Incorporation of molecular markers and targeted therapy in the treatment of advanced non–small-cell lung cancer has improved outcomes and may be beneficial in the adjuvant treatment of this disease.

Systemic and Targeted Therapies for Early-Stage Lung Cancer
Elizabeth Byron, MD, and Mary Pinder-Schenck, MD

**Background:** Even with aggressive surgical treatment, relapse rates remain high for patients with resectable non–small-cell lung cancer (NSCLC). In an effort to improve survival in these patients, numerous clinical trials have evaluated neoadjuvant and adjuvant chemotherapy.

**Methods:** The authors reviewed the results of the prospective randomized clinical trials that have established adjuvant chemotherapy as the standard of care for patients with surgically resected NSCLC. In addition, the authors summarize data on predictive and prognostic markers for patients with early-stage NSCLC and discuss novel therapies and clinical trials currently underway in early-stage NSCLC.

**Results:** Three large randomized clinical trials and two meta-analyses have demonstrated a survival benefit for adjuvant cisplatin-based chemotherapy compared with surgery alone in patients with early-stage NSCLC. As a result, adjuvant cisplatin-based chemotherapy is recommended as the standard of care in these patients. Numerous promising biomarkers and agents have been developed in the metastatic setting and are currently being evaluated in the adjuvant setting.

**Conclusions:** While adjuvant chemotherapy has improved survival for patients with early-stage NSCLC, the prognosis for early-stage lung cancer remains poor. Incorporation of molecular markers and targeted therapies into the management of patients with advanced NSCLC has improved outcomes. Development of these strategies in the adjuvant setting offers the potential to increase cure rates in patients with early-stage NSCLC.

**Introduction**
Lung cancer is the leading cause of cancer-related mortality in the developed world. Worldwide, 1.2 million new cases are diagnosed each year, and over 1 million people die of the disease annually. Almost 80% of all lung cancers are defined as non–small-cell lung cancer (NSCLC), and approximately 90% of those cases are related to tobacco exposure. The most common histological subtypes of NSCLC include adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma. Immunohistochemistry is often helpful in distinguishing between these subtypes. Squamous cell carcinomas are often TTF-1-negative and p63-positive, whereas adenocarcinomas are often TTF-1-positive. This differentiation is imperative as it can significantly affect treatment management. In recent decades, the frequency of the histological subtypes has shifted, and adenocarcinoma has surpassed squamous cell carcinoma as the most common type of lung cancer. Despite the recent advances in both screening and imaging tools, only approximately 30% of NSCLC cases...
are diagnosed at an early stage, leaving the majority diagnosed when the disease is not amenable to complete surgical resection. Even for those patients able to undergo successful surgical excision, NSCLC has a poor prognosis, with only one-third surviving at 5 years and roughly one-sixth surviving at 10 years.\(^3\)

Surgical excision has long been the standard treatment for patients with resectable stage I to IIIA NSCLC. However, as many as 40% of patients with stage I, 66% of stage II, and 75% of stage IIIA will develop recurrence and die as a result of their disease within 5 years of resection.\(^4\) Residual micrometastases are believed to be the cause of disease recurrence. In an effort to eradicate micrometastases and improve overall survival, numerous clinical trials have evaluated adjuvant and neoadjuvant chemotherapy. Several large randomized trials are discussed in detail below; based on results of these studies, the current standard of care for completely resected stage II or III NSCLC is adjuvant platinum-based chemotherapy.\(^3\)

**Adjuvant Chemotherapy Trials**

Early trials of adjuvant chemotherapy failed to demonstrate a consistent survival benefit. Many of these trials were limited by small sample sizes, heterogeneous patient populations, and the use of alkylating agents and other older chemotherapy regimens. In 1995, the NSCLC Collaborative Group published a meta-analysis that examined the role of chemotherapy in the treatment of NSCLC.\(^6\) This included 52 studies \((N = 9,287)\) conducted between 1965 and 1991. Adjuvant chemotherapy with older alkylating agents was associated with poorer survival, with an absolute decrease in survival of 5% at 5 years \((\text{combined hazard ratio } [HR] = 1.15; P = .005 \text{ for 5 trials } [N = 2,145])\). Eight trials \((N = 1,394)\) included in the meta-analysis utilized more modern cisplatin-based combination chemotherapy. The overall HR for this group was 0.87 \((P = .08)\), corresponding to a 13% reduction in risk of death and a 5% increase in survival at 5 years. While these results were not statistically significant, they were sufficiently promising to generate renewed interest and a series of adjuvant cisplatin-based chemotherapy in completely resected NSCLC (Table).\(^7\)\(^-\)\(^15\)

Three large randomized trials — the International Adjuvant Lung Cancer Trial (IALT), the National Cancer Institute of Canada Clinical Trials Group (NCIC-CTG) JBR.10, and the Adjuvant Navelbine International-

