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Review Article

International trial of adjuvant therapy in high risk stage I non-squamous cell carcinoma identified by a 14-gene prognostic signature

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Abstract: There is widespread agreement amongst clinical oncologists that more refined risk-stratification in early-stage lung cancer patients beyond conventional TNM staging is needed. Over the past decade, a number of molecular prognostic signatures have been designed to meet this need by correlating patterns in the differences in gene expression or modification to patient prognosis. Unfortunately, the majority of proposed signatures are not amenable to practical widespread implementation or have not yet undergone large-scale, rigorous clinical validation. A practical 14-gene prognostic signature that has undergone large-scale blinded independent validation is now ready for widespread clinical use. An international clinical trial is underway that has been designed to document the precise degree of benefit derived from adjuvant therapy in high-risk stage I patients identified by the 14-gene prognostic assay.

Keywords: Non-small cell lung cancer; molecular; prognostic; predictive; biomarker; signature; personalized therapy; adjuvant therapy

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Introduction

Surgical resection remains the gold standard of treatment for patients with stage I non-small cell lung cancer (NSCLC) and essentially represents the hope for cure (1,2). As compared to the high 5-year survival rates experienced by patients with the other prevalent, localized solid tumors such as breast cancer (98%), colorectal cancer (91%), and prostate cancer (100%), the 5-year survival rate after diagnosis of "localized" lung cancer is only 53% (3). Although some of these survival differences may be attributed to differences in patient demographics associated with each of these solid tumors, the wide discrepancy in outcomes also indicates that the label of "localized" disease conferred by our current TNM staging system does not adequately predict outcome or response to "complete" resection.

Current surgical standard of care dictates the complete removal of an affected lobe as opposed to sublobar resection for patients with stage I disease whenever possible (4). The poorer outcomes associated with sublobar resections is thought to be due to the presence of micrometastatic disease that has spread either locally or via the lymphatic system past the detectable margins of surgical resection. The prevalence of micrometastatic disease in lung cancer not only explains the poor outcomes associated with sublobar resections, but also the inadequacy of our current staging system that relies on tumor size, and nodal status alone to predict the likelihood of distant occult metastasis or micrometastasis and therefore patient outcome.

Molecular prognostic signatures

The desire to improve risk stratification beyond TNM staging has led to the recent development of molecular

prognostic signatures in lung cancer. A number of biomarker signatures prognostic of survival in NSCLC have been proposed (5,6). After a decade of research and dozens of proposed signatures, proof-of-principle is no longer the objective. Clinicians are now looking for a meaningful way to integrate data from a prognostic gene signature into the standard care of NSCLC patients. More accurate prognostic information, such as may be accessible through gene signatures, could contribute in a very important way to this current clinical decision making. That decision making often involves suboptimal choices that are often being weighed largely based on the current level of inadequately precise prognostic information based on TNM staging alone. For example, a more accurate prognostic picture may influence the approach toward surgical resection. Achieving complete oncologic lobar resection at the expense of pulmonary function is often a challenging balance to strike in patients with limited cardiopulmonary reserve. Often, such patients with stage IA lung cancer undergo wedge resection rather than anatomic resection because of the estimated risk of greater pulmonary volume loss. Subsequent risk analysis of the resected tumors could allow a more precise risk-benefit ratio to be estimated in the consideration of subsequent re-operation for completion lobectomy. Prognostic signatures may also contribute to decisions regarding post-operative care. Patients with high-risk tumors could be followed closely with more frequent imaging and be considered for adjuvant chemotherapy after surgical resection. Although the NCCN currently recommends administration of adjuvant chemotherapy in "high-risk" stage I patients [defined as stage IB disease and one of the following features: poorly differentiated tumors, vascular invasion, wedge resection, tumors larger than 4 cm, visceral pleural involvement, or undetermined lymph node status (Nx) (7)], these collective criteria based on conventional clinicopathologic features have never been validated to predict benefit from adjuvant chemotherapy. Conversely, patients with low-risk tumors could potentially be spared from toxic chemotherapy regimens that may be more likely to harm than help.

Until recently, the successful translation of prognostic signature bench research to the bedside has been challenging. One major barrier has been the lack of development of a prognostic signature using practical laboratory technology. Most proposed prognostic signatures have been developed using microarray technology (5,6). While microarrays are extremely powerful at surveying multiple potential prognostic gene candidates, the practical applicability of microarrays for a clinically rigorous test remains largely unproven. Microarrays typically require fresh-frozen tissue that has been snap-frozen immediately upon surgical resection (8). This creates a logistical barrier that may prove extremely difficult or even impossible to overcome in a community non-academic based setting. While new reagents such as RNAlater (Life Technologies, Foster City, CA) that preserve RNA at room temperature may one day provide a possible alternative to snap freezing, the overall robustness of such a platform, which involves a microarray approach that relies inherently upon extremely pure RNA, remains to be seen. In addition, many microarray-based prognostic signatures are platform dependent and based on complex algorithms (8), making them difficult for other groups to interpret, understand, and independently validate. A clinical trial based on the "metagene" prognostic model which utilized a complex microarray-based algorithm, for example, was recently stopped and the research paper describing the algorithm was recently retracted because of the inability of other groups to independently validate the model (9).

