The Evolving Role of Chemotherapy and Predictive Markers in Early-stage Non-small-cell Lung Cancer

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Abstract
Lung cancer continues to be the leading cause of cancer mortality worldwide. The utility of traditional chemotherapy has reached a plateau in both the metastatic and adjuvant settings. However, we have entered an era of better targeting of both traditional cytotoxic agents and newer compounds for select subsets of patients based on genetic analysis of the tumour. Substantial evidence supports the use of adjuvant chemotherapy in both stage II and stage III non-small-cell lung cancer (NSCLC), but it remains unclear which combination of either traditional or targeted therapies will work best for an individual patient. The use of chemotherapy and targeted therapies in patients with resected stage I NSCLC remains controversial because we cannot predict who benefits from its usage. This article focuses on the evolving role of predictive biomarkers in early-stage NSCLC and summarises the trials that are prospectively evaluating the use of those biomarkers to individualise treatment of NSCLC.

Keywords
Early-stage non-small-cell lung cancer, chemotherapy, predictive markers

Lung cancer is the leading cause of cancer mortality worldwide. Surgery remains the optimal treatment for early-stage non-small-cell lung cancer (NSCLC); however, five-year survival rates for resected NSCLC without additional treatment range from 20 to 40% for stage IIIA disease to 70 to 80% for stage IIA disease.1 Mortality occurs most commonly from recurrences at sites outside the lung, suggesting that the cancer had spread before surgery.

The goal of systemic therapy in patients with resected NSCLC is to eradicate the prior seeded micrometastases to improve overall survival. Several clinical trials and meta-analyses have shown improved survival in resected NSCLC with different combinations of chemotherapy.2–7 These analyses are still based on empiric treatment and do not address the question of who will benefit the most from therapy and who may be harmed. The ultimate goal of adjuvant NSCLC treatment is to predict which patients will benefit from the use of chemotherapy, targeted therapy and radiation therapy, and to individualise therapy based on the patient’s tumour characteristics, including histology and genetic analysis. Recently, trials have begun to use novel biomarkers as a first step towards individualised adjuvant therapy.

In this article, we will give a brief historical background on the use of chemotherapy in the adjuvant setting, followed by previously investigated biomarkers to guide adjuvant therapy and end with a discussion of ongoing clinical trials to address the important question of how to personalise adjuvant therapy.

History of the Use of Chemotherapy in Resected Non-small-cell Lung Cancer
Initial use of chemotherapy in NSCLC was only for metastatic disease. In 1991, an international panel recommended against the routine use of adjuvant chemotherapy outside of clinical trials.8 Four years later a meta-analysis was undertaken to investigate the role that chemotherapy after surgery had played in trials up to that point.9 Fourteen trials were evaluated and the meta-analysis showed a 5% improvement in five-year survival but failed to reach statistical significance. Owing to this encouraging result and the established benefit of adjuvant chemotherapy in multiple other solid tumours, several follow-up studies were initiated.

The follow-up clinical trials validated the use of adjuvant chemotherapy in resected NSCLC.10 The major adjuvant trials are beyond the scope of this article; however, they are highlighted in Table 1.