<table>
<thead>
<tr>
<th>Trial</th>
<th>No. of Patients</th>
<th>Stage</th>
<th>Regimen</th>
<th>Survival Benefit (5 yr)</th>
<th>Hazard Ratio (95% CI)</th>
<th>(P) Value</th>
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<tbody>
<tr>
<td>ECOG 3590(^7)</td>
<td>488</td>
<td>II-IIIA</td>
<td>Cisplatin Etoposide</td>
<td>0%</td>
<td>0.93 (0.74–1.18)</td>
<td>.56</td>
</tr>
<tr>
<td>ALPI(^8)</td>
<td>1,209</td>
<td>I-IIIA</td>
<td>Cisplatin Mitomycin Vindesine</td>
<td>3%</td>
<td>0.96 (0.81–1.13)</td>
<td>.589</td>
</tr>
<tr>
<td>ANITA(^9)</td>
<td>840</td>
<td>IB-IIIA</td>
<td>Cisplatin Vinorelbine</td>
<td>9%</td>
<td>0.80 (0.66–0.96)</td>
<td>.017</td>
</tr>
<tr>
<td>NCIC-CTG JBR.10(^10),(^14)</td>
<td>482</td>
<td>IB-II</td>
<td>Cisplatin Vinorelbine</td>
<td>15%</td>
<td>0.69 (0.52–0.91)</td>
<td>.04</td>
</tr>
<tr>
<td>IALT(^11),(^13)</td>
<td>1,867</td>
<td>I-IIIA</td>
<td>Cisplatin plus Vindesine or Vinblastine or Vinorelbine or Etoposide</td>
<td>4%</td>
<td>0.91 (0.81–1.02) (0.86 (0.76–0.96)) (10^) (0.03)</td>
<td></td>
</tr>
<tr>
<td>BLT(^12)</td>
<td>381</td>
<td>I-IIIA</td>
<td>Cisplatin plus Vinorelbine or Vindesine Cisplatin plus Mitomycin or Ifosfamide or Vinblastine</td>
<td>2%</td>
<td>1.02 (0.77–1.35)</td>
<td>.90</td>
</tr>
<tr>
<td>CALGB 9633(^15)</td>
<td>344</td>
<td>IB</td>
<td>Carboplatin Paclitaxel</td>
<td>4%</td>
<td>0.83 (0.64–1.08)</td>
<td>.125</td>
</tr>
</tbody>
</table>

\(^4\) Updated results.

AlPI = Adjuvant Lung Project Italy, ANITA = Adjuvant Navelbine International Trialists Association, BLT = Big Lung Trial, CALGB = Cancer and Leukemia Group B, CI = confidence interval, ECOG = Eastern Cooperative Oncology Group, IALT = International Adjuvant Lung Trial, NCIC-CTG = National Cancer Institute of Canada Clinical Trials Group.
al Trialist Association (ANITA) trial — demonstrated a statistically significant survival benefit for cisplatin-based adjuvant chemotherapy and led to the adoption of adjuvant cisplatin-based chemotherapy as the standard of care in resected NSCLC. The IALT randomized 1,867 patients with completely resected stages I to III NSCLC to adjuvant cisplatin (combined with either vinblastine, vindesine, vinorelbine, or etoposide).13 Postoperative radiation therapy was performed at the discretion of individual centers. Although target enrollment was 3,300 patients, the trial was discontinued early due to slow accrual. At a median follow-up of 56 months, an absolute benefit of 4.1% (HR = 0.86; 95% confidence interval [CI] = 0.76–0.98, P < .03) was observed for patients assigned to adjuvant chemotherapy. An updated analysis performed after a median follow-up of 90 months demonstrated a loss of statistical significance for overall survival (HR = 0.91; 95% CI, 0.81–1.02, P = .10).11 A higher rate of late noncancer-related deaths was observed in the group assigned to adjuvant chemotherapy and was proposed as an explanation for the lack of an overall survival benefit at this time point. Among the causes for an increase in noncancer-related deaths in the patients who received adjuvant chemotherapy were secondary malignancies and cardiopulmonary disease. Interestingly, none of the deaths were documented as secondary to chemotherapy.

The NCIC-CTG JBR.10 trial included 482 patients with completely resected stages IB to II NSCLC.10 Patients were randomly assigned to chemotherapy with cisplatin at 100 mg/m² every 4 weeks with weekly vinorelbine. Patients did not receive adjuvant radiation therapy. Overall survival was significantly improved at 5 years in the chemotherapy arm compared with observation (94 vs 73 months, HR = 0.69; 95% CI, 0.52–0.91, P = .04). The investigators presented an updated analysis in 2009: the benefit of chemotherapy was preserved with longer follow-up (HR = 0.78; 95% CI, 0.61–0.99, P = .04).14 There were no differences in noncancer deaths between the chemotherapy and observation arms, and no long-term unexpected toxicities of chemotherapy were observed. The ANITA trial randomized 840 patients with stage IB-IIIA NSCLC to adjuvant chemotherapy with cisplatin and vinorelbine vs observation. Postoperative radiation therapy was performed at the discretion of individual centers. After 7 years of follow-up, the investigators reported an overall survival benefit of 8.4% (HR = 0.80; 95% CI, 0.66–0.96, P = .017) in favor of adjuvant chemotherapy.8

The Cancer and Leukemia Group B trial (CALGB 9633) randomized patients with resected stage IB NSCLC to adjuvant chemotherapy with carboplatin and paclitaxel or to observation.10 While 3-year survival was higher in patients who received adjuvant chemotherapy, a significant survival benefit was not sustained at longer follow-up for adjuvant chemotherapy in this population (HR = 0.83; 95% CI, 0.64–1.08, P = .125). These findings are consistent with the results of NCIC-CTG JBR.10, ANITA, and the IALT, which also failed to demonstrate a survival benefit for cisplatin-based chemotherapy in the stage IB subgroup. An exploratory analysis of CALGB 9633 demonstrated an improvement in survival for the subgroup of patients with tumors larger than 4 cm in diameter. While adjuvant chemotherapy may be considered for this subgroup of patients with stage IB NSCLC, current guidelines do not recommend routine use of chemotherapy in patients with stage I NSCLC.

