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Improved outcomes and staging in non-small-cell lung cancer guided by a molecular assay

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There remains a critical need for improved staging of non-small-cell lung cancer, as recurrence and mortality due to undetectable metastases at the time of surgery remain high even after complete resection of tumors currently categorized as 'early stage.' A 14-gene quantitative PCR-based expression profile has been extensively validated to better identify patients at high-risk of 5-year mortality after surgical resection than conventional staging – mortality that almost always results from previously undetectable metastases. Furthermore, prospective studies now suggest a predictive benefit in disease-free survival when the assay is used to guide adjuvant chemotherapy decisions in early-stage non-small-cell lung cancer patients.

Lay abstract: There is a need for improvement in the way early-stage non-small-cell lung cancers are staged and treated because many patients with 'early-stage' disease suffer high rates of cancer recurrence after surgery. In recent years, a specialized test has been developed to allow better characterization of a tumor's risk of recurrence based on the genes being expressed by tumor cells. Use of this test, in conjunction with standard staging methods, is better able to identify patients at high risk of cancer recurrence after surgery. Evidence suggests that giving chemotherapy to patients at high risk of recurrence after surgery reduces recurrence rates and improves long-term patient survival.

Lung cancer remains the leading cause of cancer-related mortality in the United States. It is estimated that 228,220 new cases of lung cancer are diagnosed nationally each year, leading to approximately 140,000 annual deaths [1]. Over the past several decades, even with improvements in our understanding, diagnosis and treatment of lung cancer, there has been only a modest improvement in the 5-year survival for the disease. In 1975, the 5-year survival rate for all lung cancers was 12%; today, it is estimated to be 19.9% [2].

The 5-year survival for lung cancer is dependent upon several variables, including anatomic factors – such as tumor size, local obstruction, nodal involvement and distant metastasis – and a variety of patient, immunologic and molecular factors [3]. From an anatomic standpoint, low rates of lung cancer survival reflect a substantial proportion of patients (57%) with known metastatic disease at the time of diagnosis, for whom the expected 5-year survival is only 5%. Earlier diagnosis through enhanced screening of patients at high risk of lung cancer therefore represents an opportunity for improvement via increased diagnosis and treatment before metastasis has occurred. However, even the diagnosis of smaller, earlier tumors would not completely solve the problem of high lung cancer mortality since an unusually high percentage of these small, 'early-stage' lung cancers already have the potential for metastasis [4–7]. As a result, only half of patients with disease that is believed to be localized at the time of diagnosis and surgical resection survive 5 years [1].

Future
Medicine

Clinically, the most widely utilized tool for predicting survival of patients with lung cancer is the tumor, node, metastasis (TNM) staging system [8]. These clinicopathological descriptors of tumors, including size, local invasion and presence of nodal or distant metastasis, were first integrated into the TNM staging system by Denoix in 1953 [9]. The eighth edition of the TNM staging system (hereinafter 'eighth edition') for non-small-cell lung cancer (NSCLC), the most common form of lung cancer, was released in 2018 and categorizes patients into one of 11 distinct clinical stages: IA1, IA2, IA3, IB, IIA, IIB, IIIA, IIIB, IIIC, IVA, IVB [10]. Despite the eighth edition's extensive training dataset, validation studies have demonstrated disappointing results in adequately risk-stratifying patients with stage IA–IIB disease. In these validation studies, the 5-year overall survival for patients with pathological stage I NSCLC remains as low as 55% [11,12]. Even after complete surgical resection of the earliest stage IA lung cancers, the 5-year survival is only approximately 70%, demonstrating that TNM staging alone does not adequately identify patients at high risk of undetected metastasis and therefore does not predict patient outcome [13,14].

