Non-Small-Cell Lung Cancer mRNA Expression Signature Predicting Response to Adjuvant Chemotherapy

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Despite undergoing an apparent complete resection of non-small-cell lung cancer (NSCLC) with curative intent, 33% of patients with pathologic stage IA and 77% with stage IIIA disease die within 5 years of diagnosis, most because of metastatic disease present at the time of surgical resection. Several randomized trials showed that adjuvant chemotherapy (ACT) directed against this microscopic metastatic disease improves survival of patients with resected NSCLC.1-6 However, the effect of ACT on prolonging overall and disease-free

24. Smith-Warner SA, Spiegelman D, Yaun SS, et al: Alcohol and breast cancer among initially healthy women in the Fuchs et al study falls squarely in the range of 5.0 to 14.9 g/d, which represented the optimum balance between increased breast cancer mortality and decreased cardiovascular mortality among initially healthy women in the Fuchs et al study.18 The fact that most previous studies of alcohol and breast cancer survival used total mortality as the end point explains the previous null results.
25. The study by Kwan et al1 is important because it elucidates a mystery in the literature concerning lifestyle factors that affect breast cancer survival. Their data showed the expected decrease in survival when recurrence and breast cancer death were used as end points, and no decrease in overall survival with alcohol intake. Of interest, the cut point of < 6.0 and ≥ 6.0 g/d used in the Kwan et al study falls squarely in the range of 5.0 to 14.9 g/d, which represented the optimum balance between increased breast cancer mortality and decreased cardiovascular mortality among initially healthy women in the Fuchs et al study.18 The fact that most previous studies of alcohol and breast cancer survival used total mortality as the end point explains the previous null results.
26. The study by Kwan et al1 is also important because it gives guidance to women living with breast cancer and their clinicians. Their decision is the same as that facing women without breast cancer; the answer is not a clear-cut yes or no. Moderate consumption of alcohol is likely to increase a woman’s risk of dying as a result of breast cancer while decreasing her risk of dying as a result of heart disease. Whether to consume alcohol is an individual decision and is dependent on each woman’s evaluation of and comfort level with those risks.

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survival is modest, with 4% to 15% improvement in 5-year survival, and often ACT is associated with serious adverse effects. Therefore, prospectively identifying the subgroup(s) of patients who will most likely benefit from any or a specific type of ACT would be of substantial clinical benefit.

Currently, a patient’s TNM stage is the main clinical variable that provides prognostic information to suggest which patients need ACT. However, the TNM information (or the specific tumor histopathologic subtype) does not predict which patients within a TNM-stage category will derive survival benefit from ACT. A prognostic biomarker signature (whether it is derived from studies of the tumor or other patient materials, such as blood) separates a population with respect to the outcome of interest, irrespective of treatment, and can be used to estimate disease-related patient trajectories. Subramanian and Simon, in a review of 16 studies on tumor gene expression–based prognostic signatures in lung cancer, suggested guidelines for the design, analysis, and evaluation of prognostic signature studies. By contrast, a predictive biomarker signature separates a population with respect to the outcome of interest in response to a particular treatment and can be used to predict the usefulness of a given treatment in a specific patient. Because predictive signatures directly address the question of which patients are more likely to benefit—or not—from a specific treatment, they have more direct impact on the clinical decisions concerning treatment selection. In interactions with individual patients, although it is great to be able to tell a patient that he or she has a tumor with a profile that augurs for a good prognosis and that no further treatment is needed, it does little good to describe molecular studies of the patient’s tumor showing that the patient’s prognosis is poor, and that there is no specific treatment approach. By contrast, it is obviously more satisfying to the physician and patient to be able to indicate that, although a patient has a tumor with a poor prognosis, the tumor’s characteristics suggest that it is most likely to benefit from a specific type of treatment. Thus, prognostic and predictive biomarker signatures need to be integrated into clinical care. Individual biomarkers have been investigated as predictive markers for ACT response. The International Adjuvant Lung Cancer Trial (IALT Bio) study analyzed 761 NSCLC tumors from a large clinical trial and showed that patients with completely resected NSCLCs (by clinical pathologic stage) whose tumors did not express ERCC1 (ERCC1-negative tumors) benefited from cisplatin-based adjuvant chemotherapy, whereas patients with ERCC1-positive tumors did not. Other potentially predictive tumor biomarkers, including RRM1, p53, and RAS, gave inconsistent results.