Three randomized trials evaluating adjuvant chemotherapy — the Big Lung Trial (BLT), the Adjuvant Lung Project Italy (ALPI), and Eastern Cooperative Oncology Group (ECOG) 3590 — failed to demonstrate a survival benefit for adjuvant chemotherapy.7,8,12 The BLT and ALPI trials were included, along with ANITA, IALT, and JBR.10, in the Lung Adjuvant Cisplatin Evaluation (LACE) meta-analysis.16 This pooled analysis of individual patient data from 4,584 patients found a 5.4% absolute survival benefit (HR for death = 0.89; 95% CI, 0.82–0.96, P = .005) in favor of adjuvant chemotherapy. Subset analyses revealed that the benefit of chemotherapy appeared significant only in patients with stages II and III NSCLC, and the benefit was independent of other prognostic factors. The authors concluded that cisplatin-based adjuvant chemotherapy improved overall and disease-free survival in patients who underwent surgery for NSCLC.

**Neoadjuvant Chemotherapy Trials**

In an effort to improve the numbers of overall survival in NSCLC despite adequate surgical resection, neoadjuvant clinical trials have also been performed in addition to the adjuvant trials described above. From the studies performed, both benefits and disadvantages to receiving chemotherapy in the neoadjuvant setting were observed. Several potential advantages of neoadjuvant chemotherapy over adjuvant therapy included earlier introduction of systemic therapy to address micrometastatic disease, improved drug delivery and patient tolerance in the preoperative setting, enhanced evaluation of the biological effects of conventional and novel agents, and facilitation of more limited surgical resections. Potential disadvantages included imprecise initial staging, the possibility of progression on therapy that may preclude surgical resection, and the risk of increased surgical morbidity or mortality following neoadjuvant chemotherapy.

The early history of neoadjuvant chemotherapy for NSCLC illustrated both the pitfalls and the promise of this strategy. In 1990, a randomized phase II trial was published that compared initial surgery to induction chemotherapy with cisplatin, cyclophosphamide, and
induced by surgery. The trial was stopped prematurely after accruing only 26 patients because 4 patients on the chemotherapy arm experienced disease progression, ultimately precluding resection in 2 of those patients. However, later in the 1990s, two randomized phase III trials reported a significant survival benefit for patients receiving neoadjuvant chemotherapy followed by surgical resection compared with those who underwent surgery alone. These trials prompted further evaluation of the use of neoadjuvant chemotherapy in patients with early-stage NSCLC.

The first of these trials compared surgery alone to induction chemotherapy with 3 cycles of cisplatin, etoposide, and cyclophosphamide followed by surgery in 60 patients with clinical stage IIIA NSCLC. Radiotherapy was not administered in either arm, and patients who had a response to induction chemotherapy received 3 additional cycles postoperatively. When the results were initially reported, chemotherapy was associated with a highly significant survival benefit; patients treated with chemotherapy had a median survival of 64 months compared with 11 months for those who underwent surgery \( P < .008 \). However, with longer follow-up, the magnitude of this benefit diminished. Median survival was 21 months in the chemotherapy arm compared with 14 months in the control arm \( P = .056 \). The second trial also included 60 patients with stage IIIA NSCLC, and they were randomized to surgery followed by radiotherapy vs induction chemotherapy with mitomycin, ifosfamide, and cisplatin followed by surgery and radiotherapy. Median survival was 26 months for those who received chemotherapy compared with 8 months for those who received surgery alone \( P < .001 \). Both of these trials were stopped early because of positive interim analyses. Although criticized for their small sample sizes and shorter than expected survival in the control arms, these trials nevertheless generated substantial interest in the neoadjuvant approach.

For further analysis of the potential role of neoadjuvant chemotherapy, Burdett et al performed a meta-analysis of randomized trials comparing induction chemotherapy followed by surgery to surgery alone. With 7 trials and 988 patients, the combined results showed a significant increase in survival associated with neoadjuvant chemotherapy. The absolute improvement in survival was 6% at 5 years \( P = .02 \), with an 18% relative reduction in the risk of death \( HR = 0.82; 95\% CI, 0.69–0.97 \). The largest studies included in this meta-analysis were the French Thoracic Cooperative Group and the Southwest Oncology Group (SWOG) S9900 trials.

The French Thoracic Cooperative Group trial involved 355 patients with stages I (except T1N0), II, or IIIa NSCLC who were randomized to either primary surgery or preoperative chemotherapy with 2 cycles of cisplatin, mitomycin, and ifosfamide. Median survival was 37 months in the chemotherapy arm and 26 months for the surgery-alone arm \( P = .15 \). At baseline, there was an excess of patients with clinical N2 disease in the chemotherapy group \( 72 \text{ vs } 50 \text{ patients in the primary surgery group; } P = .065 \). Sixteen postsurgical deaths occurred in the preoperative chemotherapy group compared with 9 in the group randomized to primary surgery \( P = .16 \). In an unplanned subgroup analysis, patients with clinical stages I and II NSCLC appeared to benefit from preoperative chemotherapy \( HR = 0.68; 95\% CI, 0.49–0.96; P = .027 \), while patients with stage IIIA disease did not \( HR = 1.04; 95\% CI, 0.68–1.60; P = .85 \). Patients with an objective response to preoperative chemotherapy were eligible to receive postoperative chemotherapy; 84% of those patients actually received chemotherapy after surgery. Postoperative radiotherapy was administered to patients in both groups with pathological T3 or N2 disease or those with incomplete surgeries.

