Predictive Molecular Markers: Has the Time Come for Routine Use in Lung Cancer?

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Meta-analyses and randomized studies have shown that platinum-based therapy prolongs survival and improves symptom control and quality of life in patients with advanced non-small cell lung cancer (NSCLC) compared with those receiving supportive care alone.1–3 However, despite the introduction of new chemotherapeutic drug classes over the past 10 years, a therapeutic plateau appears to have been reached with respect to the primary endpoint of overall survival. Phase III trials in the United States examining platinum doublets show median survival times clustered around 8 months. Although some European trials have reported median survival of over 9 months, these trials have had a higher percentage of stage III patients, who typically have longer survival. If available, incorporation of reliable predictive markers into the therapy selection process for individual patients would undoubtedly lead to improved results. One way to improve outcomes beyond what is seen with current platinum doublets is the addition of molecularly targeted agents. Targeted agents from multiple drug classes have been combined with platinum regimens in recent phase III trials in advanced NSCLC including epidermal growth factor tyrosine kinase inhibitors (ZD1839 or Iressa, OSI-774 or Tarceva), antisense oligonucleotides (ISIS 3521), matrix metalloproteinase inhibitors (BMS 275291), and the hypoxic cytotoxin tirapazamine. Unfortunately, the results of these studies have been uniformly negative despite preclinical evidence of increased activity of the combinations. They stand in sharp contrast to studies in breast cancer, in which HER2-positive patients achieved increased survival with trastuzumab (Herceptin) in combination with chemotherapy.
Obviously, lack of patient selection with a predictive marker for the targeted agent may have masked benefit in a sensitive subset of patients. Some researchers have argued that no further large randomized trials of similar design should be conducted without prospective use of a reliable predictive marker for the agents in question.4

Pharmacogenomics, described as the intersection between the fields of pharmacology and genetics, is an investigation of the effects of inherited genetic variations on an individual’s response to drugs. Polymorphisms are natural variations of genomic DNA. Single-nucleotide polymorphisms (SNPs) are the most commonly studied type of marker for pharmacogenomics and are defined as DNA base substitutions that occur in at least 1% of the population.5 In general, the single base change leads to an amino acid substitution that may or may not affect the function of the enzyme. Large-scale efforts are underway to catalog SNPs and characterize their relationship to drug activity.

In addition to SNPs, which can be examined in any cell in the body, tumor-specific molecular markers also are being examined. Altered expression of genes that contribute to carcinogenesis may also influence response to therapy. Tumor-specific molecular markers may be assessed directly in tumor biopsies by analysis of DNA (gene amplification or deletion, or point mutations such as found in KRAS), RNA (expression level differences) or protein (expression level differences and pathway activation). The advantages of pharmacogenomics and tumor marker analysis to cancer therapy lie in the potential for oncologists to customize treatment regimens for individual patients. Future clinicians, armed with patient genetic profiles and tumor-specific expression information, may be able to minimize toxicity while optimizing doses and scheduling of targeted agents and chemotherapy in those patients most likely to benefit. Although many potential predictive markers are being studied (Table 1), this discussion focuses on selected promising markers, including those thought to play roles in response to DNA damaging chemotherapy (excision repair cross-complementing [ERCC1], xeroderma pigmentosum group D [XPD]), taxane resistance (β-tubulin III), antimetabolite therapy (RRM1), irinotecan metabolism (UGT1A1), and epidermal growth factor receptor (EGFR) pathway inhibition (EGFR, HER2).

