Prognostic signature of early lung adenocarcinoma based on the expression of ribonucleic acid metabolism–related genes

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ABSTRACT

Objective: The current staging system for lung cancer is not sufficient to accurately identify those patients with early-stage tumors who would benefit from postsurgery chemotherapy. The objective of this study was to validate a prognostic signature based on the expression of 5 RNA (ribonucleic acid) metabolism-related genes.

Methods: Five lung cancer microarray datasets, 3 from adenocarcinomas and 2 from squamous cell carcinomas, were analyzed. Kaplan-Meier survival curves and Cox proportional hazards models were used to evaluate the relationship between the classifier and recurrence and survival.

Results: Statistically significant differences in relapse-free survival and overall survival were observed when lung adenocarcinoma patients were divided into 3 risk groups. The prognostic information provided by the signature was independent from other demographic and disease variables, including stage. Significant differences in survival were observed between low- and high-risk groups in stage-IB patients: 5-year survival rates ranged from 83% to 100% in the low-risk groups, and from 30% to 71% in the high-risk groups, depending on the dataset. The RNA metabolism score additionally displayed an association with the benefit of adjuvant chemotherapy ($P < .001$), suggesting that those patients in the low-risk group are not good candidates for this treatment.

Conclusions: The RNA metabolism signature is a prognostic marker that may be useful for predicting survival and optimizing the benefit of adjuvant chemotherapy in patients with lung adenocarcinoma. (J Thorac Cardiovasc Surg 2015;150:986-92)

The RNA score accurately predicts overall survival in stage IB lung adenocarcinoma patients.

Central Message

Expression of 5 RNA metabolism–related genes predicts survival, and benefit of adjuvant therapy in resectable lung adenocarcinomas.

Perspective

More than half of patients with early-stage lung cancer have a recurrence after surgery, and prognostic factors are needed to select those patients who would benefit from adjuvant therapy. We describe a prognostic signature, based on the expression of RNA metabolism–related genes, that accurately classifies adenocarcinoma patients, independently of stage, and predicts benefit from adjuvant chemotherapy.

See Editorial Commentary page 993.

Supplemental material is available online.

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Lung cancer is the leading cause of cancer mortality, representing >25% of all cancer deaths.¹ Non–small cell lung cancer (NSCLC) accounts for approximately 80% of all lung cancer cases. Currently, only 15% to 20% of NSCLC cancer patients present with localized, potentially curable disease.² These numbers are expected to increase in the near future, owing to the implementation of screening programs based on the results of the National Lung Screening Trial.³ Surgical resection is the treatment of choice for early-stage NSCLC, but several studies have demonstrated that adjuvant chemotherapy can improve survival.^{4–6} Based on these studies, adjuvant chemotherapy has become the standard of care for resected stage II, but its use is controversial in stage IB, and is not recommended in stage IA.^{7,8} Nevertheless, data suggest that adjuvant chemotherapy is effective for selected patients with stage-I NSCLC.⁹

Abbreviations and Acronyms

CI	= confidence interval
DCC	= Director's Challenge Consortium (for the Molecular Classification of Lung Adenocarcinoma)
NSCLC	= non-small cell lung cancer
RNA	= ribonucleic acid

Several studies have reported clinicopathologic and molecular prognostic factors in patients with early-stage NSCLC.¹⁰⁻¹² In search of RNA (ribonucleic acid) metabolism-related genes with altered expression in lung adenocarcinoma, we previously identified a 5-gene categorical classifier with prognostic potential.¹³ The biological importance of RNA metabolism in lung adenocarcinomas is evidenced by the identification of aberrant RNA transcripts associated with somatic mutations in genes encoding splicing factors.¹⁴ The main objective of the present study was to validate the prognostic performance of the RNA metabolism signature in independent cohorts of patients with early-stage NSCLC. Our study reveals a substantial capacity of the signature to classify adenocarcinoma patients into various risk groups. In addition, the signature shows an association with the benefit of adjuvant chemotherapy.

METHODS

Patient Cohorts

Five independent NSCLC microarray datasets were used to assess the prognostic capacity of the RNA metabolism signature: 3 with expression data from adenocarcinoma patients, and 2 from patients with squamous-cell carcinoma. Tumor specimens were collected under approval from the respective institutional review boards. All datasets were retrieved from the Gene Expression Omnibus repository (<http://www.ncbi.nlm.nih.gov/geo/>), except for the files from the Director's Challenge Consortium for the Molecular Classification of Lung Adenocarcinoma (DCC) (<https://array.nci.nih.gov/caarray/home.action>).