It is in this context that Zhu et al report that an NSCLC tumor–derived 15–gene expression signature is both prognostic for survival of untreated patients and predictive for survival after ACT. The gene expression signature was first derived using microarray analyses of frozen tumor tissue from 62 patients who were in the observation group of the National Cancer Institute of Canada Clinical Trials Group JBR.10 randomized controlled clinical trial of adjuvant navelbine plus cisplatin versus observation. The authors identified the minimum number of genes required to provide mRNA expression levels that enabled the classification of these patients into good and poor prognosis groups. The authors found this signature to be prognostic independent of clinically available variables (histology, stage, age, and sex); in addition, they found it to be prognostic in four other published NSCLC microarray data sets (resected tumors without adjuvant therapy) and in other observed patients enrolled onto the JBR.10 trial. They were also able to test the same samples by the more quantitative methodology for mRNA expression patterns, quantitative real-time polymerase chain reaction, which confirmed the microarray findings. However, the real surprise came when Zhu et al tested the signature on patients enrolled onto the JBR.10 trial who received ACT. They found that patients whose tumors were predicted to have a poor prognosis but who received ACT exhibited significantly (and dramatically) better survival than the observed patients (those who did not receive ACT) whose tumors had a poor prognosis signature. By contrast, if a patient’s tumor showed a good prognosis signature and ACT was administered, the patient did significantly worse than patients with a good signature who were just observed. Thus, the tumor biomarker information benefited patients in several ways: for patients whose tumors showed a good prognosis, it not only suggested that the patients did not need ACT, but that it might in fact be harmful. For patients with tumors with poor prognoses, the tumor biomarker information suggested that they needed additional therapy, and that they were likely to gain survival benefit from ACT. Thus, ACT would be worth the treatment toxicity for these patients.

Although numerous gene expression prognostic signatures have been identified for lung cancer in the last few years, the study by Zhu et al is one of the first to address both the prognostic and predictive values of a gene expression signature. A strength of the study is that appropriate patients were selected for the signature development. The JBR.10 trial was a randomized clinical trial designed to test the efficacy of adjuvant chemotherapies in patients with resected NSCLCs; therefore, it provides an ideal patient population for developing a predictive signature for ACT. The National Comprehensive Cancer Network recommends several important clinical and pathologic risk factors for considering ACT for patients with lung cancer. These factors include higher stage, involved lymph node status, poor tumor differentiation, the presence of vascular invasion, use of a wedge resection, and presence of minimal tumor margins. Quantifying the prognosis values of these readily available clinical variables and comparing and integrating these with molecular prognosis signatures will be necessary to fully assess the value of molecular studies of tumors in the care of patients with lung cancer.

To formally define a predictive biomarker signature, four groups of patients are needed: treated and untreated groups, as well as positive and negative biomarker groups (or high- and low-risk signature groups). Different approaches can be used to identify potentially predictive biomarker signatures. First, predictive signatures can be identified on the basis of biologic or functional knowledge and molecular mechanisms. For example, the identification of ERCC1 as a predictive marker for chemotherapy response was based on the molecular mechanisms of chemotherapy and DNA repair pathways. Second, predictive signatures can also potentially be identified by preclinical experiments, for instance, using human tumor cell lines and xenograft models that show different responses to chemotherapy to generate biomarker signatures associated with these different preclinical therapy response phenotypes. This approach has the potential to prospectively identify predictive signatures for new drugs. Of course, although such signatures may predict for the response of other preclinical models (other tumor cell lines), it is crucial to test whether they also predict for tumor responses in patients. Third, as shown in the study by Zhu et al, a prognostic signature may also have predictive value. Thus, it was of interest that the authors studied other prognostic signatures from the literature. According to Zhu et al, out of eight such
signatures, they were only able to show the six-gene signature of Boutros et al\textsuperscript{18} to have prognostic value in the patients under observation in the JBR.10 trial, and, also, to be predictive of survival in patients with a poor prognosis according to molecular markers who received ACT in the JBR.10 trial. Likewise, the three-gene signature of Lau et al\textsuperscript{19} showed survival benefit in the patients enrolled onto the JBR.10 trial with a poor prognosis on the basis of molecular markers who received ACT. (It should be noted that both the studies by Boutros et al\textsuperscript{18} and Lau et al\textsuperscript{19} involved the same authors and probably the same patient treatment facilities as the study by Zhu et al\textsuperscript{14}). By contrast, the other six signatures (derived at other institutions) were neither prognostic (in the patients observed in the JBR.10 trial) nor predictive of benefit in patients who received ACT in the JBR.10 trial.\textsuperscript{20-25} Fourth, predictive signatures can be identified on the basis of genome-wide testing to determine which genes have interactions between expression level and treatment. This is the most direct way to identify predictive signatures, but requires a large cohort of samples with detailed treatment information, clinical outcomes, and frozen tissues. Therefore, this approach has been rarely implemented in practice. Clearly, the availability of multiple large tumor microarray data sets with clinical annotation regarding patient treatment (particularly those from randomized controlled trials), similar to the JBR.10 microarray data set published with the study by Zhu et al, will be an important resource for future research.