The SWOG S9900 trial was stopped prematurely when the positive results from the adjuvant chemotherapy trials were reported. The trial enrolled only 354 of the planned 600 patients, prior to closing the accrual. Patients with stage IB-IIIa NSCLC (excluding clinical N2 disease) were randomized to either primary surgery or 5 cycles of carboplatin and paclitaxel followed by surgery. The results of the study were updated at the 2009 World Conference on Lung Cancer (WCLC) and revealed a 41% response rate to chemotherapy with a median follow-up of 64 months \( HR \text{ for death } = 0.80; 95\% CI, 0.61–1.04; P = .11 \) in favor of chemotherapy. These results were not statistically significant; however, the authors did point out that the trial was closed to accrual early.

Finally, the largest randomized trial published that compared neoadjuvant chemotherapy to surgery alone was the Medical Research Council (MRC) LU22/Dutch Society of Pulmonologists (NVALT) 2/European Organisation for Research and Treatment of Cancer (EORTC) 08012 trial. This trial enrolled 519 patients and compared 3 cycles of platinum-based preoperative chemotherapy followed by surgery to primary surgery in patients with stages I to III NSCLC. A wide variety of chemotherapy regimens were permitted, with the most commonly used ones being cisplatin/vinorelbine and cisplatin/gemcitabine. Postoperative complications were not increased in the group receiving chemotherapy, and the overall response rate was 49%. However, the use of preoperative chemotherapy did not improve survival \( HR = 1.02; 95\% CI, 0.80–1.31; P = .86 \). At 55 months, median survival was better than expected in the surgery-alone arm.

Neoadjuvant chemotherapy trials in NSCLC are a heterogeneous group, with many differences in methods of staging, use of chemotherapy combinations,
and use of postoperative radiotherapy or chemotherapy. These differences make it challenging to compare results across trials or to utilize these data to identify patients most likely to benefit from neoadjuvant chemotherapy. Some of the trials did show promising results and, therefore, further investigation of the role of neoadjuvant chemotherapy in early-stage NSCLC remains viable. However, current guidelines recommend adjuvant chemotherapy as the standard of care for patients with resectable NSCLC.

Prognostic and Predictive Markers
In an effort to understand which patients with resected NSCLC will benefit from adjuvant chemotherapy, several prognostic and predictive markers have emerged from retrospective analyses. The term “prognostic” refers to a marker that is useful for estimating a patient outcome (such as survival) independent of therapeutic decisions, while a predictive marker is useful in making therapeutic decisions. These markers will be important in designing future trials in NSCLC and are already being incorporated into prospective clinical trials in the adjuvant and metastatic setting. Some of the main markers of interest at this time include excision repair cross complementation group 1 (ERCC1), ribonucleotide reductase messenger 1 (RRM1), breast cancer gene 1 (BRCA1), MutS homologue 2 (MSH2), and thymidylate synthase (TS).

DNA repair mechanisms are important in the resistance of NSCLC to treatment with cisplatin. The destruction of cells by cisplatin requires the binding of the drug to DNA and the creation of platinum-DNA adducts. Some of these adducts establish covalent cross-linking between DNA strands, thereby inhibiting DNA replication. Nucleotide excision has a central role in DNA repair and is associated with resistance to platinum-based chemotherapy. ERCC1 plays a rate-limiting role in the nucleotide excision repair pathway that recognizes and removes cisplatin-induced DNA adducts. Throughout the years, small retrospective studies have reported an association between low levels of expression of ERCC1 mRNA in several solid tumors, including NSCLC, and improved clinical outcomes with treatment with platinum-containing therapy. These results prompted further investigation into the potential predictive value of ERCC1 and a patient’s response to adjuvant platinum-based chemotherapy.

The IALT Biology (IALT Bio) study was subsequently designed to further explore whether the tumor markers could be used to predict a survival benefit from adjuvant cisplatin-based chemotherapy. This study examined patients who had enrolled in the IALT trial previously. The authors used immunohistochemical analysis to determine the expression of the ERCC1 protein in 761 paraffin-embedded tumor samples. The samples included 389 (51%) who were assigned to chemotherapy and 372 (49%) to the control group. The results showed that ERCC1 expression was positive in 335 patients (44%) and negative in 426 patients (56%). Expression of the enzyme was significantly correlated with age (P = .03; less common in patients < 55 years of age than in patients aged 55–64 years), with histological type (P < .001; less common in adenocarcinomas than in squamous cell carcinoma), and with pleural invasion (P = .01; less common in the absence than in the presence of pleural invasion). A benefit from cisplatin-based adjuvant chemotherapy was associated with the absence of ERCC1 (P = .009). Adjuvant chemotherapy, when compared with observation, also significantly prolonged overall survival among patients with ERCC1-negative tumors (HR for death = 0.65; 95% CI, 0.50–0.86, P = .002) but not among patients with ERCC1-positive tumors (adjusted HR for death = 1.14; 95% CI, 0.84–1.55, P = .40). Of note, the 5-year overall survival rates among patients with ERCC1-negative tumors were 47% in the chemotherapy group (95% CI, 40%–55%) and 39% in the control group (95% CI, 32%–47%). However, in the control group, the 5-year overall survival rate was significantly higher among patients with ERCC1-positive tumors than among patients with ERCC1-negative tumors (HR = 0.66; 95% CI, 0.49–0.90, P = .009). This suggests that the presence or absence of ERCC1 may be useful to determine the sensitivity of NSCLC cells to platinum-based therapy in the future.