Surgical resection is the primary treatment option for patients with stage I and II disease and represents the best opportunity for cure [15,16]. In patients with 'completely resected' stage I lung cancer (i.e., without evidence of distant or regional spread), recurrence typically occurs as a result of the presence of undetectable, often microscopic metastatic disease that is present at the time of surgery [17]. The 5-year recurrence rate of resected stage I NSCLC has been reported to be as high as 35–40%, demonstrating that the presence of micrometastases is a common phenomenon [18]. This is particularly staggering when compared with 5-year recurrence statistics for other types of stage I cancers, including breast cancer (3.1%) [19] and colon cancer (4.6%) [20]. In a randomized prospective trial of patients with stage I lung cancer undergoing lobectomy versus segment or wedge resection, it was shown that patients who had lobectomy developed less locoregional recurrence [21]. It is thought that this change is related to more complete excision of occult micrometastases. It has become paramount, therefore, to accurately predict which patients with early-stage disease are more likely to have micrometastases that are missed by current imaging and pathological modalities.

Over the past 15 years, substantial effort has been applied toward identifying prognostic indicators that signify aggressive NSCLC tumor biology on a molecular level. Early efforts in this arena focused on profiling oncoproteins and other cellular products that were being transcribed at a cellular level to ascertain the risk of a particular tumor [22]. More contemporary efforts have focused on genomic and gene expression models to provide additional prognostic information for risk stratification beyond that provided by the TNM staging system alone. Although multiple models have been proposed and developed, this review will focus on the only genetic model that is currently in clinical use today.

A 14-gene prognostic & predictive molecular assay for early-stage NSCLC

Tumorigenesis is thought to be promoted by both genetic and epigenetic changes within a precancerous cell and changes in the tumor microenvironment [23–25]. For example, it is clearly established that alteration in the level of expression of certain genes is causative of cancer [26]. Several groups previously developed gene expression analysis tools that predicted patients with higher than expected mortality after resection of early-stage lung cancer [17,27–35]. Many of these early gene signatures were based on a microarray platform that utilized frozen tissue samples, making their use impractical in clinical settings because of difficult reproducibility, excessive cost and lack of widespread availability [36]. This early research underscored the need for a more practical, available and reliable molecular assay to fill the need for risk stratification in patients who have early-stage disease by conventional TNM criteria but who may have a high risk of treatment failure following resection.

Development & technical validation of the 14-gene lung cancer assay

The 14-gene lung cancer assay utilizes quantitative reverse transcription PCR (qRT-PCR) for analysis of readily available and highly stable formalin-fixed, paraffin-embedded (FFPE) tissue samples [37]. The assay was designed at the University of California San Francisco (UCSF) using a training cohort of samples from 361 patients with nonsquamous NSCLC. Notably, patients with squamous cell lung carcinoma were excluded from assay development, as prior investigations have suggested fundamental differences in the molecular makeup between squamous cell and nonsquamous cell lung carcinoma [38]. Four target genes were selected from over 200 cancer-related gene candidates using L1-penalized Cox proportional hazards modeling in an initial pilot study [37]. For each sample, RNA was extracted from FFPE tissue blocks, and the relative expression of individual genes was measured using qRT-PCR. In later studies, L2-penalized Cox proportional hazards modeling was used to develop a more