Although not all the trials were positive, a large meta-analysis of the data has reaffirmed the use of adjuvant chemotherapy. The Lung adjuvant cisplatin evaluation (LACE) meta-analysis was conducted to identify treatment options for post-operative chemotherapy.11 Data from 4,584 patients were pooled from the five largest adjuvant trials (Adjuvant lung project Italy [ALPI],12 Adjuvant International Trialist Association [ANITA],13 Big lung trial [BLT],14 International adjuvant lung cancer trial [BALT] and ICR 1.0). The overall hazard ratio (HR) was 0.89, corresponding to a five-year overall survival benefit of 5.4% from...
regardless of therapy. Examples of this include tumour stage, tumour size
as a prognostic marker is one that indicates survival benefit (or detriment)
prognostic and predictive markers that are promising emerging prognostic and predictive markers that are concerning. Although we still have a lot to learn about the basic
non-cancer mortality. In this large trial, initial analysis indicated an overall
long-term IALT data indicating potential harm in the form of increased
survival, we will need to identify those patients most at risk for
highlight the fact that we do not yet understand who will be most helped
chemotherapy, whereas those with high ERCC1 levels were less likely
to benefit. The rationale was that DNA damaged by cisplatin was unable
to undergo repair, resulting in greater tumour cell death. In another
form of increased non-cancer mortality: in this large trial, initial analysis indicated an overall
survival benefit, but with longer-term follow-up to 7.5 years, the
significance of the benefit was lost and there was an indication of excess
non-cancer mortality in the chemotherapy arm. This increased non-
cancer death and loss of benefit over time has not been seen in other
adjuvant trials, including JBR.10 and ANITA, but the IALT long-term data
evaluated and ERCC1 expression was positive in 426 (56%). A benefit of cisplatin-based adjuvant chemotherapy was associated with the absence of ERCC1 (test for interaction; p=0.009). Adjuvant chemotherapy, as compared with observation, significantly prolonged survival among patients with ERCC1-negative tumours (HR 0.65; 95% confidence interval [CI], 0.50–0.86; p=0.002) but not among patients with ERCC1-positive tumours (HR 1.14, 95% CI 0.84–1.55; p=0.40). Patients with ERCC1-positive tumours who did not receive adjuvant chemotherapy survived longer than those with ERCC1-negative tumours (adjusted HR for death 0.66, 95% CI 0.49–0.90; p=0.009). The result suggested that completely resected ERCC1-negative NSCLC would benefit from a cisplatin-based adjuvant chemotherapy, whereas those with high ERCC1 levels were less likely to benefit. The rationale was that DNA damaged by cisplatin was unable to undergo repair, resulting in greater tumour cell death. In another study, Simon et al. evaluated the effect of intratumoural ERCC1 expression on survival in NSCLC patients who underwent surgical resection for cure. Using realtime polymerase chain reaction (RT-PCR) analysis of ERCC1 normalised to 18S ribosomal RNA expression, an ERCC1 value of 50 revealed a statistically significant difference in median survival for patients with ERCC1 expression of >50 (94.6 months) compared with patients with ERCC1 expression of <50 (35.5 months, p=0.01). The value of ERCC1 has yet to be tested prospectively, but there are several randomised prospective clinical trials (Southwest Oncology Group [SWOG] S0720, Tailored post-surgical therapy in early-stage NSCLC [TASTE] and International tailored chemotherapy adjuvant [ITACA]) testing the utility of ERCC1 as a biomarker in adjuvant NSCLC treatment (see below).

### Ribonucleotide Reductase M1
The regulatory subunit of ribonucleotide reductase M1 (RRM1) is essential for nucleotide excision repair. RRM1 expression was studied

### The Evolving Role of Biomarkers for Choosing Chemotherapy in Resected Non-small-cell Lung Cancer
The limited benefit of using adjuvant chemotherapy seen so far in trials, and the lack of benefit in certain subsets, such as most stage IB tumours, highlight the fact that we do not yet understand who will be most helped by treatment and which agents to use. To make further improvements in survival, we will need to identify those patients most at risk for recurrence and determine which chemotherapy will work the best in each person. In addition, we will need to identify those at low risk to avoid the unnecessary toxicity of chemotherapy, a point highlighted by the long-term IALT data indicating potential harm. In this large trial, initial analysis indicated overall survival benefit, but with longer-term follow-up to 7.5 years, the significance of the benefit was lost and there was an indication of excess non-cancer mortality in the chemotherapy arm. This increased non-cancer death and loss of benefit over time has not been seen in other adjuvant trials, including JBR.10 and ANITA, but the IALT long-term data are concerning. Although we still have a lot to learn about the basic biology of NSCLC, which is a very heterogeneous disease, there are some promising emerging prognostic and predictive markers that are beginning to be incorporated into our decisions for adjuvant therapy. A prognostic marker is one that indicates survival benefit (or detriment) regardless of therapy. Examples of this include tumour stage, tumour size and patient sex. A predictive maker is one that predicts for differential benefit from a particular therapy. Although we will not discuss prognostic markers here in much detail, there have been several early-stage prognostic biomarkers published (see Table 2). The predictive markers and how they fit into the ongoing clinical trials are discussed below. Although there is a great need for biomarkers in NSCLC, each biomarker must be rigorously tested and validated before it is implemented.