Characteristics of the datasets used in this study are shown in Table 1. Briefly, the DCC dataset was based on expression data from 302 lung adenocarcinoma patients.¹⁵ Four institutions formed the DCC: the University of Michigan Cancer Center, Moffitt Cancer Center, Memorial Sloan-Kettering Cancer Center, and Dana-Farber Cancer Institute. None of the patients received preoperative chemotherapy or radiation. We divided the patients in the DCC database into those who did not receive any adjuvant therapy ($n = 213$), and those who received adjuvant chemotherapy ($n = 89$). The GSE31210 dataset contained data from 204 lung adenocarcinoma patients who did not receive postoperative therapy from the National Cancer Center Hospital in Japan.¹⁶ The GSE13213 dataset was obtained from 117 lung adenocarcinoma patients who underwent potential curative resection at Aichi Cancer Center in Japan.¹⁷ None of them received adjuvant chemotherapy.

The GSE17710 dataset contained information on 56 primary tumors surgically extracted at the University of North Carolina from treatment-naïve patients with lung squamous cell carcinomas.¹⁸ The fifth NSCLC dataset (GSE4573) contained expression data of resected lung squamous cell carcinomas collected from 129 patients at the University of Michigan Hospital.¹⁹ No information was found about the use of adjuvant therapy in these patients.

Description of the RNA Metabolism Signature

The RNA metabolism signature was derived from a comparison of normal lung tissues with lung adenocarcinomas.¹³ Five genes were selected for the prognostic classifier: *ADAR2*, *MARS*, *RAE1*, *SNRPB*, and *SNRPE*. The median expression of each of these genes was used to dichotomize tumors as high or low expressers (the probesets used to assess gene expression are specified in Table E1). *MARS*, *RAE1*, *SNRPB*, or *SNRPE* are overexpressed in lung adenocarcinomas¹³; therefore, tumors with high expression of these 4 genes received 1 risk point for each elevated gene. In the case of *ADAR2*, this gene is down-regulated in lung tumors and may function as a tumor suppressor.¹³ Consequently, 1 risk point was assigned to those tumors with low expression of *ADAR2*. The combined risk score was calculated as the sum of the 5 individual scores (ranging from 0 to 5). Patients were divided into 3 risk groups based on the combined risk score: 0: low risk; 1 to 3: medium risk; 4 or 5: high risk.

Statistical Analysis

The clinical endpoints of the cohorts are shown in Table 1. Overall survival was estimated by the Kaplan-Meier method, and differences between curves were compared with the log-rank test. Kaplan-Meier estimates of survival, with their 95% confidence intervals (CIs), are shown in Tables E2-E6. Multivariate analyses were performed using the Cox proportional hazards model. Based on univariate Cox significance levels ($P < .2$), variables were incorporated into Cox models for multivariate analysis. The assumption of proportional hazard was tested by introducing time-dependent covariates into the models. The interaction between the RNA metabolism signature and adjuvant chemotherapy was additionally evaluated, using the Cox proportional hazards model. All statistical analyses were 2-sided. All tests were performed using STATA/IC 12.1 software (Stata Corporation, College Station, Tex).

RESULTS

Lung adenocarcinoma patients included in the DCC dataset showed important differences in prognosis when divided into low-, medium-, and high-risk groups based on a 5-gene RNA metabolism signature.¹³ To validate this observation, we used 2 independent microarray datasets of lung adenocarcinoma patients: GSE31210 and GSE13213, based on Affymetrix, Inc and Agilent technologies (both Santa Clara, Calif), respectively. Demographics and clinical characteristics of these cohorts are summarized in Tables E7 and E8.

In the GSE31210 dataset (204 lung adenocarcinoma patients), the RNA metabolism score was associated with risk of recurrence and death (Figure 1, A and B). Cox regression analyses for relapse-free survival and overall survival are shown in Tables E9 and E10, respectively. The prognostic information provided by the RNA metabolism score was independent of other demographic and disease variables, including stage (Table 2). The 5-year survival rates were 95.8% (95% CI: 73.9%-99.4%) for the low-risk group; 89.6% (95% CI: 81.4%-94.3%) for the medium-risk group; and 73.8% (95% CI: 60.7%-83.2%) for the high-risk group.

