Introduction
Lung cancer remains the most common cause of cancer-related death in the United States. Based on estimates published by the Surveillance, Epidemiology, and End Results (SEER) database, 160,340 American men and women died of a malignancy of the lung or bronchus in 2012. Deaths attributable to lung cancer accounted for 29% of all male-related cancer mortality and 26% of all female-related mortality in 2012. At best, patients with localized disease have a 5-year survival rate of 52.2%, whereas patients with metastatic disease have a 5-year survival rate of only 3.7%. These survival rates include patients with both small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Although significant advances have been made in the treatment of NSCLC with the use of molecular targeted therapies such as erlotinib and crizotinib, median overall survival (OS) for patients with advanced NSCLC (stage IIIB or IV) treated with epidermal growth factor receptor (EGFR)-targeted therapy averages only 24 months. To make significant improvements in median OS and progression-free survival (PFS) for both SCLC and NSCLC, novel therapies are needed.

Immunotherapeutics can be defined as a broad class of therapies designed to elicit immune-mediated responses. Several types of lung cancer may respond to immunotherapeutic approaches.
destruction of tumor cells. In some circumstances, antitumor responses can be generated by administering a vaccine containing a unique allogeneic tumor antigen to individuals in order to stimulate humoral immune response against the specific antigen. At least four phase III clinical trials using this approach for the treatment of NSCLC are underway.4-6 A second approach to immunotherapy involves the use of immunomodulators, which stimulate innate and cell-mediated antitumor effects. For example, talactoferrin (TLF) is a protein that modulates innate and cell-mediated antitumor responses through its interaction with immature dendritic cells in bowel mucosa.7 Autologous cellular therapies with a design similar to sipuleucel-T for the treatment of castrate-resistant metastatic prostate cancer are in progress for the treatment of NSCLC.8,9 Lastly, monoclonal antibodies such as ipilimumab directed against inhibitory signals on activated T cells have shown promise in inducing objective responses in patients with advanced, previously treated NSCLC.10-12

This article reviews recent clinical trials in NSCLC and SCLC treatments that utilize direct antigen-mediated cell death through vaccines, cellular therapies, immunomodulator molecules, and monoclonal antibodies to induce antitumor effects. Additionally, ongoing research efforts at our center to utilize cellular-based therapies for the treatment of both NSCLC and SCLC are highlighted.

### Non-Small Cell Lung Cancer

The identification of unique antigens present on malignant cells has led to significant interest in vaccine development, which can target malignant cells for immune-mediated destruction. This section reviews recent studies of allogeneic cancer vaccines that are the subject of ongoing phase III clinical trials: BLP-25 anti-MUC1, TG4010 (modified virus of Ankara [MVA]–mucin 1 [MUC1]–interleukin 2 [IL-2]), CIMAvax EGF, and the melanoma antigen encoding gene A3 (MAGE-A3). The autologous therapeutic belagenpumatucel-L (BGPT-L), a cellular therapy directed against transforming growth factor β2 (TGF-β2) expression, has shown promise in early clinical trials and is the subject of a current phase III clinical trial.8 The immunomodulator protein TLF was the subject of two recently published phase II clinical trials, one of which demonstrated a statistically significant benefit in OS when TLF was combined with a standard platinum doublet in first-line chemotherapy.13

**Table. — Summary of Non-Small Cell Lung Cancer Immunotherapy Trials**

<table>
<thead>
<tr>
<th>Immunotherapy</th>
<th>Immune Target</th>
<th>No. of Patients</th>
<th>Stage</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLP-25</td>
<td>MUC1</td>
<td>171</td>
<td>IIIB/IV after first-line therapy</td>
<td>3-yr OS 31% for BLP-25 and 17% for BSC (P = .035)14,15</td>
</tr>
<tr>
<td>TG4010</td>
<td>MUC1</td>
<td>65</td>
<td>IIIB/IV with first-line chemotherapy</td>
<td>MS 12.7 mos for TG4010 with concurrent chemotherapy and 14.9 mos for TG4010 with sequential chemotherapy16</td>
</tr>
<tr>
<td>TG4010</td>
<td>MUC1</td>
<td>148</td>
<td>IIIB/IV with first-line chemotherapy</td>
<td>6-mo PFS 43.2% for TG4010 and 35.1% for chemotherapy alone (P = .307)6</td>
</tr>
<tr>
<td>CIMAvax EGF</td>
<td>Epidermal growth factor</td>
<td>80</td>
<td>IIIB/IV after first-line chemotherapy</td>
<td>MS 11.7 mos with GAR and 3.6 mos with PAR17</td>
</tr>
<tr>
<td>MAGE-A3</td>
<td>MAGE-A3</td>
<td>182</td>
<td>IB/II after complete resection</td>
<td>HR for PFS .73 (95% CI: 0.45–1.16) with MAGE-A3 compared with standard of care18</td>
</tr>
<tr>
<td>Belagenpumatucel-L</td>
<td>TGF-β2</td>
<td>75</td>
<td>II/IIIA/IIIB/IV after completion of therapy</td>
<td>MS 581 days with high vaccine doses and 252 days with low vaccine doses (P = .0186)6</td>
</tr>
<tr>
<td>Talactoferrin</td>
<td>Nonspecific</td>
<td>110</td>
<td>IIIB/IV in combination with first-line chemotherapy</td>
<td>RR 47% with TLF and 29% with placebo13 (P = .05)</td>
</tr>
<tr>
<td>Talactoferrin</td>
<td>Nonspecific</td>
<td>100</td>
<td>IIIB/IV in combination with first-line chemotherapy</td>
<td>OS 3.7 mos with BSC and 6.1 mos with talactoferrin19 (P = .04)</td>
</tr>
<tr>
<td>Ipilimumab</td>
<td>CTLA-4</td>
<td>204</td>
<td>IIIB/IV or recurrent disease in combination with first-line chemotherapy</td>
<td>Immune-related PFS 5.7 mos for ipilimumab + chemotherapy vs 4.6 mos for placebo + chemotherapy (HR = .72; P = .05)11</td>
</tr>
<tr>
<td>BMS-936558</td>
<td>PD-1</td>
<td>296 (122 with NSCLC)</td>
<td>IV after completion of first-line chemotherapy</td>
<td>OR in 6 of 18 with squamous cell histology and 7 of 56 with nonsquamous cell histology12</td>
</tr>
<tr>
<td>BMS-936559</td>
<td>PD-L1</td>
<td>207 (75 with NSCLC)</td>
<td>IV after completion of first-line chemotherapy</td>
<td>OR in 4 of 49 with nonsquamous cell histology and 1 of 49 with squamous cell histology10</td>
</tr>
</tbody>
</table>