### Table 1: Post-1995 Meta-analysis – Non-small-cell Lung Cancer Randomised Adjuvant Platinum Trials

<table>
<thead>
<tr>
<th>Trial</th>
<th>Stage</th>
<th>n</th>
<th>Chemotherapy</th>
<th>Significant?</th>
<th>Survival Benefit</th>
<th>Long-term Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>E3590</td>
<td>II–IIA</td>
<td>488</td>
<td>Cis/VP16</td>
<td>No</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>ALPI</td>
<td>II–III</td>
<td>1,209</td>
<td>Cis/MVd</td>
<td>No</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>BLT</td>
<td>II–III</td>
<td>381</td>
<td>Cis/4 options</td>
<td>No</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>IALT</td>
<td>II–III</td>
<td>1,867</td>
<td>Cis/Vinca or VP16</td>
<td>Yes</td>
<td>4% at 5 years</td>
<td>Benefit lost at 7 years</td>
</tr>
<tr>
<td>JBR.10</td>
<td>IB–II</td>
<td>482</td>
<td>Cis/Vinca</td>
<td>Yes</td>
<td>11% at 5 years</td>
<td>Benefit retained &gt;9 years</td>
</tr>
<tr>
<td>CALGB</td>
<td>IB</td>
<td>344</td>
<td>Carbo/Pac</td>
<td>Yes</td>
<td>20% at 4 years</td>
<td>Benefit lost at 57 and 74 months</td>
</tr>
<tr>
<td>ANITA</td>
<td>I–IIIA</td>
<td>840</td>
<td>Cis/Vinca</td>
<td>Yes</td>
<td>8.6% at 5 years</td>
<td>Benefit retained at 7 years</td>
</tr>
</tbody>
</table>

**ANITA = Adjuvant Navelbine® international trialist association trial; ALPI = Adjuvant lung project Italy; BLT = Big lung trial; CALGB = Cancer and leukemia group B; Carbo = carboplatin; Cis = cisplatin; IALT = International adjuvant lung cancer trial; MVI = mitomycin C and vindesine; Pac = paclitaxel; Vinca = vinca alkaloid; VP16 = etoposide.**

### Table 2: Early-stage Non-small-cell Lung Cancer Prognostic Biomarkers

<table>
<thead>
<tr>
<th>Marker</th>
<th>Trial</th>
<th>n</th>
<th>HR Survival</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSH2</td>
<td>IALT</td>
<td>768</td>
<td>HR 0.66; p=0.01</td>
<td>Kamal et al., 2010⁶⁶</td>
</tr>
<tr>
<td>ERCC1</td>
<td>IALT</td>
<td>761</td>
<td>HR 0.66; p=0.009</td>
<td>Olaussen et al., 2006⁶⁶</td>
</tr>
<tr>
<td>MRP1</td>
<td>IALT</td>
<td>782</td>
<td>HR 1.37; p=0.007</td>
<td>Filipits et al., 2007⁷⁹</td>
</tr>
<tr>
<td>p53 expression</td>
<td>JBR.10</td>
<td>253</td>
<td>HR 1.89; p=0.03</td>
<td>Tsaö et al., 2007⁷⁹</td>
</tr>
<tr>
<td>β-Tubulin III</td>
<td>JBR.10</td>
<td>256</td>
<td>HR 1.72; p=0.04</td>
<td>Seve et al., 2007⁷⁹</td>
</tr>
<tr>
<td>Ras mutation</td>
<td>JBR.10</td>
<td>253</td>
<td>Not prognostic</td>
<td>Tsaö et al., 2007⁷⁹</td>
</tr>
<tr>
<td>MET</td>
<td>Retrospective</td>
<td>447</td>
<td>HR 0.66; p=0.04</td>
<td>Tsaö et al., 2007⁷⁹</td>
</tr>
<tr>
<td>BRCAl</td>
<td>Retrospective</td>
<td>126</td>
<td>HR 1.98; p=0.02</td>
<td>Rosell et al., 2007⁷⁹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>58</td>
<td>HR 2.4; p=0.04</td>
<td></td>
</tr>
</tbody>
</table>