### DNA Damaging Agents: Platinum Compounds

Although clinical resistance to platinum compounds is most likely multifactorial, emerging evidence suggests that alterations in DNA repair capacity play a significant role. Cytotoxicity from cisplatin principally stems from the formation of bulky intrastrand platinum-DNA adducts and intrastrand crosslinks; removal of these adducts is mediated by the nucleotide excision repair (NER) pathway. ERCC1 is a major component of this pathway, responsible for initiating the cleavage of the damaged DNA strand.6 The function of XPD, also known as ERCC2, is to unwind DNA in preparation for either NER or gene transcription.7 Genetic polymorphisms of the DNA repair genes, ERCC1 and XPD, are associated with variable DNA repair capacity (DRC) and have been proposed as an explanation for differences in survival and response seen in clinical trials of platinum-based therapy. In preclinical models, increased DNA repair is associated with platinum resistance, whereas reduced repair capacity is a marker of sensitivity. High tumor ERCC1 expression has previously been reported to confer resistance to platinum and fluorouracil-based therapies in gastric and ovarian cancers.8,9

In 56 patients with advanced-stage NSCLC receiving gemcitabine and cisplatin, we analyzed ERCC1 mRNA levels (isolated from paraffin-embedded tumor tissue with laser microdissection). Because gemcitabine is known to inhibit repair of platinum-induced DNA damage, we anticipated that this effect would abrogate

<table>
<thead>
<tr>
<th>Gene</th>
<th>Abnormality</th>
<th>Drug</th>
<th>Response</th>
</tr>
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<tbody>
<tr>
<td>p53</td>
<td>mutation</td>
<td>multiple</td>
<td>↓</td>
</tr>
<tr>
<td>K-ras</td>
<td>mutation</td>
<td>multiple</td>
<td>↓</td>
</tr>
<tr>
<td>Her-2</td>
<td>inc expression</td>
<td>multiple</td>
<td>↓</td>
</tr>
<tr>
<td>p27</td>
<td>dec expression</td>
<td>multiple</td>
<td>↓</td>
</tr>
<tr>
<td>MAPK</td>
<td>inc expression</td>
<td>vincas/EGFR</td>
<td>↑</td>
</tr>
<tr>
<td>Beta tubulin</td>
<td>multiple alterations</td>
<td>taxanes</td>
<td>↓</td>
</tr>
<tr>
<td>ERCC1</td>
<td>inc expression</td>
<td>platinum</td>
<td>↓</td>
</tr>
<tr>
<td>RRM1</td>
<td>inc expression</td>
<td>gemcitabine</td>
<td>↓</td>
</tr>
</tbody>
</table>

inc: increased; dec: decreased.
the impact of ERCC1 overexpression. Nevertheless, patients with low levels of ERCC1 had an increased response rate and longer survival (Fig. 1).

In a second trial, patients were randomized to receive one of three gemcitabine-containing regimens, two of which included cisplatin. Low ERCC1 mRNA levels showed trends toward improved time to progression (TTP; \( P = .07 \)) and median survival (MS; \( P = .19 \)).

A number of retrospective analyses have examined XPD polymorphisms at codons 751 and 312 in peripheral blood mononuclear cells and correlated results with outcome. For advanced NSCLC patients treated with gemcitabine/cisplatin having the variant 751, TTP was 9.6 months compared with 4.2 months for common alleles (\( P = .01 \)). For patients with variant alleles at both 751 and 312, TTP was 9.6 months compared with 4.3 months (\( P = .03 \)) for patients with both common alleles and presumably better DRC.

In contrast with these findings, others have found that the common allele of XPD 312 and XRCC1 399 is associated with longer survival and that the greater the number of variant alleles, the poorer the overall survival (20.4 months for no variant alleles vs. 6.8 months for the presence of 3 variant alleles; \( P = .009 \); Table 2).

Thus, the exact role polymorphisms of DNA repair genes have on treatment outcomes is not clear but certainly warrants further study.