BSC = best supportive care, GAR = good antibody response, HR = hazard ratio, MS = median survival, NSCLC = non-small cell lung cancer, OR = objective response, OS = overall survival, PAR = poor antibody response, PFS = progression-free survival, RR = response rate.
humanized anti–CTLA-4 monoclonal antibody, as well as BMS-936558, an anti–PD-1 monoclonal antibody, and BMS-936559, an anti–PD-L1 monoclonal antibody, have shown promise in early clinical trials to augment T-cell–mediated antitumor responses in NSCLC (Table). In addition, the results of a phase III clinical trial that studied the Toll-like receptor 9 (TLR-9) agonist PF-3512676 were recently published and shown to have no effect on OS while increasing toxicity when combined with chemotherapy. This study by Hirsh et al is not reviewed in this article.

**Allogeneic Cancer Vaccines**

**BLP-25 Anti-MUC1 Vaccine**

Mucin 1 is a glycoprotein that facilitates cellular adhesion via ICAM-1 and is expressed by many types of epithelial tissue. When expressed by malignant cells, MUC1 has been shown to differ structurally from its nonmalignant counterpart. BLP-25 is a liposomal vaccine preparation that targets the exposed peptide core of MUC1 expressed by malignant cells. In a preclinical study by Samuel et al, liposomal MUC1 successfully generated measurable anti-MUC1 T-cell proliferation, anti-MUC1 antibodies, and interferon-γ to suggest a T helper 1 (Th1)-mediated immune response in mice. BLP-25 has been studied in all phases of trial development.

Palmer et al conducted a phase I study that demonstrated the safety of BLP-25 administration in patients with advanced NSCLC (stage IIIB or IV). The vaccine was delivered via two subcutaneous injections to the upper arm 3 days after infusion of cyclophosphamide 300 mg/m² (given to inhibit suppressor T cells). Toxicity was graded according to the Cancer and Leukemia Group B (CALGB) Expanded Common Toxicity Criteria. The most common reactions were grades 1–2 including injection-site erythema (9 patients), grades 1–2 liver enzyme elevations (6 patients), myalgia/arthralgia (5 patients), and fatigue (5 patients). One patient from each of the two dose arms developed grade 3 lymphopenia. The study also quantified cytotoxic T-cell responses using a 51Cr-release assay. Of 12 patients, 5 demonstrated in vitro cytotoxic T-cell responses when exposed to MUC1 antigen. However, none demonstrated a detectable humoral response. The median OS between the two dose arms was not statistically significant due to the underpowered nature of the analysis but favored the group receiving the highest vaccine dose. At week 13, 4 patients achieved stable disease and were eligible to receive additional vaccine treatments on a compassionate basis, whereas the remaining 8 patients showed evidence of disease progression.

Building on the data from Palmer et al, a phase IIIB study was conducted across 17 sites in the United Kingdom and Canada and published in 2005. This study, conducted by Butts et al, randomized 171 patients with stage IIIB or IV NSCLC who experienced “stable disease or an objective response” at the conclusion of first-line chemotherapy to receive best supportive care (BSC) or the BLP-25 vaccine. BLP-25 was administered in a fashion similar to that in the phase I study, with four subcutaneous injections given 3 days after cyclophosphamide 300 mg/m² was administered. Study participants with stage IIIB disease were further stratified by the presence of locoregional disease or malignant pleural effusions based on the American Joint Committee on Cancer (AJCC 6th edition). The primary endpoint was survival measured from the day of randomization to the date of death. After a median follow-up of 26 months, the median OS for patients who received BLP-25 did not differ statistically from that for patients who received BSC (17.4 months and 13 months, respectively; hazard ratio [HR] = .739; 95% confidence interval [CI]: 0.509–1.073; P = .112). For patients with locoregional disease (n = 65), the OS approached statistical significance, with a 2-year survival rate of 60% in the BLP-25 arm and 36.7% in the BSC group. The median OS in the locoregional BLP-25 group was not reached at the conclusion of the initial study. Cytotoxic T-lymphocyte responses were measured in patients receiving the BLP-25 vaccine. Of 78 patients, 16 demonstrated a T-cell response to in vitro MUC1 antigen exposure. The median OS for those patients with a T-cell response was 27.6 months compared with 16.7 months in patients without a measurable T-cell response (statistics not performed).