	Table 1. Algorithm genes utilized in the University of California San Francisco molecular assay.					
Gene	Name	Chromosome	Reference sequence	Protein location	Relevant biological functions and pathways	Role in algorithm
BAG1	BCL2-associated athanogene	9p12	NM_004323	Cytoplasm	Apoptosis; cell surface receptor linked signaling	Prognosis
BRCA1	Breast cancer 1, early onset	17q21–q24	NM_007294	Nucleus	Induction of apoptosis; protein ubiquitination; regulation of G2/M transition DNA damage checkpoints; regulation of DNA repair	Prognosis
CDC6	Cell division cycle 6 homolog	17q21.3	NM_001254	Nucleus	Regulation of transcription involved in G1/S phase of mitotic cell cycle; regulation of cell proliferation	Prognosis
CDK2AP1	Cyclin-dependent kinase 2-associated protein 1	12q24	NM_004642	Nucleus	Regulation of S phase of mitotic cell cycle; DNA-dependent DNA replication	Prognosis
ERBB3	V-erb-b2 erythroblastic leukemia viral oncogene homolog 3	12q13	NM_001982	Plasma membrane	Regulation of cell adhesion; transmembrane receptor protein tyrosine kinase signaling; regulation of phosphoinositide 3-kinase cascade; regulation of cell proliferation	Prognosis
FUT3	Fucosyltransferase 3	19p13.3	NM_000149	Cytoplasm	Carbohydrate metabolism; protein amino acid glycosylation	Prognosis
IL11	Interleukin 11	19q13.3–q13.4	NM_000641	Extracellular	Regulation of cell proliferation; B-cell differentiation; megakaryocyte differentiation; regulation of peptidyl-serine phosphorylation; regulation of MAPKKK cascade	Prognosis
LCK	Lymphocyte-specific protein tyrosine kinase	1p34.3	NM_001042771	Cytoplasm	Induction of apoptosis; activation of caspase activity; regulation of T-cell receptor signaling; leukocyte migration	Prognosis
RND3	Rho family GTPase 3	2q23.3	NM_005168	Cytoplasm	Cell adhesion; small GTPase mediated signal transduction; actin cytoskeleton organization	Prognosis
SH3BGR	SH3 domain-binding glutamic acid-rich protein	21q22.3	NM_007341	Cytoplasm	Protein complex assembly	Prognosis
WNT3A	Wingless-type MMTV integration site family, member 3A	1q42	NM_033131	Extracellular	Canonical Wnt receptor signaling; multicellular organismal development; regulation of cell proliferation and differentiation; regulation of catenin protein nuclear translocation	Prognosis
ESD	Esterase D	13q14.1–q14.2	NM_001984	Cytoplasm	Recycling of sialic acids; serine hydrolysis	Reference
TBP	TATA box binding protein	6q27	NM_003194	Nucleus	Transcription initiation from RNA polymerase II promoter; RNA elongation from RNA polymerase II promoter; transcription from RNA polymerase III promoter	Reference
YAP1	Yes-associated protein 1	11q13	NM_006106	Nucleus	Hippo signaling cascade; regulation of transcription; contact inhibition	Reference

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elaborate scoring system based on the expression of 11 active genes [39]. Gene expression profiles were translated into risk scores that reflected the biological aggression of a tumor. The resultant risk scores from the training cohort of patient samples were divided into thirds to stratify patients at low, intermediate and high risk of mortality after surgical resection of lung cancer. After the assay was completely specified, technical validation was completed in line with Clinical Laboratory Improvement Amendments (CLIA) guidelines [40].

The final assay includes 11 cancer-related target genes and three reference genes (Table 1). Several of the genes in the 14-gene lung cancer assay, including *BAG1*, *BRCA1*, *CDC6*, *ERBB3* and *WNT3A*, are known participants in established oncogenic pathways. The 11 target genes are all implicated in molecular lung cancer pathways, including *KRAS*, *BRAF*, *EGRF*, *HER2*, *ALK* and *p53*. Five of the genes overlap with prognostic gene signatures previously identified in NSCLC (*CDC6* [27,33,34], *ERBB3* [29], *FUT3* [27,33,35], *LCK* [29] and *RND3* [33]).

One weakness of previously reported genetic prognostic models in lung cancer was the lack of external blinded validation in large independent cohorts [36]. In fact, the only study that included a blinded independent validation was published by a consortium of major academic institutions in response to a National Cancer Institute director's challenge. In this study, the best performing genomic classifier failed blinded validation without the use of clinical covariates [34]. By contrast, the 14-gene lung cancer assay was externally validated by two large-scale, multicenter,

blinded studies with entirely independent cohorts and included international patients. The first external validation was completed by the Kaiser Permanente Division of Research in a cohort of 433 patients with stage I nonsquamous NSCLC. The results from the molecular assay were compared with the known patient outcomes by Kaiser Permanente Division of Research, and the 5-year lung cancer-specific survival was 84.6% (95% CI: 74.4–91.0) in the low-risk group, 70.3% (95% CI: 60.6–78.0) in the intermediate-risk group and 63.3% (95% CI: 55.8–69.8) in the high-risk group. After multivariate analysis (adjusting for patient age, sex, smoking history, tumor cell histology and tumor size), it was demonstrated that both high-risk and intermediate-risk groups were statistically significant predictors of patient mortality. A second validation involved an international, large-scale trial of the molecular assay with a cohort of 1006 Chinese patients who had undergone resection of early-stage disease at one of several participating institutions in the China Clinical Trials Consortium. Similarly, it was shown on multivariate analysis that high-risk and intermediate-risk designation remained statistically and clinically significant predictors of survival [37].

