVAX and Lucanix compare favorably with existing second-line chemotherapy or EGF receptor inhibitor therapy in patients with lung cancer, with positive 1-year survival rates and overall survival duration (Table 1, page 14).^{2,4,5-7}

A phase 3 trial of Lucanix (STOP) is now recruiting, with 231 of the projected 700 patients accrued as of June 2011.⁸ The multicenter trial involves 92 sites. Eligibility criteria include stage IIIB/IV NSCLC, with response or stable disease following first-line chemotherapy or chemoradiotherapy. Patients with a response are randomized to Lucanix plus best supportive care or best supportive care alone.

Figure. Lucanix: Overall Survival Dose Response



A dose-dependent effect on overall survival was observed in advanced-stage patients receiving 3 different doses of Lucanix.

Dose Cohort 1: 1.25×10^7 cells/injection; Dose Cohort 2: 2.5×10^7 cells/injection; Dose Cohort 3: 5×10^7 cells/injection

Source: Nemunaitis J, et al. *J Clin Oncol*. 2006;24:4721-4730. Reprinted with permission. © 2008 American Society of Clinical Oncology. All rights reserved.

TAG

The successes in activating the immune system and blocking inhibitors with separate vaccines raises the question of whether both effects can be combined in a single vaccine. Although there were initial concerns about overstimulating the immune system, researchers have developed a vaccine that essentially combines the mechanisms employed in GVAX and Lucanix. The TGF β 2 Antisense + rhGMCSF tumor-associated glycoprotein (TAG) vaccine uses an expression

plasmid that coexpresses GMCSF and TGF β 2 antisense nucleotide sequences, incorporated into autologous tumor tissue.^{9,10}

The phase 1 trial of TAG recruited 38 patients, of whom 22 were treated. Two of these patients had NSCLC.⁹ Patients were in-

fused with either 1×10^7 (n=7) or 2.5×10^7 (n=15) cells. The median number of vials constructed per patient was 11 (range 7 to 26). Cell viability was 79% to 99% (median 92%). Median GMCSF expression was 394 pg/million cells. Median TGF β 2 knockdown was 54%, and there was minimal TGF β 1 inhibition, as expected. There was little evidence of adverse events, apart from injection site pain.

Stable disease of 3 or more months' duration was observed in 15 patients, and appeared to be quite prolonged in a follow-up study. One complete response occurred in a patient with stage IVa malignant melanoma, and there was a partial response in another individual, while 2 patients

The successes of activating the immune system and blocking inhibitors with separate vaccines raises the question of whether both effects can be combined in a single vaccine.

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Table 1. Therapeutic Options of ≥ 1 Prior Treatment for NSCLC

Agent	n	Response	Survival (months)	1-year Survival
Docetaxel	104	6% PR	7	29%
Placebo	100	0	5	19%
Docetaxel	250	9% PR	6	26%
Gemcitabine	124	0–19% PR	7.9	–
Erlotinib ¹	427	8% PR, 1% CR	6.7	28%
Placebo ¹	211	1% PR	4.7	18%
Pemetrexed ²	265	9% PR	8.3	30%
Docetaxel ²	276	9% PR	7.9	30%
GVAX ³	33	9% CR	12	44%
Lucanix ⁴	40	13% PR	15.9	62%
Lucanix ⁵	21	0% PR	15.5	72%

Key: CR — complete response; GMCSF — granulocyte-macrophage colony-stimulating factor; PR — partial response

Sources: 1. Shepherd FA, et al. *N Engl J Med.* 2005;353:123-132; 2. Hanna N, et al. *J Clin Oncol.* 2004;22:1589-1597; 3. Nemunaitis J, et al. *J Natl Cancer Inst.* 2004;96:326-331; 4. Nemunaitis J, et al. *J Clin Oncol.* 2006;24:4721-4730; 5. Nemunaitis J, et al. *Cancer Gene Ther.* 2009;168:620-624.