Validation of potential predictive signatures/biomarkers in independent data sets is a crucial step toward the application of these signatures in clinical practice. Because of its direct impact on clinical decisions with respect to treatment options, predictive signature validation will likely require a similar standard of evidence as that needed to adopt a new therapeutic intervention.\textsuperscript{26-27} Therefore, prospective randomized controlled clinical trials are the best strategy to validate predictive signatures. Biomarker/signature-based clinical trials generally require large sample sizes,\textsuperscript{28} and novel clinical designs for testing the predictive markers/signatures have been recently proposed.\textsuperscript{26-27,29,30} This emphasizes the importance of clinical and molecular data sets to enable the use of data collected from previously conducted randomized clinical trials to test new predictive signatures. For this type of analysis of retrospective data, it is important to prospectively state the testing hypothesis, analysis techniques, and patient population, and to precisely describe the algorithm, parameters, and scoring system for use of the molecular data. Most importantly, the data used to test the signature should be independent of the data used to derive the signature. Thus, a limitation of the study by Zhu et al is that the same cohort of patients (62 patients in the JBR.10 observation group) was used both as a training data set to derive the 15-gene signature, and part of the testing data set to test the interaction between the signature and ACT. In general, training and testing sets should be completely independent; otherwise, the conclusion concerning the interaction between the signature and treatment may be the consequence of data mining steps used for deriving the signature. Thus, another independent data set is necessary to validate the predictive value of the 15-gene signature described by Zhu et al.

Although the majority of published molecular signatures for lung cancer prognosis are based on measurements of mRNA expression, other molecular signatures, such as gene copy numbers, protein expression levels, inherited polymorphisms, and micro RNA signatures, are also being tested. The same study design, analysis, and validation principles apply to these signatures. Finally, integrating these different types of molecular signatures and clinical-pathology variables efficiently will be challenging but important for developing a final, clinically useful predictive model for ACT response in patients with lung cancer.

Overall, the study by Zhu et al is a step in the right direction toward personalized treatment in lung cancer. However, much work needs to be performed before a molecular signature is applied to care of patients with lung cancer. First, the 15-gene signature described by Zhu et al may only be predictive for the specific adjuvant chemotherapy (vinorelbine plus cisplatin) used in the JBR.10 trial; other signatures will need to be developed for commonly used combinations, such as carboplatin plus taxanes, cisplatin and carboplatin plus gemcitabine, or cisplatin and carboplatin plus pemetrexed. Molecular signature studies will need to be performed by a Clinical Laboratory Improvement Amendments–certified laboratory test to ensure that the data is of suitable quality for clinical decision making. The requirement of frozen tumor samples for microarray and most quantitative real-time polymerase chain reaction molecular profiling largely limits sample availability for developing and validating prognosis and predictive signatures. Therefore, analytic approaches that can be applied to formalin-fixed, paraffin-embedded samples will facilitate the clinical use of molecular signatures. Finally, because of the complex nature of analytic procedures involved in the development and validation of molecular signatures, it is critical to follow good practice for statistical analysis and presentation of results. Subramanian and Simon\textsuperscript{10} and Minna et al\textsuperscript{16} provided detailed guidelines on statistical analysis and results interpretation. Considering recent analyses that indicate that it may not be possible to reproduce genome-wide data analyses,\textsuperscript{31} we want to emphasize the importance of crystal-clear descriptions of all analytic details (including methods and parameters used in each step, documented by computing programs such as Sweave\textsuperscript{23}) and the full description of the predictive models to ensure that others are able to reproduce the findings using the same data. Finally, although such predictive signatures are of great interest and potentially useful, actual identification of the functional causes for individual tumor sensitivity or resistance to a particular therapy will provide not only biomarkers for therapy prediction, but also potential new targets to enhance therapeutic response.

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Small Tumors, Intermediate Models, Big Hopes

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See accompanying article on page 4425

In his 1987 legendary article “An Odyssey in the Land of Small Tumors” on the management of neuroendocrine tumors, Moertel1 concluded with a vision for tumor-specific therapy that refrains from empiricism. This vision, in part, has been fulfilled by a better understanding of genetic and molecular aberrations that are relevant in the pathogenesis of neuroendocrine tumors.2 Based on this knowledge, many molecularly targeted therapies have undergone preclinical and clinical evaluations in this disease. Two classes of targeted agents, namely the vascular endothelial growth factor receptor (VEGFR) inhibitors and the mammalian target of rapamycin (mTOR) inhibitors, are the furthest along in their clinical development. Sunitinib, a multitkinae VEGFR small molecular inhibitor, has demonstrated in the phase III setting an improvement in progression-free survival over placebo in patients with advanced and progressive pancreatic neuroendocrine tumors.3 A phase III trial (RAD001 in Advanced Neuroendocrine Tumor-3; RADIANT-3) in a similar patient population has also found a statistically significant improvement in progression-free survival favoring the rapamycin derivative everolimus plus best supportive care over best supportive care alone.4

The next frontiers in the systemic treatment of neuroendocrine tumors with molecular targeted agents will involve the exploration of new targets, as well as rational combinations of active compounds to counteract resistance or promote synergy. Preclinical evaluation of novel combinations in representative animal models can be informative as an intermediate step to guide clinical studies. In this issue of Journal of Clinical Oncology, Chiu et al5 performed an interesting set of in vivo experiments using a combination of the mTOR inhibitor rapamycin and the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor erlotinib in the RIP-Tag2 transgenic mouse model, which develops pancreatic neuroendocrine tumors. Significant increases in apoptosis, reduction in proliferation, and prolongation in survival were observed in the animals treated with the targeted combination versus vehicle or either agent alone.

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