Differences in survival were additionally observed among the 117 lung adenocarcinoma patients from the GSE13213 dataset (Figure 1, C; and Table E11). A multivariate analysis showed the prognostic importance of the score

TABLE 1. Summary of the datasets used in the study

Dataset	Cancer type	Array platform	Number of patients	Median follow-up (mon)	Clinical endpoint
DCC	Lung ADC	Affymetrix U133A	213 + 89	48	RFS and OS
GSE31210	Lung ADC	Affymetrix U133 Plus 2.0	204	58	RFS and OS
GSE13213	Lung ADC	Agilent G4112F	117	68	OS
GSE17710	Lung SCC	Agilent 44K Custom	56	22	RFS and OS
GSE4573	Lung SCC	Affymetrix U133A	129	34	OS

For DCC, number of patients indicates 213 patients without adjuvant therapy, and 89 with adjuvant chemotherapy. Affymetrix and Agilent are based in Santa Clara, Calif. DCC, Director's Challenge Consortium (for the Molecular Classification of Lung Adenocarcinoma); ADC, adenocarcinoma; RFS, relapse-free survival; OS, overall survival; SCC, squamous cell carcinoma.

as well (Table 2). The 5-year survival rates were: 90.0% (95% CI: 47.3%-98.5%) for the low-risk group; 72.4% (95% CI: 60.8%-81.0%) for the medium-risk group; and 41.9% (95% CI: 24.7%-58.3%) for the high-risk group. We additionally tested whether the prognostic signature was applicable to patients with lung squamous cell carcinoma. We used data from the GSE17710 (56 patients) and GSE4573 (130 patients) datasets, which used Agilent and Affymetrix, Inc, technologies, respectively. The signature was unable to classify patients with lung squamous cell carcinoma into groups with differing prognoses (Figure E1).

We next evaluated the usefulness of the score within separated early stages in the 3 datasets of adenocarcinoma patients. The RNA metabolism signature maintained its prognostic capacity when patients were divided by stages (Figure 2). Data from the individual datasets are shown in Figures E2-E6. In stage IB, important differences in 5-year survival rates were observed between low- and high-risk groups: 100% in the low-risk group, and 54.6% (95% CI: 32.1%-72.4%) in the high-risk group for patients in the DCC dataset; 100% in the low-risk group, and 71.4% (95% CI: 40.6%-88.2%) in the high-risk group for patients in the GSE31210 dataset; and 83.3% (95% CI: 27.3%-97.5%) in the low-risk group, and 30.0% (95% CI: 7.1%-57.8%) in the high-risk group for patients in the GSE13213 dataset. The negative predictive value of the signature in stage-IB patients was 95% (only 1 of the 21 patients in the low-risk group relapsed).

The potential association between the prognostic groups derived from the signature, and the benefit of adjuvant chemotherapy was evaluated in the DCC cohort, which contains clinical outcome data from 213 patients with no adjuvant therapy, and 89 patients treated with adjuvant chemotherapy (demographics and clinical characteristics of these patients are summarized in Table E12). The RNA metabolism score displayed interactive effects with adjuvant chemotherapy ($P = .013$), indicating differential responses to chemotherapy among groups. In particular, differences were found in overall survival between patients treated and untreated (in favor of the untreated group) in the low- and medium-risk groups (Figure E7, A and B); whereas no differences were found between treatment arms in the high-risk group (Figure E7, C).

A characteristic of the DCC dataset, likely due to a bias in patient selection, is the poorer outcome of those stage-I patients receiving adjuvant chemotherapy.²⁰ This fact would explain the increase we observed in the survival of untreated patients in low- and medium-risk groups, and the absence of effect in the high-risk group. Therefore, to better evaluate the beneficial effect of adjuvant therapy in the various risk groups, we analyzed the association between the prognostic score and adjuvant chemotherapy in stages I and II/III separately ($n = 196$ and $n = 103$, respectively). The interaction between the RNA metabolism score and adjuvant chemotherapy was retained in both groups ($P = .024$ and $P = .045$, respectively). In stage-I patients, differences in favor of the untreated group were still observed, especially in low- and medium-risk groups (Figure 3, A-C). In contrast, in stage-II/III patients, in the low- and medium-risk groups, no differences in survival between patients treated or untreated were found, whereas in patients in the high-risk group, a clear survival improvement after treatment with chemotherapy was observed (Figure 3, D-F). The 5-year survival rate for stage-II/III patients with a high risk of progression was 9.4% (95% CI: 0.7%-31.8%) in the untreated group and increased to 41.7% (95% CI: 15.2%-66.5%) in the group treated with adjuvant chemotherapy.

DISCUSSION

This study extensively validates the prognostic capacity of a signature based on the expression of RNA metabolism-related genes in patients who have early-stage lung adenocarcinoma. The signature is composed of 5 genes: *MARS*, *RAEI*, *SNRPB*, *SNRPE*, and *ADAR2*, the first 4 of which are up-regulated in lung adenocarcinomas and seem to be associated with cell proliferation or oncogenic potential; the 5th (*ADAR2*) is down-regulated and may function as a tumor suppressor.¹³ In accordance with other markers specifically developed for lung adenocarcinomas,²¹⁻²³ our signature was unable to predict survival in patients with lung squamous cell carcinoma, likely reflecting the fundamental molecular and biological differences among these histologic subtypes.