A randomized phase III trial prospectively examined the impact of ERCC1 expression in patients with advanced NSCLC and found that the response rate was improved in the group that received customized chemotherapy based on ERCC1 expression compared with the control group (treated with cisplatin plus docetaxel regardless of ERCC1 expression). There was no difference in overall survival between the two groups. ERCC1 is now being incorporated into adjuvant chemotherapy trials as a prospective biomarker. If the results are positive, ERCC1 may become an established predictive marker in the care of patients.

RRM1 is a gene that encodes the regulatory subunit of ribonucleotide reductase, and it is involved in tumor invasiveness and metastasis. It is located on chromosome segment 11p15.5, a region with a frequent loss of heterozygosity in NSCLC and is associated with poor survival in early-stage patients. Phosphatase and tensin homologue (PTEN) is a bifunctional phosphatase that regulates the cellular signaling, survival, and migration and is thought to mediate these effects of RRM1. Increased expression of RRM1 was shown to decrease the formation of metastases, inhibit the development of carcinogen-induced lung tumors, and prolong survival in tumor-bearing mice.
A large cohort of patients with NSCLC were studied by Zheng et al. to validate RRM1 as a marker of clinical outcome in this subset of patients. The study evaluated 187 patients with early-stage NSCLC who had received only surgical treatment. High expression of RRM1 protein was noted to be associated with a better outcome. The median disease-free survival (DFS) exceeded 120 months in the group of patients with tumors that had high expression of RRM1 and was 54.5 months in the group with low expression of RRM1 (HR for disease progression in the high-expression group = 0.46; *P* = .004). The OS was more than 120 months for patients with tumors with high expression of RRM1 and 60.2 for those with low expression of RRM1 (HR for death = 0.61; *P* = .02). Ongoing randomized phase III trials will compare customized chemotherapy based on ERCC1 and RRM1 expression to standard platinum-based chemotherapy.

BRCA1 was identified in 1990 and first sequenced in 1994 as the 81-kDA gene, localized on chromosome 17q21. Deleterious BRCA1 mutations are associated with bilateral breast cancer and earlier age at onset compared to sporadic cancers. BRCA1 is a tumor suppressor gene, and the BRCA1 protein plays key roles in DNA damage detection and repair, transcriptional regulation, cell cycle control, ubiquitination, and chromatin remodeling. Although the number of patients with cancers related to germline mutations in BRCA1/2 is small, there is now ample evidence that a wider group of sporadic tumors may have BRCA-like phenotypes. BRCA expression has been reported in NSCLC and noted to be a prognostic and predictive marker. Low levels of mRNA expression were associated with prolonged survival in patients with surgically resected disease. Additionally, patients with low levels of BRCA expression had a higher likelihood of responding to cisplatin. Conversely, high BRCA expression in NSCLC was associated with platinum resistance and taxane sensitivity.

Based on its differential impact in response to platinum and taxanes, prospective clinical trials have been designed to corporate BRCA1 as a biomarker for treatment selection. The Spanish Lung Cancer Group recently reported their feasibility study utilizing BRCA mRNA expression to determine adjuvant therapy in patients with resected stage II-III NSCLC. Assignment of patients to customized chemotherapy was shown to be feasible, with no detrimental effect of treating patients with high BRCA1 levels with docetaxel alone. A randomized phase III trial utilizing the same design, the Spanish Customized Adjuvant Trial (SCAT), is ongoing. When completed, these studies are expected to add to our understanding of BRCA1 as a biomarker in NSCLC.

The enzyme TS generates thymidine monophosphate, which is subsequently phosphorylated to thymidine triphosphate for use in DNA synthesis and repair. TS is inhibited by a variety of chemotherapeutic agents, including 5-fluorouracil and pemetrexed. Pemetrexed inhibits multiple enzymes involved in purine synthesis, including TS, dihydrofolate reductase, and glycaminide ribonucleotide formyltransferase. In preclinical models, low levels of these enzymes correlated with sensitivity to pemetrexed. Differences in TS expression across histological subtypes of NSCLC have been proposed as a molecular explanation for the effect of histology on outcomes in pemetrexed-treated NSCLC patients. Indeed, in tumor specimens from NSCLC patients, TS protein and mRNA levels are significantly higher in squamous cell carcinomas compared with adenocarcinomas, suggesting that TS expression rather than histology may serve as a more reliable marker of pemetrexed sensitivity. Among the molecular markers used as potential predictors of a survival benefit from adjuvant chemotherapy, ERCC1, RRM1, and BRCA1 are the most well-established. Further investigation is necessary to confirm how influential they will be in directing the care of patients in the future.

### Molecularly Targeted Therapies

Substantial progress has been made over the past decade in the characterization of molecular targets and predictive biomarkers for selection of targeted therapy. As we know, lung tumors are the result of a multistep process in which normal lung cells accumulate multiple genetic and epigenetic abnormalities and evolve into cells with malignant biological capabilities. Recent advances in the understanding of the complex biology of NSCLC, particularly the activation of oncogenes by mutation, translocation, and amplification, have provided new treatment targets and allowed the identification of subsets of tumors with unique molecular profiles that can predict response to therapy in this disease. The identification of specific genetic and molecular abnormalities in tumor tissue specimens and the administration of specific inhibitors to those targets are the basis of personalized cancer treatment.