The 14-gene lung cancer assay is unique compared with its predecessors based on a variety of metrics. Although previously developed gene signatures prognostic of lung cancer survival were based on analysis of snap-frozen specimens [17,36], the 14-gene lung cancer assay utilizes FFPE tissues for analysis. Although snap-frozen tissues yield extremely high-quality RNA, they are not routinely collected, are difficult to handle and are expensive to transport. Conversely, the use of FFPE tissue samples, which are widely available, makes this assay relevant to the routine management of patients with lung cancer in a global community setting. Additionally, the assay utilizes qRT-PCR-based signatures, whereas older models used primarily microarray-based molecular profiles; qRT-PCR has a substantial advantage over microarray-based assays, including widespread availability, reduced cost, model simplicity, significantly greater reproducibility and the ability to use stored paraffin-embedded versus snap-frozen tissues [41–43].

Importantly, the 14-gene lung cancer assay is an analytically precise and reproducible tool, as validated in a CLIA-certified laboratory [40]. The quantitative PCR primer and probe data showed a linear input RNA range extending between 2^{10}- and 2^{15}-fold, which is paramount, as it enables analysis of tumors from the FFPE specimens with a wide range of RNA yields. The assay has high PCR efficiency, with a median efficiency of 91.2%, which is on par with previously published reports of FFPE qPCR-based primer and probe sets [44]. The reproducibility, tested by repeating the assay on 15 samples representative of a wide range of risk scores for five independent experiments, was also exceptionally high [40]. This demonstrated reproducibility and reliability of the UCSF molecular assay, which is a critical component for validation of the clinical utility of the test. Blinded, large-scale, independent clinical validation; the ability to run the assay on FFPE tissue specimens; the use of well-established quantitative PCR; and the high reliability and reproducibility of the assay are the major reasons the 14-gene lung cancer assay remains the only prognostic and predictive lung cancer assay in clinical use today.

Staging refinement using the 14-gene lung cancer assay

It has been previously established that biological molecular signatures can refine prognosis in patients with other types of malignancies [45,46], though such molecular predictors have yet to be formally incorporated into the established TNM staging system for lung cancer [17,36]. The utilization of the 14-gene lung cancer assay as a complement to conventional staging has been evaluated, and it has been shown to improve survival predictions [47,48].

In the initial study, a novel staging system that incorporated the 14-gene lung cancer assay was developed and validated [47]. Within the UCSF training cohort, each tumor was analyzed with the molecular assay to determine the phenotype of its biological risk profile [37]. Through a supervised reclassification staging system that integrated the outcome of the prognostic assay with the eighth edition, the patient's stage was downgraded by one stage, for low risk; upgraded by one stage, for high risk; or unchanged, for intermediate risk [47]. This newly developed staging system, renamed TNM biology (TNMB) (Table 2), was subsequently applied to the independent Kaiser Permanente Division of Research and China Clinical Trials Consortium validation cohorts (n = 1373).

In a Kaplan–Meier analysis of overall survival, the survival curve separation for TNMB staging was superior to conventional staging by either the seventh or eighth edition (Figure 1) [47]. Numerically, this is represented by the chi-square statistic of the log-rank test, which was 159 according to the seventh edition versus 155 according to the eighth edition. By contrast, the chi-square statistic improved to 194 for the TNMB staging system, which indicates improved survival discrimination with the TNMB staging system.