Table 2. FANG Vaccine/TAG Vaccine Characterization/Comparison [Mean \pm SD] — All Patients

Parameter	TAG	FANG
1.0 or 2.5 x 10 ⁷ cells	16/22	15/15
Doses manufactured per patient	9 (range 1-29)	9 (range 1-18)
Cell viability (%)	92.0 \pm 5.4	94.0 \pm 4.0
GMCSF expression (pg/million cells)	1,324.8 \pm 1,964.8	927.3 \pm 1,481.0
TGFB2 \downarrow (%)	69.3 \pm 29.3	94 \pm 0.13
TGFB1 \downarrow (%)	15 \pm 18	92 \pm 0.13

Key: GMCSF — granulocyte-macrophage colony-stimulating factor; TGF — transforming growth factor

Sources: Olivares J, et al. *Clin Can Res.* 2011;17(1):183-192; Trial of bi-shRNA-furin and granulocyte macrophage colony stimulating factor (GMCSF) augmented autologous tumor cell vaccine for advanced cancer (FANG). <http://www.clinicaltrials.gov/ct2/show/NCT01061840>. Accessed August 11, 2011.

progressed. Three of the patients were not evaluable. Median survival to that time was 465 days, in a population with a life expectancy of between 4 months and 6 months at baseline. Nine patients remained alive as of June 2011, and follow-up continues. There was a correlation in this study between immune response, as determined by

ELISPOT results showing activated T-cell expression, and response to TAG. The immune response appeared to increase from day 0. An immune response was not observed in patients who did not demonstrate prolonged survival or stable disease.

To summarize the findings with TAG, no additional toxicity was observed with the combination of the 2 components in 1 vector. This suggests that it may be possible to combine vaccines as well as separate therapeutics in the same patient. There was evidence of anti-tumor activity with this vaccine. The GMCSF expression appeared to be consistent with GVAX, and the TGF β 2 activity was comparable to the Lucanix level of knockdown.

Furin Vaccine

The enzyme furin is responsible for lysing the pro-TGF β molecule into TGF β 1, β 2, and β 3.¹¹ Researchers constructed an RNA interference molecule (RNAi) that prevented furin expression in tumor cells, and as a consequence all TGF β s were knocked down to a level of 90% to 95% (Table 2).

This RNAi was then placed into a vector with GMCSF and used to create adjuvant bi-shRNA furin and GMCSF augmented autologous tumor cell vaccine (FANG, Gradalis, Inc). A phase 1 study was initiated with 45 patients initially involved in this ongoing trial.¹² The dosage regimen

was 1×10^7 vs. 2.5×10^7 cells/intradermal injection, once per month for 12 months. Results are pending completion of the phase 1 trial.

Summary

The evidence obtained thus far from clinical trials of cancer immunotherapy clearly shows that vaccines vary widely in their effectiveness for individual patients. One of the challenges that researchers face is identifying markers that will predict the susceptibility of a given patient's tumor cells to the treatment. TG4010 is a novel interleukin (IL)-2, MUC1 gene-based vaccine largely developed in Europe that is entering phase 2/3 lung cancer trials in the United States, focusing on stage IV patients. The activity of this vaccine correlates significantly with the patient's levels of NK cells, thus NK cell levels appear to pre-identify patients that are more sensitive to the vaccine. A laboratory test is under development to aid in patient selection.

In conclusion, vaccine therapy is already a standard of care in prostate cancer and melanoma. Early studies have demonstrated the safety of this approach in NSCLC, as well as preliminary evidence of efficacy. Vaccination is therefore likely to find a role in the treatment of NSCLC as data from the ongoing phase 2 and 3 trials become available.

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DISCUSSION

Would you simultaneously initiate vaccine and non-vaccine immunotherapy?

Ronald B. Natale, MD: Development of the vaccine therapy and of the non-vaccine therapy are currently processes of “one step at a time.” However, I believe that in the future there certainly will be a role for combining different approaches in combination immunotherapy trials. The first step is to develop each of the components of what will comprise those combinations downstream.

What is the role of chemotherapy with immunotherapy? Is there a concern that if you have a patient who has had a great deal of chemotherapy, they may fail to mount an immune response?

Natale: The way the current clinical trials are designed, I think it is rational to use a sequential approach. Early on in the development it is going to be important to look at the contribution of the immunomodulatory agent or the vaccine separately from chemotherapy. There may be a possibility at some time in the future to begin to combine them, although it probably will require increased understanding of the impact of chemotherapy on the immune system that we are trying to manipulate through the use of the immunomodulatory proteins or the vaccines.

What tissue is needed to test for MAGE expression on the tumor and is this test validated?

Charles A. Butts, MD: For the MAGRIT trial at least, there is a central laboratory that conducts the assessments, and I believe it is a reverse transcription polymerase chain reaction (RT-PCR) technique utilized to measure MAGE-A3, which is performed on the tumor cells themselves. In fact, there have been reports of circulating tumor cells, or bone marrow-detected tumor cells that have been MAGE-A3-positive, so it can be detected even at small sample size. But, for the study, it is the primary tumor or lymph nodes that are assessed using RT-PCR.