The successful development of personalized therapy depends on the identification of a specific molecular target that drives cancer growth, subsequent validation of a clinically applicable biomarker, and development of a clinically sound and rational end-point, coupled with the understanding of the molecular mechanisms associated with the tumor's resistance. In lung adenocarcinoma, at least two different major pathways have been identified in its pathogenesis. Those 2 pathways include a smoking-associated activation of KRAS signaling and a nonsmoking-associated activation of epidermal growth factor receptor (EGFR) signaling. Lung adenocarcinomas arising in
never-smokers or light smokers are characterized by markedly higher frequencies of a series of targetable oncogene abnormalities, including EGFR and HER2 tyrosine kinase domain-activating mutations and EML4-ALK translocation. Squamous cell carcinoma of the lung has been less histologically and molecularly studied than adenocarcinoma. Squamous cell also harbors genetic abnormalities, resulting in activation of oncogenes, including EGFR-vIII and DDR2 mutations and fiberblast growth factor receptor 1 (FGFR1) gene amplification.

EGFR belongs to a family of receptor tyrosine kinases that includes EGFR/ERBB1, HER2/ERBB2, HER3/ERBB3, and HER4/ERBB4. Binding of ligands, including epidermal growth factor, phosphorylates EGFR and results in a number of downstream effects such as cell growth, proliferation, and survival. These effects are mediated primarily through the PI3K-AKT-mTOR and RAS-RAF-MEK-ERK signaling pathways. Mutations of EGFR occur in approximately 24% of adenocarcinomas and up to 60% in tumors from never-smokers. The mutations are limited to the first 4 exons of the tyrosine kinase domain, exons 18 to 21, with the most frequent mutations occurring as in-frame deletions in exon 19 (44% of all mutations) and missense mutations in exon 21 (41% of all mutations). Several trials, including the International Tarceva vs Chemotherapy trial (EURTAC), have helped support EGFR mutation status as a powerful predictive marker for response to EGFR tyrosine kinase inhibitors (TKIs) in metastatic NSCLC. The National Comprehensive Cancer Network (NCCN) has recommended testing all advanced NSCLC adenocarcinomas for EGFR mutations. In EGFR-positive patients, EGFR TKI therapy is recommended in the first-line setting, based on the superior response rates and progression-free survival (PFS), as well as a more favorable toxicity profile observed across multiple clinical trials. The role of EGFR TKI therapy in the adjuvant setting remains under investigation, and several clinical trials incorporating EGFR mutation status into adjuvant treatment assignment are underway.

In lung cancer, ALK translocations have been identified in a subset of adenocarcinomas, and this abnormality consists of the formation of a fusion transcript with cell-transforming activity, which is the product of an inverted translocation of EML4 gene located at chromosome 2p21 and the ALK gene located at 2p23. EML4-ALK translocation has been detected in 7% of lung adenocarcinomas, particularly in patients with a history of being never-smokers or light smokers, and is associated with early onset of tumor. The standard method to assess EML4-ALK fusion in lung cancer tumors is fluorescence in situ hybridization (FISH). Crizotinib is an oral, small molecule inhibitor of ALK, with proven preclinical and clinical activity in NSCLC harboring ALK fusions. The drug was evaluated in the phase I setting, with an expansion cohort at the recommended phase II dose for patients with ALK activation. In the 82 NSCLC patients enrolled in the molecular expansion cohort, the overall response rate was 60.8%, and an additional 22% had stable disease. The US Food and Drug Administration (FDA) approved crizotinib in 2011 for the treatment of NSCLC with evidence of ALK translocation as determined by FISH.

KRAS mutations are more common in lung adenocarcinoma than other NSCLC histological types and are more frequently found in tumors from patients with a smoking history (approximately 30%). In lung cancer, KRAS mutations are found in codons 12, 13, and 61, which are mainly GGT to TGT transversions that produce glycine to cysteine amino acid changes. KRAS mutations are rarely detected in EGFR-mutant tumors. Although RAS has proven a challenging molecular target, recent studies have evaluated downstream molecules in the RAS/RAF/MEK pathway as potential therapeutic targets in lung cancer. In the metastatic setting, the MEK1 inhibitor, selumetinib, combined with docetaxel, demonstrated improved PFS and a trend toward improved overall survival compared with docetaxel alone. Numerous clinical trials of agents targeting this pathway are currently underway in the metastatic setting.

BRAF is a serine-threonine protein kinase that functions in the RAS/mitogen-activated protein kinase signaling pathway. BRAF is downstream of KRAS and directly phosphorylates MEK. Subsequent phosphorylation of ERK activates genes involved in proliferation and survival. Mutant BRAF proteins have increased kinase activity and are transforming in vitro. BRAF mutations have been described in multiple cancer types, including NSCLC, where 1% to 3% of cancers are affected. In NSCLC, BRAF mutations are seen almost exclusively in adenocarcinomas and, in contrast to EGFR and ALK, appear to be more common in current and former smokers. However, the number of patients described to date is small.

In malignant melanoma, BRAF mutations occur in over 50% of patients, and the vast majority of mutations occur at valine 600 (v600) within exon 15 of the kinase domain. In lung cancer, several different BRAF mutations, including V600E, have been described. Preclinical work has suggested that mutant BRAF plays a role in lung adenocarcinoma initiation and maintenance. Clinically, BRAF inhibitors have been found to be effective in BRAF-mutated melanoma, and the FDA recently approved the BRAF inhibitor vemurafenib in V600E-mutated melanoma. This drug and a number of other BRAF inhibitors are currently being tested in patients with BRAF-mutant NSCLC. Importantly, non-V600E BRAF-mutant lung cancer cell lines exhibited resistance to vemurafenib.
HER2 (ERBB2) is a member of the ERBB family of receptor tyrosine kinases, which also includes EGFR (ERBB1), HER3 (ERBB3), and HER4 (ERBB4). Growth factor binding results in heterodimerization of HER2 and another member of the ERBB family. Activation of HER2 in this way initiates the PI3K-AKT-mTOR and RAS-RAF-MEK-ERK pathways, promoting cell survival and proliferation. HER2 appears to be the preferred dimerization partner of all members of the ERBB family. Deregulation of HER2 can occur through protein overexpression, gene copy number gain, or somatic mutation. While HER2 overexpression or gene copy number gains are relatively common in NSCLC, HER2 mutations are found in only 2% to 4% of NSCLC cases. The most common mutation is an in-frame insertion in exon 20. Clinically, HER2 mutations appear to be more common in women and in never-smokers with adenocarcinoma histology.