Butts et al recently published updated data from their original study. After 2 years of additional follow-up, the median OS remained statistically nonsignificant (17.2 months in the BLP-25 group and 13.0 months in the BSC group; HR = .745; 95% CI: 0.533–1.042). However, 3-year survival rates did reach clinical significance (31% for BLP-25 and 17% for BSC; P = .035). With the additional 2 years of follow-up, the stage IIIB locoregional subgroup reached a median OS of 30.6 months in the BLP-25 arm and 13.3 months in the BSC arm (HR = .548; 95% CI: 0.301–0.999), with 3-year survival rates of 49% and 27%, respectively (P = .070). The original phase IIIB study was underpowered to detect differences among the subgroups of stage IIIB disease (wet vs locoregional). Further clinical trials to analyze BLP-25 in these predefined subgroups are needed.

Two phase III placebo-controlled trials (NCT00409188 and NCT01015443) are currently underway to further assess BLP-25. The START trial is an international phase III placebo-controlled trial that will compare survival among patients with unresectable stage III NSCLC (stage IV excluded) who have completed first-line chemoradiotherapy and are randomized to receive BLP-25 or BSC. The INSPIRE trial is similar to the START trial but is ongoing in Asian countries among patients with unresectable stage III NSCLC who have completed primary chemoradiotherapy (defined as 2 or more cycles of chemotherapy sequentially or concurrently with radiotherapy of at least 50 Gy). As with the START trial, patients with
stage IV disease are excluded from the INSPIRE trial. The results of these studies are eagerly awaited.

**TG4010 (MVA–MUC1–IL-2) Vaccine**

TG4010 is an immunotherapy designed to target the MUC1 antigen expressed on malignant cells. However, instead of directly targeting a MUC1 epitope like the BLP-25 vaccine, TG4010 utilizes a recombinant vaccinia virus (modified virus of Ankara, or MVA) that encodes for human MUC1 and IL-2. Preclinical work showed that the transduction of peripheral blood mononuclear cells obtained from healthy donors with MVA–MUC1–IL-2 induced expression of MUC1 on dendritic cell surfaces.20,27

Because several malignancies are known to overexpress MUC1, Rochlitz et al performed a phase I clinical trial in 13 patients with various solid tumors. Patients with an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, histologically proven cancer with MUC1 tissue expression greater than 50%, and incurable disease were eligible for trial enrollment. TG4010 was administered in three dose cohorts via at least two subcutaneous injections once every 3 weeks for cohorts 1 and 2 and weekly for cohort 3. Mild adverse events attributable to TG4010 vaccination were injection-site pain (4 patients), influenza-like syndrome (2 patients), vertigo (1 patient), and rash (1 patient). Three patients who received TG4010 had proven NSCLC, with 1 patient experiencing a significant reduction in tumor size following administration of five doses of TG4010. The patient’s first two imaging studies during the trial showed evidence of tumor progression; however, 6 months after the last TG4010 injection, the patient had a mean reduction in tumor volume of 64% compared with his pretrial imaging.8

Two phase II studies have since reported data examining the use of TG4010 in combination with first-line chemotherapy in patients with advanced NSCLC (stages IIIB and IV). Ramlau et al conducted a randomized, open-label study that compared TG4010 combined with cisplatin and vinorelbine doublet therapy (arm A) with TG4010 given as monotherapy followed by TG4010 plus cisplatin and vinorelbine upon disease progression (arm B). Sixty-five patients with an ECOG performance status of 0–1 were randomized in a 2:1 fashion: 44 to the TG4010 plus platinum doublet upfront (arm A) and 21 to the TG4010 followed by TG4010 plus the platinum doublet chemotherapy. The median survivals in arms A and B were 12.7 and 14.9 months, respectively. CD8 T-cell responses were evaluable in 31 of 65 patients, and subgroup analysis demonstrated that patients whose tumors produced a detectable immune response against MUC1 antigen had a longer time to tumor progression and longer median survival than those who did not have a detectable CD8 T-cell response.