**BRCAl = breast cancer 1; ERCC1 = excision repair cross-complementation group 1; HR = hazard ratio; IALT = international adjuvant lung cancer trial.**

### Excision Repair Cross-complementation Group 1
The excision repair cross-complementation group 1 (ERCC1) protein functions in the nucleotide excision repair pathway and is required for the proper repair of DNA after damage from insults such as ultraviolet (UV) light or cisplatin. ERCC1 was one of the first proteins to be studied in a retrospective fashion using immunohistochemistry (IHC) on operative specimens taken from patients who had participated in the IALT trial. In the ‘IALT-Bio’ analysis, 761 tumour specimens were evaluated and ERCC1 expression was positive in 33% (44%) and negative in 426 (56%). A benefit of cisplatin-based adjuvant chemotherapy was associated with the absence of ERCC1 (test for interaction; p=0.009). Adjuvant chemotherapy, as compared with observation, significantly prolonged survival among patients with ERCC1-negative tumours (HR 0.65; 95% confidence interval [CI], 0.50–0.86; p=0.002) but not among patients with ERCC1-positive tumours (HR 1.14, 95% CI 0.84–1.55; p=0.40). Patients with ERCC1-positive tumours who did not receive adjuvant chemotherapy survived longer than those with ERCC1-negative tumours (adjusted HR for death 0.66, 95% CI 0.49–0.90; p=0.009). The result suggested that completely resected ERCC1-negative NSCLC would benefit from a cisplatin-based adjuvant chemotherapy, whereas those with high ERCC1 levels were less likely to benefit. The rationale was that DNA damaged by cisplatin was unable to undergo repair, resulting in greater tumour cell death. In another study, Simon et al. evaluated the effect of intratumoural ERCC1 expression on survival in NSCLC patients who underwent surgical resection for cure. Using realtime polymerase chain reaction (RT-PCR) analysis of ERCC1 normalised to 18S ribosomal RNA expression, an ERCC1 value of 50 revealed a statistically significant difference in median survival for patients with ERCC1 expression of >50 (94.6 months) compared with patients with ERCC1 expression of <50 (35.5 months, p=0.01). The value of ERCC1 has yet to be tested prospectively, but there are several randomised prospective clinical trials (Southwest Oncology Group [SWOG] S0720, Tailored post-surgical therapy in early-stage NSCLC [TASTE] and International tailored chemotherapy adjuvant [ITACA]) testing the utility of ERCC1 as a biomarker in adjuvant NSCLC treatment (see below).
in patients with early-stage NSCLC who had only undergone resection with no adjuvant chemotherapy. In this study, patients with high RRM1 expression had a median disease-free survival exceeding 120 months compared with patients with low RRM1 expression who had a median survival of 54.5 months (HR for disease progression or death in the high expression group, 0.46; p=0.004). The overall survival was more than 120 months for patients with tumours with high levels of RRM1 expression and 60.2 months for those with low levels of RRM1 expression (HR for death, 0.61; p=0.02).

RRM1 is the molecular target of gemcitabine and high levels of RRM1 have been linked to gemcitabine-resistance in advanced NSCLC. A small prospective phase II clinical trial in patients with locally advanced, non-resectionable NSCLC revealed that RRM1 expression was significantly (p=0.002) and inversely correlated (r = -0.498) with disease response to platinum plus gemcitabine. A randomised phase III trial of 170 patients who received either gemcitabine versus gemcitabine plus carboplatin revealed that low RRM1 and ERCC1 expression was significantly correlated with disease response (r = -0.41; p=0.001 for RRM1; r = -0.39; p=0.003 for ERCC1). A model for response prediction that included RRM1, ERCC1 and the treatment arm was highly predictive of the treatment response observed (p=0.0005). RRM1 expression appears to correlate with ERCC1 expression (p=0.001) in both early- and late-stage NSCLC and improves the prognostic utility. The SWOG S0720 trial listed below will use the levels of both RRM1 and ERCC1 expression in patients with resected stage I NSCLC to determine whether or not they should receive cisplatin plus gemcitabine as adjuvant chemotherapy versus observation.