### Table 2 Genetic Polymorphisms of XPD and XRCC1 Independently Predicts Survival in Advanced Stage NSCLC

<table>
<thead>
<tr>
<th>Genetic Polymorphism</th>
<th>Patients</th>
<th>Median Survival (mos)</th>
<th>Log-Rank Test</th>
<th>Hazard Ratio (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>XPD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asp/Asp**</td>
<td>50</td>
<td>16.3</td>
<td>( P = 0.003 )</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>(Asp312Asn)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asp/Asn</td>
<td>41</td>
<td>15.2</td>
<td>1.36 (0.97–1.9)</td>
<td></td>
</tr>
<tr>
<td>Asn/Asn</td>
<td>12</td>
<td>6.6</td>
<td>1.84 (1.31–2.58)</td>
<td></td>
</tr>
<tr>
<td>XRCC1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg**</td>
<td>51</td>
<td>17.3</td>
<td>( P = 0.07 )</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>(Arg399Gln)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Gln</td>
<td>42</td>
<td>11.4</td>
<td>1.45 (1.03–2.05)</td>
<td></td>
</tr>
<tr>
<td>Gln/Gln</td>
<td>10</td>
<td>7.7</td>
<td>2.11 (1.49–2.98)</td>
<td></td>
</tr>
<tr>
<td>Combined</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 variants***</td>
<td>26</td>
<td>20.4</td>
<td>( P = 0.009 )</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>1 variant allele</td>
<td>40</td>
<td>16.6</td>
<td>1.41 (1.11–1.80)</td>
<td></td>
</tr>
<tr>
<td>2 variant alleles</td>
<td>24</td>
<td>11.0</td>
<td>1.99 (1.56–2.53)</td>
<td></td>
</tr>
<tr>
<td>3 variant alleles</td>
<td>13</td>
<td>6.8</td>
<td>2.80 (2.20–3.57)</td>
<td></td>
</tr>
</tbody>
</table>

*By Cox proportional hazards model adjusted for stage and performance status.
**Homozygous wild type.
***Double homozygous wild type.
translated into low response rates to taxane therapy and poor survival, subsequent studies have shown that although such mutations are seen in the laboratory, they rarely occur in clinical specimens.16,17

More recently, studies have concentrated on differences in tubulin isotypes as a possible predictor of taxane resistance. Class III β-tubulin has been found to be less sensitive to paclitaxel-induced microtubule stabilization than other isotypes expressed in epithelial cells.18 Increased expression of β-tubulin III has been detected in paclitaxel-resistant lung and ovarian cancer cell lines; whereas in an experimental model, antisense to β-tubulin III resulted in a resensitization to paclitaxel.19

NSCLC patients participating in a randomized trial with one of three platinum-based regimens (gemcitabine/cisplatin, vinorelbine/cisplatin, or paclitaxel/carboplatin) had paraffin-imbedded tumor tissue examined for mRNA expression of β-tubulin III and other markers.21 Analysis of the arm treated with the antimicrotubule vinorelbine revealed no difference in response rates for differential expression of β-tubulin III, although patients with low β-tubulin III expression had a longer TTP (7.3 months vs. 4.1 months; P = .04). Patients on the paclitaxel/carboplatin arm with low levels of β-tubulin III expression had higher response rates (P = .05) but no difference in TTP. Our own studies have identified substantial tumor expression of β-tubulin III in 39 of 74 (53%) NSCLC patients treated on the SWOG 9509 trial.20 Preliminary analysis shows a strong trend toward improved survival in patients treated with paclitaxel/carboplatin, but the relatively small number of tumor samples available for analysis precludes statistical significance. Prospective studies evaluating this marker are ongoing.

**Gemcitabine**

Ribonucleotide reductase (RR) is the enzyme required for converting ribonucleotides into 2′-deoxyribonucleotides, the eventual substrates required for DNA synthesis and cell replication. RR is composed of two subunits (M1 and M2) that are encoded by different genes on separate chromosomes.22 Overexpression of the M1 subunit has been linked to gemcitabine resistance.23 Low RRM1 mRNA levels correlate with a significantly longer TTP and survival in patients receiving first-line therapy with gemcitabine/cisplatin but did not influence survival for patients receiving paclitaxel/cisplatin or vinorelbine/cisplatin, as expected.21 Interestingly, a correlation also has been found between the DNA repair gene ERCC1 and RRM1 levels in NSCLC patients being treated with gemcitabine and the DNA-damaging agent cisplatin. Increased mRNA expression of both was associated with a shorter overall survival for patients being treated with that regimen.24

RRM1 polymorphisms have also been examined in peripheral blood mononuclear cells of NSCLC patients treated with gemcitabine-based regimens. Preliminary reports suggest that the C/C polymorphism is associated with longer TTP and survival compared with the A/A polymorphism.12,14 These early studies, if confirmed, are consistent with the hypothesis that RRM1 levels and polymorphisms will be a useful predictive marker for benefit from gemcitabine therapy.