More recently, other, more sophisticated reclassification metrics have been utilized to better assess improvements in prognostic systems. Net reclassification improvement (NRI) is an index used to quantify how well a new model

Table 2. Tumor, Node, Metastasis eighth edition staging system versus Tumor, Node, Metastasis, Biology staging system.	
TNM eighth edition stage and molecular prognostic classifier	**TNMB stage**
Stage IA1	
Low risk	Stage IA1
Intermediate risk	Stage IA2
High risk	Stage IA3
Stage IA2	
Low risk	Stage IA1
Intermediate risk	Stage IA2
High risk	Stage IA3
Stage IA3	
Low risk	Stage IA2
Intermediate risk	Stage IA3
High risk	Stage IB
Stage IB	
Low risk	Stage IA3
Intermediate risk	Stage IB
High risk	Stage IIA
Stage IIA	
Low risk	Stage IB
Intermediate risk	Stage IIA
High risk	Stage IIB
Stage IIB	
Low risk	Stage IIA
Intermediate risk	Stage IIB
High risk	Stage IIIA
Stage IIIA	
Low risk	Stage IIB
Intermediate risk	Stage IIIA
High risk	Stage IIIB
Stage IIIB	
Low risk	Stage IIIA
Intermediate risk	Stage IIIB
High risk	Stage IIIC
Stage IIIC	
Low risk	Stage IIIB
Intermediate risk	Stage IIIC
High risk	Stage IIIC
The TNMB staging system maintains the order of the eighth edition of the TNM staging system but allows reclassification of patients based on molecular assay results. It upgrades patients by one stage for high-risk disease, downgrades patients by one stage for low-risk disease and does not change stage for intermediate-risk disease. TNM: Tumor, node, metastasis; TNMB: Tumor, node, metastasis, biology. Data taken from [48].	