Epidermal Growth Factor Receptor

The utility of single-agent tyrosine kinase inhibitors (TKIs) as first-line therapy in patients with advanced NSCLC (stage IIIb or IV) was demonstrated in the Iressa pan Asia study (IPASS) trial. IPASS was a phase III study that randomised never/light ex-smoking Asian patients who had never received therapy to receive either gefitinib or carboplatin/paclitaxel. It demonstrated a significant progression-free survival benefit for the gefitinib arm (HR = 0.74; p<0.0001). This revealed that in carefully selected patients with advanced disease, gefitinib may be superior to chemotherapy. By contrast, a phase III trial using gefitinib (Iressa survival evaluation in lung cancer [ISEL] trial) as second- or third-line therapy in patients with advanced NSCLC did not reveal a statistical advantage over placebo in an unselected population. Another TKI, erlotinib, was originally approved for second- or third-line monotherapy in advanced NSCLC after a randomised, placebo-controlled, phase III study (BR.21) demonstrated an increase in survival for these patients from 4.7 to 6.7 months. Following on from the mixed success of the use of TKIs as a therapy in patients with advanced NSCLC, their use in maintenance and adjuvant therapies was tested. The SWOG 0023 trial enrolled unresectable stage III NSCLC patients to evaluate gefitinib as a maintenance therapy following definitive concurrent cisplatin/etoposide and consolidative docetaxel. This trial was a randomised, placebo-controlled trial in an unselected patient population and resulted in an unexpected survival detriment for gefitinib. Owing to the negative gefitinib trials SWOG 0023 and ISEL, the double-blinded, prospective randomised placebo-controlled phase III trial JBR.19 using gefitinib was closed early. This study was designed to investigate the role of adjuvant gefitinib in resected stage IIB–IIB NSCLC. In this under powered study, gefitinib did not improve disease-free and overall survival. Subgroup analysis showed that KRAS mutation status, epidermal growth factor receptor (EGFR) by fluorescence in situ hybridisation (FISH) or EGFR sensitising mutation status were neither prognostic nor predictive of survival. The focus has now shifted from gefitinib to erlotinib. The Randomised double-blind trial in adjuvant NSCLC with Tarceva® (RADIANT), which requires EGFR overexpression to be detected by IHC, will investigate the role of erlotinib in the adjuvant setting. In addition, there are ongoing non-randomised studies exploring the use of adjuvant erlotinib in patients with EGFR mutations in resected NSCLC (TASTE and Massachusetts General Hospital trials discussed below). Despite the promising results seen with use of the EGFR TKIs as first-line therapy in those with EGFR mutations, their role as adjuvant therapy even in this setting remains investigational.

Although EGFR mutations are being used to predict who will respond to TKIs, the utility of evaluating EGFR expression using IHC or FISH analysis is controversial. Although there are data to suggest that increased EGFR copy number detected by FISH predicts responsiveness to antibody therapy, there is no clear predictive value of EGFR FISH for treatment with TKIs. Although FISH is not being explored in the adjuvant setting, EGFR copy number or increased EGFR expression detected by IHC are markers for poor prognosis in the JBR.10 trial in phase II biomarker validation studies for erlotinib (NCCTG-N0723 – closed) and cetuximab (SWOG S0819) in patients with advanced NSCLC.

The mutation or loss of p53 can lead to genomic instability resulting in an aggressive tumour with a poor prognosis. There have been many meta-analyses looking at both p53 protein levels and mutational status as a prognostic marker. In the IBR.10 trial, overexpression of p53 protein detected by IHC was a marker for poor prognosis but the p53 mutational status was not prognostic for survival. Overexpression of p53 protein evaluated by IHC predicted survival
Lung Cancer

**Figure 3: ECOG 1505 Trial Overview**

Eligible: n=1,500
Resected IB-IIIA
≥ Lobectomy
No prior chemotherapy
No planned XRT

Stratified:
- Stage
- Histology
- Gender
- Chemotherapy regimen:
  - Investigator choice
  - Cisplatin/lycosine
  - Cisplatin/docetaxel
  - Cisplatin/gemcitabine
  - Cisplatin/pemetrexed

Randomised
Chemotherapy x 4 cycles

Primary end-point: overall survival.
ECOG = Eastern Cooperative Oncology Group; XRT = radiotherapy.