In a larger published study by Quoix et al, TG4010 in combination with chemotherapy was compared with chemotherapy alone in 148 patients with stage IIIB (wet) or IV NSCLC (AJCC 6th edition), confirmed MUC1 tumor expression of at least 25%, and a good ECOG performance status of 0 or 1. At 6 months, the PFS did not differ significantly between those in the TG4010 with chemotherapy arm and those in the chemotherapy-alone arm (43.2% [95% CI: 33.4–53.5] and 35.1% [95% CI: 25.9–45.3], respectively, \( P = .307 \)). The median OS failed to show statistical significance: 10.7 months (95% CI: 8.8–18.0) in the TG4010 group and 10.3 months (95% CI: 8.3–12.5) in the chemotherapy-alone group. Patients who demonstrated an objective response to TG4010 based on the World Health Organization (WHO) imaging criteria had a longer median OS than those who did not have an objective response (23.3 months [95% CI: 15.9–31.5] and 12.5 months [95% CI: 8.5–20.0], respectively). The rates of serious adverse events defined by the Common Terminology Criteria for Adverse Events (CTCAE) between the two study populations did not differ significantly (52.1% with TG4010 and 47.2% with chemotherapy alone). Of interest, patients with increased numbers of activated CD16+ CD56+ CD69+ natural killer (NK) cells measured prior to treatment had a shorter median OS and increased rates of serious adverse events than did patients with normal NK cell populations. The authors postulated that increased NK cell activity may have a suppressive role against dendritic cells and effector T cells, thus explaining the increased toxicity and decreased survival observed in patients with increased numbers of activated NK cells in this trial. A larger phase III trial that accounts for pretreatment levels of CD16+ CD56+ CD69+ NK cells is in progress to better define the treatment effect and clinical benefit of upfront TG4010 use with chemotherapy (NCT01383148).

**CIMAvax EGF Vaccine**

Therapies that target the EGFR tyrosine kinase family of receptors have had a significant impact on OS in patients with NSCLC. An alternative approach to targeting EGFR is using a vaccine therapy against EGF, the cellular ligand for EGFR. CIMAvax EGF is a vaccine developed in Cuba and approved in Cuba, Peru, and Venezuela for the treatment of advanced stages IIIB and IV NSCLC after first-line chemotherapy.17,31,32 CIMAvax EGF contains human recombinant EGF conjugated to the P64K Neisseria meningitides recombinant protein.17

Gonzalez et al reported pooled data from two clinical trials that evaluated CIMAvax EGF combined with either alum as a vaccine adjuvant or emulsified with ISA51 with or without cyclophosphamide pretreatment. Similar to BLP-25, cyclophosphamide was given to reduce inhibition of T-suppressor cells. Patients underwent serial antibody measurement to EGF through an enzyme-linked immunosorbent assay (ELISA) and were stratified as good antibody responders (GARs) if they produced an antibody response to a
titer greater than 1:4,000 or poor antibody responders (PARs) if their titer was less than 1:4,000. There was no significant difference in antibody responses with the use of cyclophosphamide as pretreatment prior to EGF vaccine administration. However, there was a significant difference in the number of GARs with the use of ISA51 over alum as a vaccine adjuvant (73% and 70% for ISA51 vs 22% and 30% with alum).

Further studies have demonstrated that anti-EGF antibody production and serum EGF levels following vaccination correlate with improved survival.17,32 Ramos et al32 studied EGF vaccine with alum in 43 patients with stage IIIB or IV NSCLC. Two dose arms were studied: 71 μg (single dose) and 142 μg (double dose). Antibody titers and serum EGF levels were measured every 15 days for 60 days and then monthly thereafter. The EGF vaccine was well tolerated, with only grade 1–2 toxicities reported, all of which resolved within 48 hours of vaccine administration. Anti-EGF antibodies were detected in 38 patients, 39.5% of whom met criteria previously established for a GAR. GARs had a statistically significant increase in median OS compared with PARs (11.87 months and 7.07 months, respectively; \( P = .0095 \)). The median OS was also more statistically significant in patients with serum EGF levels less than 168 pg/mL (11.3 months if less than 168 pg/mL and 5 months if greater than 168 pg/mL; \( P = .0022 \)).

Neninger Vinageras et al37 conducted a larger phase II clinical trial in which 80 patients who completed first-line chemotherapy for the treatment of NSCLC were randomized 1:1 to receive either CIMAvax EGF or BSC. Just over half of the patients who achieved measurable anti-EGF antibodies were GARs. As was seen in the trial by Ramos et al,32 vaccinated patients who were GARs had a longer median OS (11.7 months) than did those who were PARs (3.6 months). Similarly, vaccinated patients with serum EGF levels below 168 pg/mL had a longer median OS (13 months vs 5.6 months if serum EGF levels were greater than 168 pg/mL). Patients in the vaccine arm who were younger than 60 years of age had a statistically significant increased OS over patients older than age 60 years (11.57 and 5.33 months, respectively; \( P = .0124 \)). A phase III clinical trial comparing CIMAvax EGF with BSC is ongoing outside the United States.33

**MAGE-A3 Adjuvant Immunotherapy**

MAGE-A3 is a protein that is almost exclusively expressed by malignant cells and has been demonstrated in 35% of NSCLC cases as well as in other solid organ tumors.18 The first study to demonstrate a benefit from MAGE-A3 adjuvant vaccination was a phase I study by Marchand et al,34 in which 7 of 25 patients who received a complete course of MAGE-A3 vaccination demonstrated objective tumor regression by objective imaging criteria. In 7 patients with tumor regression, 2 developed a complete disease-free state at 2 years of follow-up.