What is the histologic pathology of pseudoprogression and the mechanism?

Butts: I have seen a number of patients who actually have developed new lesions, some

quite large, intraperitoneally or in the lung, who have gone on to have a biopsy. One patient in particular had resection of a mesenteric lesion; it turned out to be a large lymph node with many lymphocytes, and showed no evidence of cancer. Biopsies of lung lesions have shown the same — lymphocytic infiltration. Some lesions may be true progression, but we should not jump to that conclusion. If the patient is well and does not have new symptoms, it is reasonable to follow up to see if the lesions actually will reduce over time.

What is your opinion concerning prophylactic vaccination studies in patients identified to be at increased risk for developing disease (ie, patients with genetic, environmental, or habitual risk factors)?

Butts: I know there have been phase 1 trials examining patients with non-invasive disease, but I am not as familiar with those. Prophylactic vaccination is a novel concept and a long way from clinical use.

What will differentiate the patients who will respond to immune intervention from those that will not?

Butts: All of the phase 3 trials include immunological marker studies or biomarker studies as a substudy within them. So, in addition to the results of START and the MAGRIT trial, we hope to learn more of exactly that — which patients respond. Is it human leukocyte-antigen (HLA)-specific to certain vaccines? Certain HLA types that respond? Are there other immune markers that can predict response?

There is another MUC1 vaccine using a Vaccinia virus for which preliminary trials suggest that patients who had high levels of activated natural killer cells in their blood may actually respond poorly to immune stimulus.

John Nemunaitis, MD: A lot of the assays used in the immunology field were developed years ago, and they are cellular-focused. We actually have found some evidence that looking at K cells is helpful in correlating with response. However, it is still a difficult surrogate parameter that we have to work with in immunology. We cannot yet identify the proper patients to enter into an immune trial, and this identification will vary from technology to technology as well.

Roy S. Herbst, MD, PhD: The problem is, where do you evaluate the markers? In the blood? Is that really what is important, or is it what is happening in the tumor micro-environment, which is more difficult to assess, that is really important?

Natale: I think that the difficulty we have had in identifying targets in squamous cell cancer gives us the opportunity to examine that important subset of patients with lung cancer. Interestingly, some of these antigens may be more strongly or more commonly expressed in patients with squamous cell cancer. I think immunological marker studies must be approached with an open mind and it should be taken into account that none of these approaches are mutually exclusive, but in the initial phases they must be developed individually.

Butts: We will learn somewhat retrospectively whether these agents may be more active in squamous vs. adenocarcinoma.

Did you use steroids as antiemetic therapy in randomized trials?

Butts: Steroids were allowed in all of these trials. In the original phase 2b trial in advanced-stage disease with Stimuvax, steroid premedication was allowed, and in the START trial steroid medication was allowed as an antiemetic. Steroids are also permitted for short-course treatment of patients who develop radiation pneumonitis; these are patients with stage III disease who have had chemotherapy and radiation. However, patients who require steroids for underlying disease or an immune problem were ineligible for the trials.

Have you seen any evidence of a repeat of encephalitis or any autoimmune problem since the clinical hold on Stimuvax?

Butts: I have not heard anything. I have seen all the assays. For the START trial, there was an interim analysis at 50% of the events that was reviewed by the Data and Safety Monitoring Board, who advised to continue the trial without any changes. A second planned interim analysis is expected to occur late in 2011.

DISCUSSION

With ipilimumab response rates relatively low, what are the response rates for PD-1?

Butts: The PD-1 trials are only phase 1. In the second phase 1 trial, there was 1 partial response and 5 stable diseases out of 11 patients.

Herbst: The Lung Group at Yale have treated a large number of patients on the expansion, and have 6 or 7 really good responses in squamous cell lung cancer. The group is small, but I think this may be an agent that goes forward in some trials.

Butts: In addition, I think the responses have been durable. The stable diseases included some lesions that had significant reduction; they just did not meet the criteria for partial response. That stable disease has been durable for many months in patients with lung cancer in a phase 1 trial in whom you would not expect to have durable stable disease for very long.

Natale: I think PD-1 is promising for the future.

Instead of autologous transduced tumor, why not make a mix of the shelf-transduced lung tumor lines, which would be easier to administer, quality-control, and prepare?

Nemunaitus: From a commercial standpoint, that would be the easier, more convenient way. The reason for the autologous tissue is that when you take the tissue out, you know that the antigens in that tissue are representative of the rest of the disease in that particular patient, which may not be the case with allogeneic cell lines.

If we make a separate vaccine for every patient, would a separate incubator be needed for every patient sample in a facility?