**Figure 4: MAGRIT Trial Overview**

Eligible: n=2,270
Resected I–IIIA
MAGE A3 Expression (35–50% of NSCLC)

Randomised
Chemotherapy

 qualitative expression was significantly associated with resistance to antimicrotubule reagents.40 Further prospective studies are needed to validate the utility of these genetic biomarkers for clinical treatment decisions.

**Insulin-like Growth Factor Receptor 1**

The prognostic role of insulin-like growth factor receptor 1 (IGFIR) expression in surgically resected NSCLC was recently undertaken.28 IGFIR expression was evaluated by IHC in tissue microarray sections. Although a positive IGFIR expression was significantly associated with squamous cell histology, there was no difference in survival between the positive and negative groups. Therefore, IGFIR expression does not appear to be a prognostic factor in resected NSCLC patients. If IGFIR targeted agents prove to have some efficacy in advanced stage NSCLC, they are likely to be further investigated in early-stage disease.

**Breast Cancer 1**

Breast cancer 1 (BRCA1) is a member of the DNA mismatch repair pathway. Overexpression of BRCA1 correlates with poor overall survival in patients with completely resected, chemotherapy-naive NSCLC.35 BRCA1 messenger RNA (mRNA) expression has been linked to resistance to DNA-damaging reagents, such as cisplatin and etoposide, while functioning as a sensitiser to antimicrotubule drugs, such as taxanes and vinca alkaloids. BRCA1 mRNA expression is being evaluated as a method to customise adjuvant chemotherapy in the Spanish customised adjuvant trial (SCAT) trial (see below).36

**Thymidylate Synthase**

In a randomised phase III trial comparing pemetrexed/cisplatin with gemcitabine/cisplatin, survival was superior in patients with non-squamous histology receiving the pemetrexed-based regimen.35,36 Using the histological classification for a therapeutic decision led the US Food and Drug Administration (FDA) to limit pemetrexed therapy to only patients with nonsquamous NSCLC. The treatment outcome difference using pemetrexed in different histologies is likely to be due to an underlying difference in thymidylate synthase (TS). TS is an enzyme that is important for DNA biosynthesis and a target of fluoropyrimidines and pemetrexed-based therapies. Low TS expression levels have been associated with a better response to pemetrexed.38,39 Although TS mRNA expression is on average lower in groups of patients with non-squamous histologies compared with squamous histologies, there is significant overlap of expression ranges in individual patient tumours. Further analysis of the relationship between chemotherapy and TS is being studied prospectively in the international ITACA phase III trial.

**Class III β-tubulin**

Tubulins are a family of globular proteins that make up microtubules in cells. They are important for many aspects of the cell, including structure, movement, mitosis and vesicular transport. The expression of class III β-tubulin (βTubIII) was studied in the JBR.10 study. IHC was performed for βTubIII on 265 out of 482 resected tumour specimens, and a high level of expression was correlated with a better relapse-free survival and overall survival in patients who received the adjuvant chemotherapy.41 A high expression of βTubIII correlates with resistance to antimicrotubule reagents.42 Further prospective studies are needed to validate the utility of βTubIII. Although to date most of the aforementioned biomarkers have not been integrated into practice, there are numerous ongoing clinical trials to address the utility of these genetic biomarkers for clinical treatment decisions.

**Genomic and Proteomic Signatures**

Several gene expression signatures containing non-overlapping genes may provide predictive information on clinical outcome. Recently, a 15-gene signature was identified in patients from the JBR.10 trial observation arm that separated the group into high-risk and low-risk subgroups with significantly different survival (HR 15.02, 95% CI 5.12 to 44.04; p<0.001; stage I HR 13.31; p<0.001; stage II HR 13.47; p<0.001). The same signature predicted survival of those patients with completely resected, chemotherapy-naive NSCLC who received adjuvant cisplatin/vinorelbine. This demonstrates the potential to select patients with early-stage (IB–II) NSCLC that are most likely to benefit from adjuvant chemotherapy with cisplatin/vinorelbine but it needs further validation. Multiple other gene signatures are under investigation. In addition to genomic signatures, studies using proteomics have been developed to classify which patients might respond to receptor TKIs.43–46 Although this is used in advanced NSCLC, the same proteomic signatures are now being applied to
early-stage NSCLC. Proteomic signatures still need validation using prospective studies.