correctly reclassifies subjects in comparison with an established model [49]. NRI ranges from -1 to 1, where -1 indicates that the new model is perfectly worse than the old one and 1 indicates the new model is perfectly better [50]. In this case, NRI showed improvement by 0.33 (95% CI: 0.24–0.41) for the TNMB system over the eighth edition [47]. The relative integrated discrimination improvement, an index that quantifies the relative improvement in the capability of a new staging system to distinguish between subjects who survive [51], showed that the TNMB system improved by 22.1% (95% CI: 8.8–35.3%) compared with the eighth edition [47]. Strikingly, in comparing the change in performance between the seventh and eighth editions, there was no improvement at all in either NRI (0.03 [95% CI: -0.01 to 0.06]) or relative integrated discrimination improvement (-2.5% [95% CI: -17.6 to 12.4%]). These findings demonstrate improved prognostic ability of the TNMB staging system in a way that has been difficult to attain with reorganization of conventional nonmolecular approaches alone.
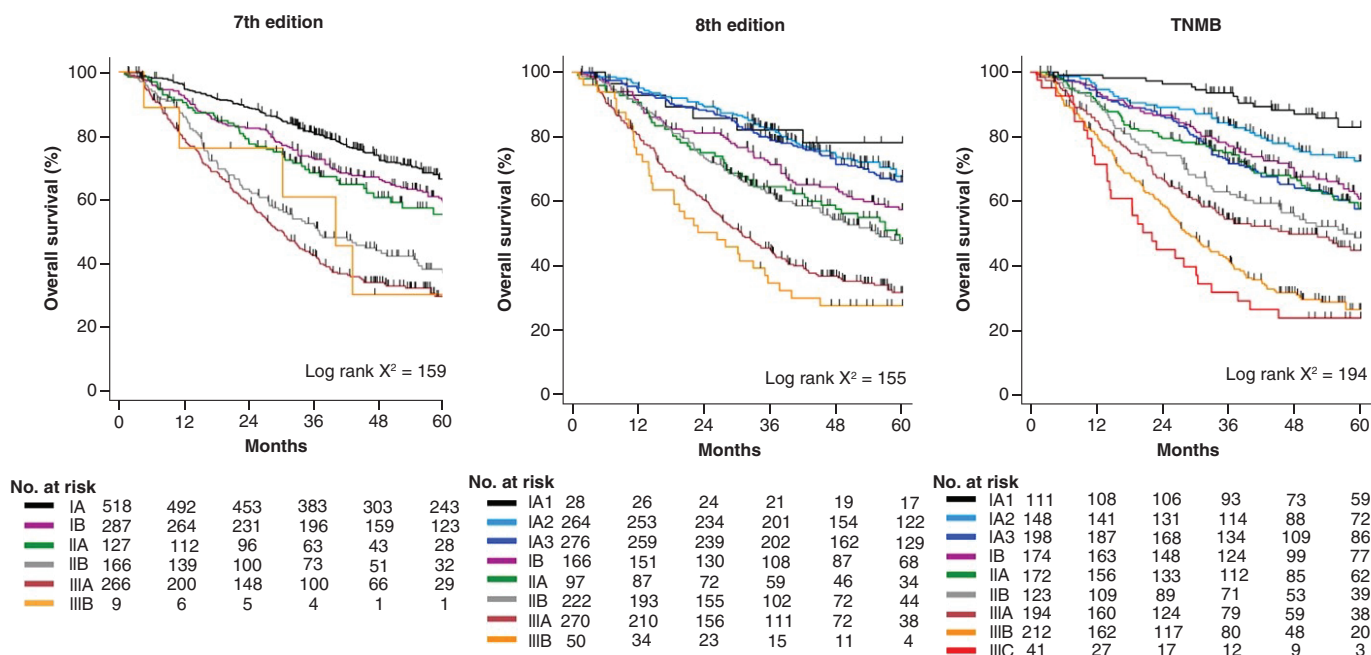
Figure 1.   Overall survival of patients with non-small-cell lung cancer after surgical resection by TNM seventh edition, TNM eighth edition and TNMB staging in the validation cohort (n = 1373). The proposed TNMB system is a novel staging system that integrates the results of the 14-gene molecular prognostic classifier into the eighth edition TNM staging. TNMB staging has distinct separation of survival by stage and a larger range of survival between early and late stages compared with conventional staging.
No.: Number; TNM: Tumor, node, metastasis; TNMB: Tumor, node, metastasis, biology.
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Following retrospective analysis, a prospective validation was performed [48]. In a prospective cohort study, 238 patients with stage I–IIIC nonsquamous NSCLC underwent surgical resection and prognostic assay evaluation at the time of cancer treatment at UCSF. All patients underwent complete R0 surgical resection of early-stage disease, including sublobar or lobar resections, with mediastinal lymph node dissection following best practice guidelines. Patients who did not undergo a residual tumor resection were excluded from the study. Patients were restaged in accordance with the seventh edition, eighth edition and TNMB staging system. Compared with the seventh edition, staging with the eighth edition did not show any improvement in classification, with NRI of 0.02 (95% CI: -0.18 to 0.21). However, with adoption of the TNMB staging system, NRI improved significantly to 0.28 (95% CI: 0.08–0.46). This prospective cohort study demonstrated that the TNMB staging system improved prognostication compared with conventional TNM staging.

Clinical utility of the 14-gene lung cancer assay in clinical practice

Several large-scale, prospective trials have previously demonstrated that adjuvant chemotherapy can decrease recurrence and increase overall survival in patients with stage II and higher NSCLC [52–55]. In these same studies, however, adjuvant chemotherapy failed to provide a benefit to patients with stage I disease even though 30–50% of these patients will die within 5 years because of disease recurrence from occult micrometastases at the time of initial resection.

Current guidelines from the National Comprehensive Cancer Network (NCCN) suggest that patients with stage IB and IIA disease who are believed by their clinicians to be at high risk of recurrence and death should receive adjuvant chemotherapy. However, these same guidelines recognize that there are no clinical or histopathological criteria that have been validated to provide adequate guidance for the determination of 'high risk' [56,57].