Many gene-expression prognostic signatures have been suggested to predict recurrence or survival in surgically

FIGURE 1. Evaluation of the prognostic capacity of the RNA metabolism signature in lung adenocarcinoma patients: (A) Relapse-free survival in the GSE31210 dataset; (B) Overall survival in the GSE31210 dataset;

TABLE 2. Multivariate Cox regression analysis in lung adenocarcinoma patients from the GSE31210 and GSE13213 databases

Database	n	Hazard ratio (95% CI)	P value
GSE31210 (n = 204)			
Relapse-free survival			
Age (y)			
≤60	99	1	.027
>60	105	1.87 (1.07-3.26)	
Smoking status			
Never-smoker	105	1	.994
Ever-smoker	99	1.00 (0.57-1.76)	
Stage			
I	162	1	.001
II	42	2.66 (1.48-4.79)	
EGFR			
Wild-type	185	1	.307
Mutated	19	0.75 (0.43-1.30)	
Prognostic score			
0	26	1	.004
1-3	110	5.53 (0.74-41.12)	
4, 5	68	12.01 (1.61-89.53)	
Overall survival			
Gender			
Female	109	1	.843
Male	95	1.10 (0.41-3.06)	
Smoking status			
Never-smoker	105	1	.684
Ever-smoker	99	1.24 (0.43-3.57)	
Stage			
I	162	1	.005
II	42	2.98 (1.40-6.35)	
EGFR			
Wild-type	185	1	.257
Mutated	19	0.65 (0.30-1.38)	
Prognostic score			
0	26	1	.132
1-3	110	2.38 (0.31-18.44)	
4, 5	68	4.61 (0.59-35.81)	
GSE13213 (n = 117)			
Overall survival			
Stage			
I	79	1	.003
II/III	38	2.38 (1.35-4.21)	
Prognostic score			
0	10	1	.002
1-3	76	2.93 (0.39-21.99)	
4, 5	31	7.46 (0.98-56.62)	

Boldface indicates statistical significance. *CI*, Confidence interval.

(C) Overall survival in the GSE13213 dataset. Patients were divided into low-, medium-, and high-risk groups based on the RNA metabolism score, as indicated in the Methods section. The *P* value of the log-rank test is shown in each graph.

FIGURE 2. Evaluation of the capacity of the RNA metabolism signature to predict overall survival in lung adenocarcinoma patients, separated by stages. Patients in the low-, medium-, and high-risk groups were combined from the separate analyses performed in the DCC, GSE31210, and GSE13213 datasets ($n = 534$). (A) Stage I ($n = 398$); (B) Stages II and III ($n = 134$); (C) Stage IA ($n = 227$); (D) Stage IB ($n = 171$). Patients were divided into low-, medium-, and high-risk groups based on the RNA metabolism score, as indicated in the Methods section. The P value of the log-rank test is shown in each graph.

treated lung cancer patients.²⁴⁻²⁶ However, a major limitation of most signatures is their inconsistent reproducibility in independent datasets.²⁶ To date, 2 profiles have been validated rigorously and are available as clinical tests.^{23,27,28} Both of them are based on the expression of cancer-related target genes (11 genes in one of the signatures, and 31 in the other) determined by quantitative polymerase chain reaction on formalin-fixed paraffin-embedded tissues. Der and colleagues²⁹ have recently validated a 15-gene classifier for patients who have stage I or II NSCLC, which seems to be predictive of improved survival after adjuvant chemotherapy.

Most others signatures have not been validated yet, or are difficult to transfer to the clinical setting because of the large number of genes or the need for sophisticated laboratory

techniques or statistical analyses. Our RNA metabolism prognostic signature, based on a straightforward analysis of only 5 genes, has been successfully validated using several independent datasets from various analytic platforms and patients with various ethnicities. Moreover, the signature has shown a clinically meaningful capability to classify patients into groups with different risks of relapse, with a high negative predictive value (ie, high accuracy in identification of patients who are not going to relapse).