The HER2 monoclonal antibody, trastuzumab, and the TKI, lapatinib, have been evaluated in NSCLC patients. Trastuzumab combined with chemotherapy in unselected patients did not result in improved outcomes compared with historical controls treated with chemotherapy alone. Similarly, single-agent lapatinib in an unselected population of patients with NSCLC demonstrated an overall response rate of only 1.3%. Individual cases of response to HER2 targeted therapy in patients with HER2 mutations have been reported. In the latter report, De Grève et al described partial responses to the pan-HER inhibitor, afatinib, in 3 heavily pretreated patients with HER2 mutations. Clinical trials targeting lung cancer patients with HER2 mutations are underway.

Angiogenesis has long been recognized as a hallmark of malignant transformation. While small tumors (< 2 mm) are able to extract oxygen and nutrients via diffusion, larger tumors require the formation of a neovasculature to sustain their growth. Secretion of proangiogenic factors by tumors results in the recruitment of endothelial precursor cells to the tumor site, proliferation of these cells, and capillary tube formation. Increased angiogenic signaling has been associated with poor prognosis in a number of malignancies, including NSCLC. The importance of angiogenesis in tumor progression and metastasis spurred efforts to develop therapies targeting components of angiogenic signaling. Bevacizumab is a humanized monoclonal antibody with a high affinity for vascular endothelial growth factor (VEGF). By binding circulating VEGF, bevacizumab inhibits binding of VEGF to its receptors, interrupting downstream proangiogenic signaling.

A randomized phase II trial in 99 patients with advanced NSCLC compared carboplatin and paclitaxel with or without bevacizumab (at a dose of 7.5 mg/kg or 15 mg/kg). Bevacizumab in combination with chemotherapy resulted in higher response rates and improved PFS (at the 15 mg/kg dose). Six severe bleeding events (4 of which were fatal) occurred on the bevacizumab arm of this trial and were associated with squamous histology, central tumor location, and cavitation. The promising results of the phase II trial led to the landmark ECOG 4599 trial, which randomized patients to either carboplatin and paclitaxel alone or in combination with bevacizumab at a dose of 15 mg/kg with continuation of bevacizumab until progression. Due to the safety concerns raised in the phase II study, only patients with nonsquamous histologies were included. Significant improvements were observed for response rates (35% vs 15%), PFS (6.2 vs 4.5 months), and overall survival (12.3 vs 10.3 months) in the bevacizumab arm. Based on the results of ECOG 4599, bevacizumab was approved by the FDA in combination with chemotherapy in the first-line setting for patients with nonsquamous NSCLC. Bevacizumab combined with chemotherapy is currently being compared to chemotherapy alone in the adjuvant setting in a large, randomized phase III study (ECOG 1505). Numerous studies combining bevacizumab with other agents are underway in advanced NSCLC, and other drugs targeting angiogenesis are also in development in NSCLC. However, initial results with small-molecule TKIs targeting angiogenesis have been disappointing in NSCLC.

FGFR1 is a transmembrane tyrosine kinase and a member of the FGFR tyrosine kinase family that comprises 4 kinases. In lung cancer, amplification of FGFR1 is important in the development of NSCLC and is predominately detected in squamous cell carcinomas (approximately 20%) compared with adenocarcinomas (1%–3%). Currently, FISH is the preferred method to assess FGFR1. Several FGFR inhibitors are in early clinical development, and these preclinical studies provide a rationale for targeting FGFR in a molecularly subset of NSCLC.

DDR2 is a tyrosine kinase, and mutations of this mechanism have been described in 4% of lung squamous cell carcinomas. DDR2 promotes cell migration, proliferation, and survival. In addition to sequencing DDR2 and describing the mutation rate in squamous cell lung cancer patient specimens, Hammerman et al demonstrated the sensitivity of DDR2 mutant cells to dasatinib in vitro. Dasatinib inhibits tyrosine kinases, including DDR2. While no clinical trials have specifically targeted patients with DDR2 mutations, prospective trials of dasatinib are anticipated in this patient population.

**Immunotherapy**

Despite advances in the treatment of NSCLC with chemotherapy and the integration of targeted therapy,
overall outcomes remain poor. While supportive care has improved significantly, toxicity of many therapies precludes long-term use.

The use of immunotherapy is under investigation as a less toxic approach to increase the treatment success rates. A better understanding of the immunology of cancer has led to novel treatment strategies, including vaccine therapy and immune checkpoint modulation.