Although MAGE-A3 has since been studied in several solid tumors, Vansteenkiste et al39 conducted a phase II, randomized, placebo-controlled trial in which MAGE-A3 was administered to 182 patients with completely resected stages IB–II NSCLC. Patients were randomized 2:1 to receive MAGE-A3 or placebo given every 3 weeks for 5 administrations and then every 3 months for 8 administrations. At a median of 28 months of follow-up, the HR for disease-free survival (DFS) and OS was 0.73 (95% CI: 0.45–1.16) and 0.66 (95% CI: 0.36–1.20), respectively, in favor of the patients who received MAGE-A3. Although not statistically significant, the trend toward improved DFS and OS in the group treated with MAGE-A3 has led researchers to conduct a large, double-blind, randomized phase III trial in patients with stage IB, II, or IIIA MAGE-A3–positive NSCLC. The trial design of this study, named the MAGRIT trial, is similar to the phase II study by Vansteenkiste et al,18 with a 2:1 randomization and the same vaccine administration schedule (NCT00480025).3

**Autologous Cellular Therapies**

**GVAX and Belangenpumatucel-L**

Several studies have examined the potential of autologous or allogeneic cellular therapies to induce antitumor effects in patients with NSCLC. One such phase I/II trial conducted by Nemunaitis et al35 used autologous cells from patients with NSCLC and combined these harvested cells with a bystander cell line generated from K562 human erythroleukemia cells previously transfected with a bacterial plasmid containing the GM-CSF gene. Of the 86 patients who underwent tumor harvesting, only 49 patients actually received the vaccine preparation, named GVAX. Seven patients achieved stable disease for at least 12 weeks following the first vaccination, but no patients achieved a complete or partial remission. A small benefit was seen in patients with a local vaccine site reaction of more than 5 mm (PFS of 141 days vs 124 days if negative; \( P = .008 \)), but no benefit was observed with respect to vaccine immune response parameters.

A second trial, also conducted by Nemunaitis et al,8 examined BGPT-L, a nonviral, allogeneic-based vaccine that targets TGF-β2 expression in NSCLC. The rationale for targeting TGF-β2 was derived in part from a study that demonstrated a correlation between high levels of TGF-β2 expression and the presence of continued disease during follow-up in patients with newly diagnosed NSCLC.56 BGPT-L is produced from four NSCLC cell lines transfected with a plasmid vector containing a TGF-β2 antisense gene. Aliquots that blocked TGF-β2 secretion by more than 35% were expanded and frozen in three dose aliquots. The trial included 75 patients with stage II, IIIA, IIIB, or IV NSCLC who had either completed therapy (n = 61) or refused prior therapy (n = 14). Patients were randomized to one of three dose cohorts of BGPT-L administered either once monthly or once every other...
month. Toxicity, immune response, and imaging for restaging were conducted at regular intervals. If a benefit was observed after 16 weeks, patients were eligible to receive up to 12 additional vaccinations. A statistically significant difference in median OS was observed between the low-dose cohort and the combined high-dose cohorts (252 and 581 days, respectively; \( P = .0186 \)). There was no significant difference in the rates of adverse events among the three dose arms of the trial (\( P = .5698 \)).

A phase III randomized, placebo-controlled trial comparing BGPT-L with BSC is ongoing (NCT00676507).

**Talactoferrin**

TFL is a recombinant form of lactoferrin isolated from *Aspergillus nigar* variant *auamori*. TFL has shown promise in possessing antitumor immune modulator properties in preclinical trials, where it was observed to inhibit the growth of squamous cell carcinoma and adenocarcinoma, enhance splenic NK cell activity, and increase the number of circulating CD4 and CD8 cells.\(^{19}\) Hayes et al\(^{7}\) conducted a dose escalation phase I clinical trial in which 10 patients with progressive cancer were given TLF. No grade 3 or 4 dose-limiting toxicities were observed among patients receiving TLF, and the majority of adverse events were rated as mild (grade 1), with the exception of 1 patient who developed grade 2 diarrhea. Interestingly, of the 3 patients with NSCLC treated with platinum/paclitaxel doublet chemotherapy as first-line treatment who participated in the study, all were alive at the end of 12 months, with 1 patient alive at 18 months. The result of this study suggests that oral TLF may have significant antitumor effects, particularly in NSCLC.

Digumarti et al\(^{13}\) recently published the results of a phase II, double-blind, placebo-controlled trial in which 110 patients with stage IIIB or IV NSCLC were randomized 1:1 to receive carboplatin/paclitaxel plus either TLF 3 g per day or placebo. Of the 110 patients enrolled in the study, 100 patients were included in a group termed the evaluable population (patients who received at least 1 dose of TLF or placebo and had at least 1 computed tomography [CT] scan after starting therapy). For the primary endpoint, the response rate defined by Response Evaluation Criteria in Solid Tumors (RECIST) among the 100 patients in the evaluable population for placebo and TLF was 29% and 47%, respectively (\( P = .05 \)). The median duration of response was 5.5 months for the placebo group and 7.6 months for the TLF group (\( P = .07 \)), the PFS in the evaluable population was 43% (placebo) and 59% (TLF; \( P = .08 \)), and the median OS in the evaluable population was 8.5 months (placebo) and 10.4 months (TLF; \( P = .11 \)). Adverse events attributed solely to TLF were rare, and fewer adverse events occurred in the TLF cohort than in the placebo cohort.