Nemunaitus: No. When the tissue comes in, it can be lysed and put into a single-cell suspension in an approximate 2-hour timeframe. Then it is placed in a flask where the gene is electroporated into the tumor tissue and incubated overnight. The next morning, samples are analyzed for the gene expression and any contaminants. Then, the samples are aliquoted and frozen. So, the process takes about

2 days, because time is needed for the gene transfer to work.

Is it ever going to be feasible to make a vaccine in this way?

Nemunaitus: We are working on constructing a “tissue disassociater,” where you core the blocks of large tumor tissue into equal-sized components, and from there the process I just described, which is currently performed by hand, is automated.

How much tissue is needed?

Nemunaitus: The amount of tissue has to be a little bit larger than a large marble, or just a little bit smaller than a golf ball.

Are there differences in sites of metastasis in terms of response?

Butts: Many of the trials looked at survival rather than response. There have been other immune agents that have shown response at multiple sites. In some of the trials, a good response in some areas was observed, while progression was detected in other areas. However, it is hard to say with certainty whether 1 site predominates in terms of response.

How are the TGF β antisense transcripts able to block TGF β in tumor cells remote from the injection site?

Nemunaitus: The vaccine is local, and the gene transfers into that local vaccination site. Once the immune system recognizes the tumor antigens that are placed locally and it is able to access the antigens without interference from the tumor inhibitors being produced by those vaccine cells, the T-cell system becomes activated. Those activated T cells are then able to penetrate the immunologic barriers of metastatic disease sites, which is demonstrated by some of the responses and some of the survival curves that we have been seeing in the patients.

Given that GVAX seems to have failed in prostate cancer, what is your rationale to believe that this approach may work in lung cancer?

Nemunaitus: There is not a clear answer on why the phase 3 trial was negative. The data

that led to the phase 3 trial was a prostate-stabilization antigen (PSA) improvement in stabilization during a prolonged period of time. Although these data are encouraging, there was not a statistically significant survival advantage in the phase 2 data. There were not any partial or complete responses in these patients. Furthermore, allogeneic cell lines were used, so there is a possibility that the antigens in these cell lines were not as well-represented in the patient population.

What is more important: overall survival, hazard ratio, or median survival? How does durability of response fit in?

Natale: Overall survival is the primary endpoint that should be achieved. The question is how that should be measured. Median survival has been used, but that is just 1 point in the overall survival curve. The advantage of hazard ratio is that it measures the differences in survival of the various arms of the study. In terms of durability, although response rates remain an important parameter to examine in clinical trials, the more important parameters are the survival outcomes.

When you give ipilimumab, do the patients have excessive response to infections? If not, why not?

Butts: Infections really have not seemed to be a significant problem, possibly because ipilimumab specifically targets the T cells, so other innate immune cells, macrophages and leukocytes, are still functioning normally.

Nemunaitus: We have treated many patients and have not experienced any problems with infections. However, it is still important to educate the patient to be aware of this possibility, as it can occur at any time within a 12-week period. Patients will respond to steroids, but if the problem is not handled it could be fatal.

Are there any trials examining the combination of vaccines that mount immune response and VEGF or EGFR treatments?

Butts: Yes. There is a trial combining the Stimuvax with bevacizumab, a VEGF inhibitor.

CME Registration Form

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PRETEST

1	2	3	4

POSTTEST

1	2	3	4	5	6	7	8	9	10

Time spent on this activity: Hours ____ Minutes ____
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EVALUATION (must be completed for your CME Quiz to be scored)

Using the scale below, circle the number that corresponds with your opinion for each item.

1 = Poor; 2 = Fair; 3 = Good; 4 = Very Good; 5 = Excellent

1. Rate the clinical usefulness of the monograph to your daily practice. 1 2 3 4 5
2. Rate the effectiveness of the teaching/learning methods. 1 2 3 4 5

Using the scale below, circle the number that corresponds with your opinion for each item.