**Prospective Clinical Trials Using Biomarkers to Predict Utility of Adjuvant Therapy**

**Cancer and Leukemia Group B 30506 Trial**

The Cancer and leukemia group B (CALGB) 30506 trial is a randomised phase III trial to determine the potential overall survival benefit of adjuvant chemotherapy in patients with NSCLC of 2–7cm without nodal involvement randomised to chemotherapy compared with observation (see Figure 7). Patients in the trial will be randomised to observation or to three cisplatin-doublet chemotherapies. High-quality fresh frozen lung cancer tumour tissue will be collected and processed from multiple institutions for gene expression array generation. The primary objective is to determine whether any gene expression patterns can be correlated with the effect of adjuvant chemotherapy for overall survival. Other biomarkers are also being investigated.

**Southwest Oncology Group S0720 Trial**

The Southwest Oncology Group (SWOG) S0720 is a phase II clinical trial using the expression of ERCC1 and RRMI to determine adjuvant therapy in patients with stage IA or IB NSCLC (see Figure 2). After surgery patients are assigned to one of two arms based on RRMI and ERCC1 gene expression analysed using automated quantitative analysis (AQUA) technology. Arm I includes patients who have high RRMI and ERCC1 expression who will be observed post-operatively. Arm II includes patients who have low RRMI and ERCC1 expression who will receive gemcitabine and cisplatin for four cycles. The primary objective is to assess the feasibility of assigning adjuvant treatment based on tumoural RRMI and ERCC1 gene expression and to estimate the two-year disease-free survival of these patients. The trial will also explore the relationship between RNA and RRMI and ERCC1 protein expression, and the relationship between RRMI and ERCC1 expression in the formalin-fixed and paraffin-embedded tumour specimens.

**Eastern Cooperative Oncology Group 1505 Trial**

The Eastern Cooperative Oncology Group (ECOG) 4599 and Avastin in lung cancer (AVAIL) phase III clinical trials for patients with advanced NSCLC demonstrated a benefit from the addition of bevacizumab to first-line chemotherapy. Based on these positive results in advanced NSCLC, the ECOG 1505 trial is an ongoing clinical trial to investigate the role of bevacizumab in the adjuvant setting for patients with resected stage IB (at least 4cm in size) to IIIA NSCLC (see Figure 3). The trial is not based on any biomarkers for enrolment stratification because none have been validated for the antivascular endothelial growth factor treatment strategies, but extensive correlates are planned and all patients will have tumour tissue collected and blood drawn and banked from multiple time-points.

**Melanoma-associated Antigen 3 Adjuvant Non-small-cell Lung Cancer Immunotherapy Trial**

The role of vaccines in NSCLC is as yet unproven and remains an area of active investigation. Melanoma-associated antigen 3 (MAGE-A3) is a tumour antigen in 30–50% of resected lung cancers. The MAGE-A3 adjuvant NSCLC immunotherapy trial (MAGRIT) is investigating the use of the MAGE-A3 vaccine in patients with completely resected stage IB, II and IIIA NSCLC that express the MAGE-A3 antigen (see Figure 4). The target accrual is 2,270 patients to find over 1,400 who will be eligible for the vaccination based on MAGE-A3 expression. The vaccination will be given 13 times over 27 months. Patients will be followed every three months over two years and will receive adjuvant chemotherapy for two years for high risk patients and one year for low risk patients. The primary end-point is disease-free survival, with the effect of adjuvant chemotherapy for overall survival. Other endpoints include biomarker correlates are planned and all patients will have tumour tissue collected and blood drawn and banked from multiple time-points.

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**Figure 6: TASTE Trial Overview**

**Figure 7: SCAT Trial Overview**

**Figure 8: ITACA Trial Overview**

*Primary end-point: feasibility followed by disease-free survival. EGFR = epithelial growth factor receptor; PO = orally; TASTE = Tailored post-surgical therapy in early-stage NSCLC.*
Lung Cancer

six months for five years and then annually until year 10. The primary end-point is disease-free survival.