The glycoprotein mucin 1 (MUC1) promotes cellular adhesion and is expressed by a number of epithelial tissues and carcinomas. MUC1 expressed in malignant cells has been shown to differ structurally from MUC1 expressed in normal tissues. BLP-25 is a liposomal vaccine preparation that targets the exposed peptide core of MUC1 expressed in malignant tissues. Promising preclinical work with the BLP-25 vaccine led to a phase I trial that demonstrated safety in patient with advanced NSCLC. Subsequently, a randomized phase IIIB study was conducted in 171 patients with stage IIIIB or IV lung cancer who had not progressed after front-line chemotherapy. While the median survival did not differ statistically, a trend was observed in favor of the vaccine, with a median overall survival of 17.2 months compared with 13 months with best supportive care (BSC; \( P = .112 \)). The study included patients with locoregional disease and, for these patients, a 2-year survival rate of 60% was observed compared with 36.7% in the BSC arm. Updated results published in 2011 demonstrated a statistically significant improvement in the 3-year survival rate: 31% for patients in the BLP-25 arm compared with 17% for those in the BSC arm.

Two randomized phase III trials have been launched to further evaluate BLP-25 in patients with NSCLC. The START trial is an international phase III placebo-controlled trial of BLP-25 in patients with unresectable stage III NSCLC who had completed chemotherapy and radiation. Preliminary results of this trial were reported recently. The study did not meet the primary end point of improved overall survival (median overall survival 25.6 months with BLP-25 vs 22.3 months with placebo [adjusted HR = 0.88; 95% CI, 0.75–1.03, \( P = .123 \)]. A predefined subgroup analysis demonstrated a statistically significant benefit for patients who received concurrent chemoradiotherapy plus vaccine, while no benefit was observed in the subgroup who received sequential chemotherapy and radiation. The ongoing INSPIRE trial has a similar design to START but is being conducted in Asia.

In the adjuvant setting, the MAGE-A3 vaccine is under evaluation. MAGE-A3 is a protein that is produced almost exclusively by malignant cells and occurs in 35% of NSCLC cases. Vansteenkiste et al conducted a phase II randomized, placebo-controlled trial in patients with completely resected stages IB and II NSCLC. A trend in favor of improved overall and disease-free survival was observed in this study. These results prompted the initiation of a randomized phase III trial, MAGRIT, in patients with MAGE-A3-positive patients with stages IB-IIIA NSCLC.

Compounds targeting CTLA-4, PD-1, and PDL-1 are under investigation in patients with advanced NSCLC. Ipilimumab is a fully humanized monoclonal antibody that augments antitumor immunity via blockade of CTLA-4, a regulatory molecule found on the surface of activated T cells and involved in immune downregulation. Ipilimumab has been shown to improve overall survival in patients with metastatic melanoma, leading to its FDA approval in this disease. In NSCLC, Lynch et al conducted a 3-arm phase II study of chemotherapy and ipilimumab in untreated patients with advanced disease. The control group received carboplatin and paclitaxel with placebo for 6 cycles. One of the experimental groups received chemotherapy plus ipilimumab for 4 cycles followed by chemotherapy and placebo for 2 additional cycles (concurrent approach), while the other experimental group received 2 cycles of chemotherapy plus placebo followed by 4 doses of ipilimumab plus chemotherapy (phased approach). A statistically significant improvement (HR = 0.7; \( P = .02 \)) in PFS was observed for patients initially treated with the phased approach; a subgroup analysis demonstrated greater improvement in PFS for patients with squamous histology who were treated with the phased approach. As a result of these findings, a phase III randomized trial comparing carboplatin plus paclitaxel with the phased ipilimumab regimen is underway in patients with squamous histology, advanced NSCLC (NCT01285609). The combination of phased ipilimumab and chemotherapy is also being evaluated in the neoadjuvant setting for patients with early-stage NSCLC (NCT01820754).

The PD-1 receptor is a coinhibitory receptor present on T cells. Binding of ligands PD-L1 or PD-L2 (produced by tumor or stromal cells) to PD-1 results in inhibition of antitumor immune responses. Topalian et al evaluated an anti–PD-1 monoclonal antibody (MDX-1106) in previously treated patients with advanced solid tumors, including NSCLC. The study enrolled 296 patients, 122 with advanced NSCLC. Of these 122 patients, 76 were included in the efficacy analysis. Objective responses were noted in 14 patients with NSCLC across the three different dose cohorts (1.0 mg/kg, 3.0 mg/kg, and 10.0 mg/kg). Six of 18 patients with squamous cell carcinoma achieved a partial or complete response by RECIST 1.0, while 7 of 56 patients with nonsquamous histology achieved an objective response. An additional 5 patients with nonsquamous histology exhibited stable disease for more than 24 weeks. Common adverse events included fatigue, rash, diarrhea, decreased appetite, nausea, and pruritus. Three deaths were at-
tributed to treatment-related pneumonitis. Brahmer et al\textsuperscript{14} reported similar results in a phase I study of a fully humanized monoclonal antibody against PD-L1 (MDX-1105). While these agents are showing promise in the metastatic setting, evaluation in earlier-stage patients remains limited at present.

Conclusions

Multiple randomized phase III trials have demonstrated an improvement in survival for patients who undergo adjuvant chemotherapy and similar results have been observed for neoadjuvant chemotherapy. However, the prognosis for non–small-cell lung cancer remains poor, with high relapse rates even when detected at the earliest stages. Furthermore, the selection of patients for adjuvant therapy remains imprecise, with only a relatively small population of patients appearing to benefit from treatment. In the metastatic setting, treatment is moving away from large trials of chemotherapy in unselected populations. Instead, trials focus early on defining subsets of patients who will benefit most from a therapy, often developing a diagnostic assay in conjunction with a new treatment. At the same time, the rapid evolution of gene sequencing technology combined with a decline in the cost of this technology is making complete molecular profiling of each patient's tumor a realistic goal.

References


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