A second phase II, double-blind, placebo-controlled trial was recently reported by Parikh et al.\(^{37}\) In this study, 100 patients with advanced-stage IIIB or IV NSCLC whose disease had progressed after first-line treatment with platinum-based chemotherapy or after second-line chemotherapy were randomized 1:1 to receive either TLF 3 g per day or placebo. After a median follow-up of 15.2 months, there was a statistically significant difference in OS (the primary efficacy endpoint) between the placebo group and the TLF group (3.7 months [90% CI: 2.8–4.9 months] and 6.1 months [90% CI: 4.7–8.4 months]), with a one-tailed \( P = .04 \) by log-rank test. The 6-month survival rates were 30% (90% CI: 20%–41%) and 52% (90% CI: 39%–63%) for placebo and TLF, respectively. The 1-year survival rates were 16% (90% CI: 9%–25%) and 29% (90% CI: 18%–41%) for placebo and TLF, respectively. The disease control rate was chosen as the secondary endpoint and was defined as the sum of complete responses, partial responses, and stable disease. The disease control rate was 23% (90% CI: 13%–32%) for the placebo group and 36% (90% CI: 25%–48%) for the TLF group (\( P = .14 \) by two-sided \( \chi^2 \) test). As in the study by Digumarti et al,\(^{13}\) TLF was shown to be well tolerated, with fewer grade 3 adverse events in patients receiving TLF than in those receiving placebo.

In both phase II randomized, double-blind, placebo-controlled trials, TLF showed promise as a well-tolerated, oral immunomodulator therapy with the potential to not only prolong OS as monotherapy in patients with NSCLC whose disease has progressed despite prior therapy, but also improve response rates in patients who are concurrently receiving first-line chemotherapy. Two phase III clinical trials are currently being conducted to further study TLF in these settings (NCT00706862 and NCT00707304).\(^{37}\)

**T-Cell–Directed Antibody Therapies**

Ipilimumab is a fully humanized monoclonal antibody directed against CTLA-4, a regulatory molecule found on the surface of activated T cells and subsets of regulatory T cells. Ipilimumab has demonstrated the ability to enhance activated T-cell antitumor responses in patients with metastatic melanoma.\(^{38}\) Two phase III clinical trials have confirmed the ability of CTLA-4 blockade to increase OS in patients with previously treated or untreated metastatic melanoma.\(^{39,40}\) These results in patients with metastatic melanoma have led researchers to investigate whether CTLA-4 blockade with ipilimumab could induce antitumor responses in patients with NSCLC.

Lynch et al\(^{11}\) recently reported the results of a phase II clinical trial that studied the effect of ipilimumab in combination with paclitaxel and carboplatin in 204 chemotherapy-naive patients with stages IIIB or IV NSCLC. Patients were assigned 1:1:1 to receive one of three regimens: (1) 4 cycles of ipilimumab plus paclitaxel and carboplatin followed by two doses of placebo plus paclitaxel and carboplatin (concurrent regimen), (2) 2 doses of placebo plus paclitaxel and carboplatin followed by 4 doses of ipilimumab plus...
paclitaxel and carboplatin (phased regimen), or (3) 6 doses of placebo plus paclitaxel and carboplatin (control regimen). Ipilimumab was administered at 10 mg/kg, paclitaxel at 175 mg/m², and carboplatin at AUC of 6 for all three treatment arms.

For the primary endpoint, immune-related PFS (irPFS), the phased regimen showed a statistically significant median irPFS compared with the control regimen (5.7 months vs 4.6 months; HR = 0.72; P = .05). No statistically significant difference was seen in the median irPFS between the concurrent regimen and the control group (5.5 months vs 4.6 months; HR = 0.81; P = .13). The median OS was 12.2 months in the phased-regimen cohort compared with 8.3 months in the control-regimen cohort (HR = 0.87; P = .23). Grades 3–4 adverse events were frequent and occurred in 37% of the control group, 41% of the concurrent group, and 39% of the phased-regimen cohort. Grades 3–4 immune-related adverse events (irAEs) included colitis, hypopituitarism, and rash, and they occurred more frequently in the concurrent and phased regimen cohorts (overall irAE rates were 20% and 15%, respectively) than in the control regimen cohort (6%).

A subgroup analysis was performed on patients in each of the three treatment arms by their tumor histology. Although the study was not powered to detect differences in irPFS among squamous cell and nonsquamous cell subgroups, patients with squamous cell carcinoma had a greater improvement in irPFS if they received the phased regimen compared to the control regimen (HR = 0.55; 95% CI: 0.27–1.12). A phase III trial is underway to determine whether OS is increased among patients with nonsquamous cell NSCLC with the combination of ipilimumab with carboplatin and paclitaxel (NCT01285609).