Prospective clinical investigation using the 14-gene lung cancer assay

In a recent prospective study, 100 consecutive patients with stage IA, IB or IIA nonsquamous NSCLC resected at UCSF were evaluated with the 14-gene lung cancer assay [58]. Based on assay results, patients with intermediate- or high-risk scores, and therefore at elevated risk of recurrence, were referred to a thoracic oncologist for discussion

of adjuvant chemotherapy. The decision of whether to pursue adjuvant chemotherapy was made on an individual basis between the patient and the primary oncologist. Patients were followed to evaluate for disease recurrence, which closely mirrors 5-year survival.

Results from the molecular assay stratified 52 (52%) patients as clinically low risk and 48 (48%) patients as high risk (intermediate risk: n = 22; high risk: n = 26). Based on NCCN guidelines, 36 (36%) patients were identified as eligible for adjuvant chemotherapy, and 13 (13%) patients actually received adjuvant chemotherapy. In all, NCCN and molecular assay recommendations for adjuvant chemotherapy were discordant in 34 (34%) patients. Ultimately, 13 (13%) patients received adjuvant chemotherapy, all of whom were molecular high risk; most cases of refusal of adjuvant chemotherapy were related to patient preference.

At 23-month median follow-up, recurrence had occurred in 11 (11%) patients. Ten (91%) of the recurrences occurred in the molecular high-risk group, with a 21% recurrence rate for high-risk patients. This was significantly higher than the 2% rate of recurrence for the low-risk group (Fisher's exact test p = 0.003) [58]. The Kaplan–Meier estimate of 5-year disease-free survival was 93.8% among molecular low-risk patients, which was significantly higher than that for molecular high-risk patients (58.8%; log-rank p = 0.006). Conversely, NCCN stratification was not as successful at predicting patient prognosis, as recurrence did not differ significantly between patients with NCCN high-risk clinicopathological features (5-year disease-free survival: 73.1%) and patients without high-risk features (75.7%; log-rank p = 0.112). On multivariate regression analysis controlling for other variables, including NCCN risk category and clinical stage, molecular high-risk status was the only significant predictor of disease recurrence (p = 0.046).

Importantly, the analysis showed that among patients who were part of the molecular high-risk group, those who received adjuvant chemotherapy had significantly better 5-year disease-free survival (91.7%) compared with those who did not (48.7%; log-rank p = 0.004). These data suggest that selective administration of adjuvant chemotherapy to early-stage patients designated as molecular high risk reduces recurrence to rates similar to those experienced by the low-risk patients who were observed.

Results from an expanded cohort of 250 patients were recently presented at the International Association for the Study of Lung Cancer 2020 North America Conference on Lung Cancer [59]. In the expanded cohort, molecular low-risk patients had freedom from recurrence (FFR) of 94.6% at median follow-up of 29 months compared with FFR of 72.4% in the high-risk group that did not receive adjuvant chemotherapy. Importantly, for the high-risk cohort that did receive adjuvant chemotherapy, FFR increased to 97.0%. This trend held true for the specific subset of 168 patients with stage IA disease, with FFR of 97.4% for low-risk, 73.2% for untreated high-risk and 100% for high-risk patients treated with adjuvant chemotherapy.

At this time, an international randomized clinical trial is underway, designed to measure the magnitude of benefit that may be derived from adjuvant chemotherapy in molecular high-risk patients [60]. Based on the technical rigor and reproducibility of the assay results coupled with a demonstration of clinical utility in a prospective study, the 14-gene lung cancer assay is now reimbursed by Medicare and many private insurers as an important component of decision-making for early-stage patients with resected NSCLC.

Opportunity for a new standard of care with higher survival for early-stage NSCLC

Patient outcomes following a diagnosis of early-stage NSCLC remain poor compared with other solid tumor types despite advances in our understanding of the molecular mechanisms of disease. Although several randomized controlled trials [52–54,61–63] have previously demonstrated improved survival in patients with stage II–IIIA NSCLC, a meta-analysis of these studies [55] failed to show definitive benefit in patients with stage I disease. Similarly, a randomized controlled trial of paclitaxel plus carboplatin in patients with stage IB lung cancer [64] demonstrated no survival benefit in comparison with observation alone.