**Irinotecan (CPT-11)**

Irinotecan is inactive until converted to its active metabolite, SN38, by carboxylesterase. SN38 then binds to and stabilizes the topoisomerase I-DNA complex, preventing re-ligation of DNA, resulting in double strand breaks and apoptosis. SN38 is further modified by glucuronidation by the enzyme UGT1A1 (uridine diphosphate glucuronosyltransferase) to form a secondary and inactive metabolite, SN38G.24 Polymorphisms of UGT1A1 appear to be important predictors for irinotecan-induced toxicity and response to therapy.

Expression of variant UGT1A1 can lead to decreased SN 38 glucuronidation and thus higher circulating levels of the active metabolite and an increased risk for toxicity.25 In particular, decreased glucuronidation has been seen in patients with the 7/7 genotype, consistent clinically with Gilberts syndrome.26 These observations have been confirmed in initial reports of patients treated with irinotecan, although the number of patients studied remains relatively small.27 Of additional interest are reports of inter-racial or ethnic differences in the incidence of UGT1A1 polymorphisms (Table 3). For example, the incidence of the 7/7 genotype is reported to average 10% to 20% in populations derived from European, African, and Hispanic populations, compared with a 2% incidence in Asian patients.27 This finding has been proposed as
an explanation for differences in toxicity and efficacy in trials of irinotecan performed in Japan and in the United States. A large phase III trial of the Southwest Oncology Group (SWOG), S0124, is prospectively examining genetic markers including UGT1A1 to predict toxicity and response to treatment of patients with extensive stage small cell lung cancer trial randomized to either etoposide/cisplatin or irinotecan/cisplatin. This study, with anticipated accrual of 620 patients, will be large enough to adequately address this question in a prospective fashion.

Epidermal Growth Factor Receptor Inhibitors

EGFR is frequently overexpressed in many solid tumors, including NSCLC, and has been reported to confer a poorer prognosis alone and in combination with HER2 (Fig. 2). EGFR is a transmembrane receptor with an internal tyrosine kinase domain, the target of small molecule inhibitors such as Iressa and Tarceva, and an external portion that is the target of monoclonal antibodies such as C225 (Cetuximab) and 2C4 (Pertuzumab). Activation (phosphorylation) of the EGFR initiates a series of intracellular events that leads to increased cell proliferation, tumor angiogenesis, metastasis, and decreased apoptosis.

Preclinical studies have shown that EGFR phosphorylation is associated with downstream intracellular events and that EGFR tyrosine kinase inhibitors potently inhibit phosphorylation. Whether expression levels of EGFR or markers of downstream pathways predict clinical benefit from EGFR inhibitors remains at issue. In preliminary reports from a study of refractory NSCLC patients treated with ZD1839 or OSI-774, tumor EGFR levels assessed by immuno-

Table 3  Racial Variations of UGT1A1 Polymorphisms: Reported As Percent Expression

<table>
<thead>
<tr>
<th>Genotype</th>
<th>European</th>
<th>Asian</th>
<th>African</th>
<th>Hispanic*</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/6 (WT)</td>
<td>34%</td>
<td>70%</td>
<td>26%</td>
<td>36%</td>
</tr>
<tr>
<td>6/7</td>
<td>55%</td>
<td>28%</td>
<td>37%</td>
<td>43%</td>
</tr>
<tr>
<td>7/7 (Gilberts)</td>
<td>11%</td>
<td>2%</td>
<td>19%</td>
<td>13%</td>
</tr>
</tbody>
</table>


Researchers have hypothesized that the levels of activated downstream molecules such as MAPK, PI3K, AKT, p27, or Stat3 may be predictive of a treatment effect, and this is being explored further. Preliminary analyses from the Southwest Oncology Group trial, S0126, demonstrate that nuclear MAPK predicts survival for patients treated with ZD1839.