**Antibodies Against PD-1 and PD-L1**

Blockade of the interaction between programmed death 1 (PD-1) ligands (PD-L1 and PD-L2) produced by stromal and tumor cells and the PD-1 receptor on activated T cells has attracted interest as promising antitumor immunotherapeutics. The PD-1 receptor is a coinhibitory receptor present on T cells that binds to either PD-L1 (B7–H1) or PD-L2 (B7–DC). Two phase I clinical trials examining the safety and clinical efficacy of monoclonal antibodies against PD-1 and PD-L1 have been published recently. Topalian et al studied the safety and efficacy of BMS-936558, a fully humanized monoclonal antibody directed against the PD-1 receptor, in patients with advanced solid tumors treated with at least one prior therapy (47% received three or more previous lines of therapy). BMS-936558 was administered as an intravenous infusion every 2 weeks, with 1 cycle defined as 4 treatments over an 8-week period. The study enrolled 296 patients, 122 of whom had advanced NSCLC. The efficacy analysis included 76 patients of these 122 patients with NSCLC (46 patients not included in the analysis either died or discontinued the study prior to assessment of a disease response). Common adverse events with BMS-936558 included fatigue, rash, diarrhea, decreased appetite, nausea, and pruritus. Grades 3–4 toxicity occurred in 41 of 296 patients, with 3 deaths attributed to treatment-related pneumonitis. Objective responses by RECIST (version 1.0) were noted in 14 patients with NSCLC across the three dose cohorts of 1.0 mg/kg, 3.0 mg/kg, and 10.0 mg/kg (response rates of 6%, 32%, and 18%, respectively). Six of 18 patients with squamous cell histology and 7 of 56 patients with nonsquamous cell histology achieved an objective response, defined as complete or partial remission. No stable disease was observed in patients with squamous cell histology, whereas 5 of 56 patients with nonsquamous cell histology had stable disease of more than 24 weeks from therapy initiation.

In a similar study, Brahmer et al published the results of a phase I clinical trial examining the safety and efficacy of BMS-936559, a fully humanized monoclonal antibody directed against PD-L1 across four dose cohorts. Patients with advanced solid tumors and disease progression after at least one prior therapy were given BMS-936559 via intravenous infusion on days 1, 15, and 29 every 6 weeks. The study enrolled 207 patients, 75 of whom had NSCLC, and 49 patients with NSCLC were included in the efficacy analysis. Adverse events were reported in 91% of patients and were mostly grade 1 or 2. Of the 207 patients, 19 experienced grade 3 or 4 adverse events. Among the 49 patients with NSCLC included in the efficacy analysis, 5 patients (4 with nonsquamous cell histology and 1 with squamous cell histology) experienced an objective response, and 6 patients experienced disease stabilization lasting at least 24 weeks.

Both of the studies by Topalian et al and Brahmer et al suggest that manipulation of T-cell activation through blockade of the PD-1 system can produce antitumor responses in NSCLC. A phase I study is currently underway to determine the safety of giving the anti PD-1 antibody BMS-936558 with platinum doublet therapy in patients with advanced NSCLC (NCT01454102). Additional studies are needed to learn whether blockade of PD-1 on activated T cells can produce meaningful improvements in OS for patients with NSCLC.

**Current Immunotherapeutic Research in NSCLC**

Research is ongoing to study the effects of a cellular-based vaccine therapy for the treatment of advanced NSCLC. The GM.CD40L bystander cell was designed at our center to enhance antitumor responses to autologous tumor cells when the two cells lines are mixed.

In an open-label, nonrandomized, phase I dose escalation study, Dessureault et al demonstrated that an autologous vaccine of excised tumor cells along with GM.CD40L bystander cells could recruit dendritic
cells and generate specific antitumor T-cell responses with an acceptable safety profile. Twenty-six patients with metastatic cancers were enrolled and underwent tumor resection and subsequent vaccine preparation. Of this group, 21 patients received at least three doses of the vaccine. Tumor regression was not observed in any of these patients, but stable disease was observed in 13 of the 21 patients at 3 months. A phase I trial examining GM.CD40L in combination with cyclophosphamide and all-trans retinoic acid (ATRA) in NSCLC has been completed at our center (manuscript in preparation with expected submission in October 2012), and a phase II study of GM.CD40L vaccine plus or minus CCL21 (a T-cell chemokine) is currently enrolling patients (NCT01433172).

**Small Cell Lung Cancer**

Although numerous immunotherapy trials are currently underway for the treatment of NSCLC, few have been conducted to examine the role of immunotherapy for the treatment of SCLC. The overall incidence of lung cancers defined as SCLC declined from 17.26% in 1986 to 12.95% in 2002. However, the incidence of SCLC in women has increased from 28% in 1973 to equal men at 50% in 2002. The 5-year all-cause survival rate for limited-stage disease increased from 4.9% in 1973 to 10% in 2002. Although a statistically significant improvement in OS has been observed for limited-stage disease, the vast majority of patients succumb to their illness within 5 years.

The BEC2/BCG (Bacillus Calmette-Guérin) vaccine was studied extensively in SCLC over the past decade and ultimately failed to achieve a significant benefit in OS.Ipilimumab is currently the subject of a phase III clinical trial to determine whether blockade of CTLA-4 in patients with extensive-stage SCLC enhances antitumor T-cell responses and prolongs OS (NCT01450761). In addition, a new vaccine targeting p53 has been developed at our center and is being studied in a phase I/II clinical trial.