The lack of rigorous clinical validation has been the second major barrier to clinical adoption. The majority of proposed prognostic gene signatures lack clinically relevant validation. While an attempt has been made by many groups to develop prognostic algorithms using large patient cohorts, the necessity of validating these algorithms on equally large independent cohorts in a blinded fashion has been underemphasized (5,6,10). As a result, it is difficult to convince the clinician that a prognostic gene signature that was developed on a specific population is universally applicable to the patient in front of them. This is an even bigger problem in tests that rely upon a large amount of quantitative data to generate a result due to statistical "overfitting". Overfitting leads to quantitative coefficients and cut-off points that are too specific to the training cohort; validation on independent datasets typically fails in these cases. As a result, tests developed by "overfitting" data to the study population cannot be generalized to other patient populations (8).

Development and validation of a 14-gene prognostic algorithm using paraffin-embedded tissues

In light of the technical challenges posed by use of a microarray-based platform and objection from the clinical community about the lack of rigorous clinical validation,

our group recently developed a quantitative PCR-based assay that measures gene expression in formalin-fixed paraffin-embedded (FFPE) lung tumor specimens (11). Quantitative PCR is robust, inexpensive, widely available, easy to interpret, and highly reproducible. Special techniques were developed to extract RNA from 361 FFPE specimens from patients who had undergone resection of stage I-IV non-squamous NSCLC at UCSF (11). The expression levels of 14 genes were measured using quantitative PCR and correlated to patient outcomes using penalized cox proportional hazards modeling (11). Three of these genes were housekeeping genes; eleven of these genes are intricately related to known canonical lung cancer pathways such as *KRAS* and *eGFR* (11). Risk scores were divided into terciles in the UCSF training cohort to yield low-, intermediate, and high-risk categories.

Once the prognostic algorithm was derived on this UCSF cohort, blinded, independent validation was performed using two large international cohorts. The first validation cohort consisted of 433 patients who underwent resection of pathologic stage I disease in the Kaiser-Permanente Northern California healthcare system. The second cohort consisted of over 1,000 patients who underwent resection of pathologic stage I-III disease at major centers of cancer care excellence that belong to the China Clinical Trials Consortium (CCTC).

Kaplan-Meier analysis demonstrated that the assay was able to successfully risk stratify patients at low, intermediate, and high-risk of mortality within 5 years of surgical resection. This risk stratification was successful not only in the Kaiser stage I validation cohort, but also within each of the stages of the CCTC validation cohort (11). Risk category was the strongest predictor of morality after adjusting for age, sex, smoking history, histology, and stage. In addition, the assay improved risk discrimination in all stage I patients (stage IA and IB) beyond the NCCN criteria (8) currently used to identify high-risk stage I patients for adjuvant chemotherapy. Furthermore, the assay was able to successfully risk-stratify patients with node-negative tumors less than 2 cm, identifying patients with almost 50% 5-year mortality despite surgical resection of these small T1a tumors (12). The numbers of small T1a tumors is expected to rapidly increase as more and more institutions and providers adopt the new lung cancer screening guidelines (8).

Of note, only one other group attempted blinded, large-scale independent validation of a molecular prognostic signature. A multi-center effort was made as part of the National Cancer Institute Directors Challenge to develop and validate a microarray-based signature using fresh frozen tissue samples collected at multiple institutions (13). Eight signatures were submitted by the participating institutions for validation. None of these signatures, however, was able to risk stratify stage I patients better than clinical covariates alone (13), highlighting the difficulty of achieving successful blinded validation of molecular prognostic signatures.

Worldwide trial of adjuvant therapy in patients with high risk stage I non-squamous cell carcinoma

The NCCN currently recommends administration of adjuvant chemotherapy in "high-risk" stage I patients. The following criteria define "high-risk" patients according to the NCCN (7):

Patients with stage IB disease and one of the following features:

(I) poorly differentiated tumors;
(II) vascular invasion;
(III) wedge resection;
(IV) tumors larger than 4 cm;
(V) visceral pleural involvement;
(VI) insufficient lymph node staging (Nx).

Notably, with the exception of tumor size, none of the above criteria have been validated to predict benefit from adjuvant chemotherapy. They have been adopted by the NCCN with the thought that these criteria are correlated with more aggressive tumors and worse survival. It has already been shown, however, that the 14-gene assay far outperforms the above NCCN criteria in identifying high-risk patients (11). The new gene signature may therefore represent a more rigorously validated tool for implementation of this current published guideline. A randomized clinical trial that has been designed to document the exact degree of benefit derived from adjuvant chemotherapy in high-risk patients identified by the assay is underway.

Conclusions

There is widespread acknowledgment of the need for more refined risk-stratification in early-stage lung cancer patients beyond conventional TNM staging. Although the development of molecular prognostic signatures has met this need, the majority of proposed signatures lack clinical relevance because they are impractical or have not yet

undergone rigorous clinical validation. A practical 14-gene prognostic signature that has undergone large-scale blinded independent validation is now ready for widespread clinical use. An ongoing international clinical trial is expected to provide additional documentation of the degree of benefit derived from this personalization of lung cancer care that is already being integrated into current practice.

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