1 = Strongly agree 2 = Agree 3 = No opinion 4 = Disagree 5 = Strongly disagree

3. The activity was presented objectively and was free of commercial bias. [Please use the "additional comments" field below to provide further information.] 1 2 3 4 5

Additional comments regarding bias: _____

4. I plan to make the following changes to my practice:

1 = Very committed	2 = Somewhat committed	3 = Not very committed	4 = Do not expect to change practice	5 = I already do this in my practice

Other - *Please explain:*

5. These are the barriers I face in my current practice setting that may impact patient outcomes:

1 = Strongly agree	2 = Agree	3 = No opinion	4 = Disagree	5 = Strongly disagree

Other - *Please explain:*

6. This activity supported achievement of each of the learning objectives. 1 2 3 4 5

Please explain:

7. I see this percent of patients per month with NSCLC:

- A. < 10%
- B. 10% to 25%
- C. 25% to 50%
- D. > 50%

8. Please list CE/CME topics that would be of value to you.

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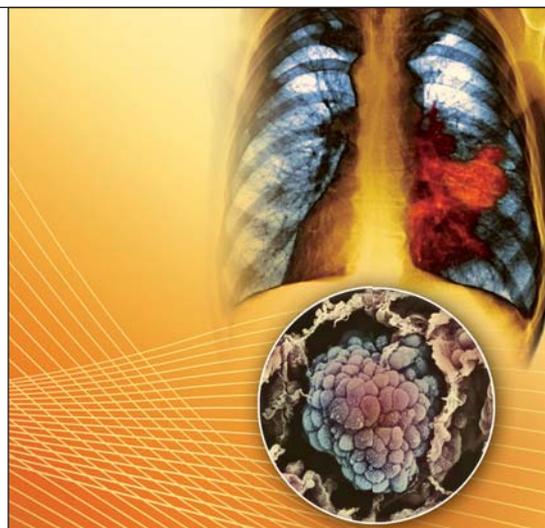
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CME Posttest

- Which of the following is a cellular-based vaccine derived from the patient's own tumor cells?**
 - Talactoferrin
 - Stimuvax
 - Lucanix
 - TAG
- Select the correct statement regarding TGF β 2:**
 - TGF β 2 stimulates the activation of T cells.
 - TGF β 2 stimulates natural killer cell function.
 - TGF β 2 inhibits activation of antigen-presenting cells.
 - TGF β 2 has no effect on B cells.
- Anticancer immunotherapy is based on the concept that:**
 - Healthy and cancerous cells generate identical surface antigens.
 - Mutated cancer cells express "non-self" surface antigens.
 - The human immune system is incapable of tumor cell cytolysis.
 - Cancer cells are incapable of generating surface antigens.
- Regarding the biology of MUC1 cancer mucin:**
 - MUC1 cancer mucin has a hydrophobic extracellular region.
 - MUC1 cancer mucin confers resistance to genotoxic agents.
 - MUC1 cancer mucin stimulates T-cell function.
 - MUC1 cancer mucin stimulates NK cell-binding.
- Which vaccine has both an activation and an anti-inhibitory effect on T cells?**
 - FANG
 - Lucanix
 - GVAX
 - Ipilimumab
- Which of the following statements is true regarding the phase 2 trial of MAGE-A3?**
 - Statistically significant improvements in the primary endpoint were observed in the vaccine group.
 - A statistically significant improvement in disease-free survival was observed in the vaccine group.
 - A 27% reduction in lung cancer recurrence was observed in the vaccine group.
 - Testing tumors for expression of MAGE-A3 was not necessary, as this protein is ubiquitously expressed in NSCLC.
- The phase 2 studies of talactoferrin demonstrated that:**
 - Talactoferrin significantly improved survival in patients with refractory NSCLC who had failed chemotherapy.
 - Talactoferrin was ineffective as a first-line agent.
 - The frequency of serious adverse events are a concern.
 - Talactoferrin was effective as a first-line and second-line agent, although the results were not statistically significant.
- Which of the following is true regarding ipilimumab?**
 - Ipilimumab mimics CTLA4 and stimulates the immune system.
 - Results of a phase 2 trial demonstrated that when ipilimumab was administered in addition to chemotherapy in a phased approach, a statistically significant improvement in progression-free survival was observed.
 - Results of a phase 2 trial demonstrated that when ipilimumab and chemotherapy were administered concurrently throughout treatment, a statistically significant improvement in progression-free survival was observed.
 - Ipilimumab had no effect on overall survival.
- Results of studies examining Lucanix demonstrated that:**
 - An immune response correlated with lack of disease progression.
 - TGF β 1 expression was significantly suppressed.
 - Survival was similar among all doses examined.
 - Efficacy of Lucanix did not compare favorably with existing second-line chemotherapy.
- Which of the following statements is true regarding GVAX?**
 - The majority of patients experienced durable responses in a phase 1/2 trial.
 - No correlation with immune function was observed.
 - Allogeneic cell lines were used to produce the vaccine in the trial of patients with NSCLC.
 - Longer survival was observed in patients receiving vaccines that secreted higher amounts of GM-CSF.

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