**BEC2/BCG Vaccine**

The discovery that the glycosphingolipid antigen GD3 is highly expressed in SCLC but rarely expressed in normal tissues led to interest in generating a vaccine that could selectively target ganglioside GD3. BEC2, a monoclonal antibody that mimics GD3, was studied by McCaffery et al in 20 patients with melanoma using two immune adjuvants to increase the immunogenicity of BEC2: BCG or QS21. When combined with BCG, 3 of 14 patients developed demonstrable antibodies against GD3, whereas no patients in the QS21 arm developed anti-GD3 antibodies. BEC2/BCG showed promising results in an early clinical trial. A study of BEC2/BCG in SCLC by Grant et al enrolled 15 patients (8 with extensive-stage disease and 7 with limited-stage disease) to receive increasing doses of BCG with an unchanged dose of BEC2. Of the 13 evaluable patients, 5 developed anti-GD3 antibodies, and all patients developed anti-BEC2 antibodies. Adverse events were deemed mild, with the exception of local skin reactions, which were grade 3 in 14 of 15 patients. The median OS was 20.5 months, and relapse-free survival was longer in patients who developed measurable anti-GD3 antibodies. For the 7 patients with limited-stage disease, the median OS was not reached at a median follow-up of 47 months. As a result of the impressive survival achieved by the small group of patients with limited-stage SCLC, a large, randomized phase III trial was initiated for patients with biopsy-proven limited-stage disease, a Karnofsky Performance Status of > 60%, and complete or partial response to at least 4 cycles of 2-drug induction chemotherapy and chest radiotherapy.

The European Organization for Research and Treatment of Cancer (EORTC) 08971-08971B (Silva study) by Giaccone et al randomly assigned 515 patients with limited-stage SCLC to receive BEC2/BCG or BSC. After a median follow-up of 35.6 months, the median OS did not differ statistically (16.4 months with BSC [95% CI: 14.6–20.3] and 14.3 months with BEC2/BCG [95% CI: 13.0–17.7]; HR = 1.12 [95% CI: 0.91–1.37]). The PFS similarly failed to achieve statistical significance between BEC2/BCG and BSC. The Silva study unfortunately failed to meet its objective of prolonging OS in patients with limited-stage SCLC who received BEC2/BCG over BSC.

**Current Immunotherapeutic Research in SCLC**

The novel immunotherapy INGN-225 (Ad.p53-DC) was developed at our center and has been studied in a phase I/II clinical trial in patients with SCLC. INGN-225 is produced from autologous peripheral blood mononuclear cells that are removed from patients and cultured in the presence of IL-4 and granulocyte macrophage colony-stimulating factor (GM-CSF) prior to incubation with a viral construct containing wild-type p53 (adenovirus Ad.p53). Preclinical work demonstrated the proof of concept that murine dendritic cells transfected with Ad.p53 were able to induce cytotoxic T lymphocytes following vaccination.

Chiappori et al conducted a phase I/II clinical trial that included patients with stable, extensive-stage SCLC and a history of chemotherapy treatment. Of the 54 patients enrolled, 14 demonstrated evidence of a positive immune response to a p53-specific EILSA. Of those 14 patients, 11 experienced a response to second-line chemotherapy. In 15 patients who did not experience an immune response based on the p53-specific spot ELISA, 5 responded to second-line chemotherapy. The median survival time was 12.6 months and 8.2 months in patients with and without an immune response, respectively (P = .131).

A randomized, phase II clinical trial is in progress at our center to study INGN-225 with or without all-trans retinoic acid (ATRA). These two study arms will be compared with BSC (NCT00617409). It is theorized that adding ATRA to INGN-225 will decrease the num-
ber of myeloid-derived suppressive cells and allow thus improved dendritic cell differentiation and function, thus improving antitumor responses when incubated with Ad.p53.

Conclusions
Numerous trials are underway internationally to determine whether novel immunotherapies can generate meaningful improvements in key clinical outcomes (such as median overall survival and progression-free survival) in patients with lung cancer. Defining patient populations that will attain the greatest benefit to treatment with immunotherapeutics and determining the best time in a patient’s treatment course to administer immunotherapy remain open questions that need further exploration in phase III clinical trials.

One area for improvement in vaccine development is how best to design vaccines that generate both an immune response and a correlative clinical response. For BLP-25 and TG-4010, overall survival was increased in the subset of patients with detectable cytotoxic CD8 lymphocyte responses. However, only a fraction of patients in each of the phase II studies had detectable responses. Similarly, a little over half of patients treated with CIMAavax EGF had antibody titers that met criteria for a good antibody response. Good antibody responders experienced an improvement in overall survival compared with poor antibody responders. These observations need to be studied in prospectively defined subgroups in ongoing and future clinical trials. Efforts to enhance the ability of a vaccine to generate immune responses in a greater percentage of patients and to identify patient factors that will predict a greater likelihood of achieving a measurable immune response are necessary to maximize the vaccine immunotherapy’s ability to improve patient outcomes.

Cellular therapies that target both small cell lung cancer and non-small cell lung cancer are in development at our center and have been studied in small phase I clinical trials. To see cellular therapies proceed to the next clinical level, their safety, efficacy, and ease of administration need to be assessed in future trials. Studies conducted to date have demonstrated that immunotherapy has the potential to make significant improvements in overall survival when combined with current lung cancer treatment approaches.

Appreciation is expressed to Rasa Hamilton for her invaluable help in preparing this manuscript for publication.

References