Randomised Double-blind Trial in Adjuvant Non-small-cell Lung Cancer with Tarceva Trial

The RADIANT trial is a phase III clinical trial designed to evaluate the role of erlotinib in the adjuvant treatment of patients with resected stage IB-IIIA NSCLC (see Figure 5). The adjuvant trial is a double-blind, placebo-controlled study that randomised 945 patients to either two years of daily oral erlotinib therapy at 150mg/day or placebo. Only patients with EGFR-positive tumour tissue detected by either FISH or IHC were randomised. Before randomisation, patients were allowed to receive up to four cycles of platinum-based adjuvant chemotherapy. Correlates of the study include EGFR Fish and mutational status. RADIANT has completed accrual and we are awaiting results.

Tailored Post-surgical Therapy in Early-stage Non-small-cell Lung Cancer Trial

The TASTE trial is enrolling patients for a randomised phase II/III adjuvant trial, evaluating the feasibility of standard versus customised treatment in stage I or IIIA non-N2, non-squamous NSCLC (see Figure 6). Patients are randomised to receive either standard chemotherapy consisting of cisplatin plus pemetrexed for four cycles (Arm A) or a customised drug treatment in Arm B. In Arm B, patients harbouring EGFR mutations will be treated with erlotinib. If they have wild-type or an undetermined EGFR status, patients will be tested for their levels of ERCC1. Those that have high ERCC1 levels will receive no treatment and be observed and those that have low ERCC1 levels will receive cisplatin plus pemetrexed for four cycles. The hypothesis is that patients receiving tailored adjuvant therapy will have better disease-free survival rates than patients in the control arm receiving standard chemotherapy therapy.

Spanish Customised Adjuvant Trial

The Spanish customised adjuvant (SCAT) trial uses the BRCA1 mRNA levels to customise treatment in patients with completely resected stage I-IIIA NSCLC (see Figure 7). The rationale for the trial is that patients with low BRCA1 mRNA levels have survived longer when treated with cisplatin-based chemotherapy,14 whereas patients with high BRCA1 levels have longer survival when treated with taxane-based therapy.15 Early-stage NSCLC patients with high BRCA1 levels had significantly worse survival.16 The trial was designed to give patients with high BRCA1 levels docetaxel, patients with intermediate BRCA1 levels docetaxel/cisplatin, and patients with low BRCA1 levels cisplatin/gemcitabine. An interim analysis presented at the American Society of Clinical Oncology in 2008 showed that single-agent docetaxel had no detrimental effect on survival compared with docetaxel/cisplatin.

International Tailored Chemotherapy Adjuvant Trial

The international tailored chemotherapy adjuvant (ITACA) trial is a pharmacogenetic-driven phase III study based on both ERCC1 and TS testing (see Figure 8). The study selects adjuvant therapy for patients with resected stage II and IIA NSCLC based on RT-PCR expression of ERCC1 and TS. ERCC1 and TS are scored as either high or low and the four possible combinations of expression levels are treated with different chemotherapy. The four chemotherapy possibilities are taxane (ERCC1 high and TS high), cisplatin/gemcitabine (ERCC1 low and TS high), cisplatin/pemetrexed (ERCC1 low and TS low). Each of the four customised chemotherapy regimens is being tested against a control arm consisting of a cisplatin-doublet of the investigator’s choice.

Future Directions

The use of chemotherapy for the treatment of patients with resected NSCLC became the standard of care less than a decade ago; however, we appear to have reached a plateau in efficacy. The benefits of empiric treatment were modest with a 5–10% improvement in overall survival at five years. Increased interest in identifying biomarkers has led to retrospective analysis of biomarkers from completed trials. Close analysis of the adjuvant chemotherapy trials revealed that subgroups of patients carrying differential expression or mutation of a particular gene resulted in different outcomes. Those biomarkers are now being used in prospective randomised controlled clinical trials to determine if they have utility as predictive biomarkers. In addition, combinations of genes are being explored as a genetic profile for individualising therapy. With improved understanding of the basic biology behind NSCLC and more drugs targeting pathways actively contributing to tumour growth, we are beginning to enter the era of personalised adjuvant therapy. The results of these ongoing trials will hopefully lead to improved care for our patients in the near future.