In the majority of patients who die after resection of stage I disease, the inciting factor is distant disease recurrence [65]. This suggests that many patients are incorrectly identified as having localized disease by conventional eighth edition criteria, when in reality they harbor occult, likely micrometastatic disease at the time of the attempt at curative surgery. It is probable that the inability of prior studies to demonstrate benefit in these patients with early-stage disease is due to the inability to select patients most likely to harbor these micrometastases, leading to a dilution of treatment effect from overtreating patients with favorable tumor biology (i.e., likely cured in the operating room). This possibility is supported by results of a retrospective review [66] that assessed several tumor gene profiles and found that patients at high risk of treatment failure would have benefited from adjuvant treatment.

High recurrence rates after surgery for NSCLC underscore the need for a practical molecular assay to more reliably identify early-stage patients with increased risk of micrometastases and recurrence after surgery. The 14-gene lung cancer assay is practical on a large scale, in that it utilizes widely available FFPE tissue blocks and highly reliable qRT-PCR to measure expression of a small number of important cancer-related genes. The assay has been rigorously validated in two large, international patient cohorts using a blinded study design [37,40]. Compared with conventional staging alone, this robust assay successfully improves our ability to identify patients at increased risk of death.

The TNMB staging system has proved more robust in distinguishing between patients with and without disease recurrence compared with conventional TNM staging [47]. This observation supports the notion that a substantial and clinically relevant improvement over conventional staging can be attained through the incorporation of molecular classifiers. The incorporation of this fourth category representing tumor biology is easy for clinicians to understand and apply and allows for the flexibility to integrate future refinements from novel characterizations of tumor biology.

Conclusion

A rigorously validated molecular assay has been shown to significantly improve identification of patients at highest risk of disease recurrence and mortality after resection of early-stage nonsquamous NSCLC. This risk classifier can be incorporated into conventional TNM staging in a clinically intuitive way and significantly improves risk discrimination beyond conventional staging. The assay has been shown to predict benefit from adjuvant chemotherapy in a prospective cohort of patients. An international randomized controlled trial investigating the magnitude of benefit of adjuvant chemotherapy in high-risk patients is underway.

Future perspective

A large, multicenter, international, randomized trial is underway to measure the magnitude of benefit derived from adjuvant chemotherapy in patients with molecular high-risk early-stage lung cancer. Adoption of the TNMB staging system may meaningfully identify patients who would benefit from additional therapy to target micrometastatic disease. Overall, clinical use of this assay may lead to substantive improvement in survival for patients with early-stage NSCLC.

Executive summary

- Despite unacceptably high rates of recurrence and mortality in patients diagnosed with early-stage non-small-cell lung cancer, prior studies have failed to demonstrate benefit from adjuvant chemotherapy in patients with stage I disease after surgery.

Development & technical validation

- A Clinical Laboratory Improvement Amendments-certified, commercially available and reimbursed 14-gene molecular prognostic assay has been developed and validated in two large international patient cohorts.
- The prognostic assay utilizes quantitative reverse transcription PCR-based analysis of readily available and formalin-fixed, paraffin-embedded tissue blocks rather than snap-frozen tissue samples, as used in prior-generation assays.

Staging refinement using the 14-gene lung cancer assay

- The molecular assay reliably identifies patients at low, intermediate and high risk of mortality after surgery for early-stage nonsquamous non-small-cell lung cancer.
- The molecular assay improves prognostic accuracy beyond conventional tumor, node, metastasis staging.

Prospective clinical investigation using the 14-gene lung cancer assay

- The molecular assay predicts benefit from adjuvant chemotherapy in patients with molecular high-risk disease.
- Patients with high-risk disease have significantly lower freedom from recurrence than low-risk patients, and high-risk patients treated with adjuvant chemotherapy have significantly improved freedom from recurrence, on par with patients with low-risk disease.
- An international randomized clinical trial is underway, designed to measure the magnitude of benefit that may be derived from adjuvant chemotherapy in molecular high-risk patients.

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