HER2, a predominant dimerization partner of EGFR, is most commonly examined in breast cancer to predict response to the monoclonal antibody, trastuzumab (Herceptin). Studies performed to date suggest that HER2 expression is of prognostic value in NSCLC. Although trastuzumab has been evaluated in NSCLC, it appears to have little clinical activity, probably because of the small percentage of patients with high levels of expression. HER2 expression has also been examined as a predictive marker for response to EGFR inhibitors in NSCLC. Although results of preclinical studies are conflicting about the relationship between HER2 expression and response to small molecule inhibitors of EGFR, testing in clinical studies have yet to show a relationship.

Iressa was approved for the third-line treatment of advanced NSCLC in the United States in May 2003. Overall response rates of 10% in phase II studies (IDEAL 1 and 2) suggest the need for a predictive marker to aid in the selection of patients most likely to benefit from this therapy. This remains an important but elusive goal. Ongoing trials with EGFR-targeted agents (both small molecules and monoclonal

Figure 2  HER2 and EGFR mRNA combined as prognostic factors for survival in surgically resected NSCLC. (Reprinted with permission from Brabender et al, Clin Cancer Res 2001;7:1850–1855.)
antibodies) continue to examine tumor tissue and blood samples for activity of downstream markers and the presence of EGFR polymorphisms, respectively, that may predict response.

Conclusions
The potential use of molecular markers in lung cancer is far-reaching, including screening high-risk patients, differential diagnosis, prognostication, and prediction of response to therapy. The recently reported International Adjuvant Lung Trial (IALT), indicating a 4% absolute survival benefit at five years for adjuvant platinum-based therapy, highlights the need for reliable predictive markers of response to select those patients who will benefit most and spare others needless toxicity.

For potential predictive molecular markers to be of practical benefit within the clinic, several criteria should be met. (1) Expression should be altered in a substantial proportion of patients; (2) the marker should correlate with response or survival; (3) the biologic specimen (tumor, plasma, or peripheral blood mononuclear cells) needed for analysis should be easily obtainable; (4) methods for analysis should be reliable and generalizable outside research centers; and (5) test results must be available within a reasonable time frame and for a reasonable cost.

The success of chemotherapy in NSCLC has been limited by an incomplete understanding of the molecular pathogenesis of cancer and its impact on the efficacy of chemotherapy as well as inter-patient and intra-racial variation in response and toxicity, as discussed in this report. Studies of potential predictive markers evaluated in lung cancer to date are retrospective, and the hypotheses generated from these trials require prospective validation. In addition, many of these trials have examined only one or two markers in relation to one drug. In reality, patients are treated with combinations of drugs, increasing the level of complexity in establishing the practical application for these markers. Despite these limitations, pharmacogenomics and predictive tumor markers offer not only the hope of improved efficacy, but also reduced toxicity and increased cost-effectiveness by rational drug selection.

Less invasive methods of analyzing tumor-specific molecular markers are being investigated, including circulating tumor proteins and shed-tumor DNA in patient plasma. These developing techniques increase the likelihood that positive findings will be incorporated into clinical practice. An additional benefit of obtaining predictive information from plasma is the capability to obtain serial samples to measure changes over time under the influence of therapy.

Gene discovery projects employing proteomics and microarray increase the likelihood of finding new markers. Ultimately, a better understanding of the molecular basis of lung cancer and inter-patient genetic variations in drug metabolism will lead to better drug selection and hopefully better patient outcomes. Although the time has not come for the routine clinical application of predictive molecular markers in lung cancer, the future holds considerable promise that one day this will be a reality.

References