Clinically useful prognostic signatures should improve therapeutic decisions, leading to patient benefit.²⁰ A prognostic test could be considered helpful in cases in which the use of adjuvant chemotherapy is not clearly indicated after consideration of conventional prognostic markers (eg, stage). At present, most patients who are in stage I receive

FIGURE 3. Association between the prognostic capacity of the RNA metabolism signature and the benefit of adjuvant chemotherapy in lung adenocarcinoma patients of the DCC dataset. (A) Low-risk group of patients with stage I. (B) Medium-risk group of patients with stage I. (C) High-risk group of patients with stage I. (D) Low-risk group of patients with stage II or III. (E) Medium-risk group of patients with stage II or III. (F) High-risk group of patients with stage II or III. Patients without any events were censored at 60 months. Patients were divided into risk groups based on the RNA metabolism score, as indicated in the Methods section. The *P* value of the log-rank test is shown in each graph.

only surgical resection, despite the fact that a substantial proportion of them will relapse. Therefore, stage-I patients could benefit the most from a strong prognostic marker.

Our RNA signature was able to identify a subgroup of stage-I patients with a high risk of recurrence. This finding is especially relevant for stage-IB patients, where treatment selection criteria are very poorly defined. In our analysis, only half of the patients in stage IB who were classified as being in the high-risk group survived (compared with 95.5% of patients in the low-risk group). We tentatively suggest that this type of classifier may be used to help decide whether adjuvant therapy is required in patients who are in stage IB. In fact, we have found an interaction between risk group and benefit of adjuvant chemotherapy. To date, few signatures have demonstrated the ability to predict which patients who have resectable NSCLC will gain a survival benefit with adjuvant chemotherapy.^{22,30,31} In our case, based on the high negative predictive value of the RNA metabolism score, and the differential treatment effects in the various risk groups, we can conclude that those lung adenocarcinoma patients in the low-risk group are not good candidates for adjuvant chemotherapy.

One limitation of our study is the analytic platform on which the validation has been conducted. Although

prognostic signatures based on microarray technologies have been successfully applied in the clinic, quantitative polymerase chain reaction is considered the gold standard for messenger RNA expression analysis. This technique requires smaller quantities of tumor tissue and has the advantage of being highly sensitive, reproducible, specific, and easily applicable in the clinical setting.²⁵ The results obtained with the RNA metabolism signature justify further validation studies based on polymerase chain reaction technologies. Applying the method to formalin-fixed paraffin-embedded tissues would additionally broaden its potential applications. The added value of the signature to clinical prognostic factors, such as adenocarcinoma subtype,³² differentiation, or lymphovascular invasion,¹¹ should be evaluated.

Finally, the analysis of the interaction between the risk groups and the benefit of chemotherapy has to be considered preliminary, given that only 1 of the 3 adenocarcinoma datasets used in our study contained patients receiving adjuvant chemotherapy. Additional evaluation of the relationship between the classifier and adjuvant treatment efficacy using appropriate trials is warranted. Moreover, potential associations between the risk score and aspects such as the chemosensitivity of the tumors and the influence of genetic factors merit further evaluation.

In conclusion, we have validated an RNA metabolism prognostic signature able to classify patients who have early-stage lung adenocarcinoma into risk groups, independently of stage. This signature is based on a straightforward 5-gene score, has shown robust prognostic value in various platforms and cohorts, and has a high negative predictive value in very early stages. The use of this signature may improve the management of lung cancer patients and minimize unnecessary treatment.

Conflict of Interest Statement

Authors have nothing to disclose with regard to commercial support.

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Key Words: RNA metabolism, prognosis, adjuvant chemotherapy, lung cancer, adenocarcinoma, squamous cell carcinoma

FIGURE E1. Evaluation of the prognostic capacity of the RNA metabolism signature in lung squamous cell carcinoma patients: (A) Relapse-free survival in the GSE17710 dataset; (B) Overall survival in the GSE17710 dataset; (C) Overall survival in the GSE4573 dataset. Patients were divided into low-, medium-, and high-risk groups based on the RNA metabolism score, as indicated in the Methods section. The *P* value of the log-rank test is shown in each graph. *CI*, Confidence interval.

FIGURE E2. Evaluation of the capacity of the RNA metabolism signature to predict relapse-free survival in lung adenocarcinoma patients of the DCC dataset, divided by stage: (A) Stage I; (B) Stages II/III; (C) Stage IA; (D) Stage IB. Patients without any events were censored at 60 months. The P value of the log-rank test is shown in each graph. *CI*, Confidence interval.

FIGURE E3. Evaluation of the capacity of the RNA metabolism signature to predict overall survival in lung adenocarcinoma patients of the DCC dataset, divided by stage: (A) Stage I; (B) Stages II/III; (C) Stage IA; (D) Stage IB. Patients without any events were censored at 60 months. The *P* value of the log-rank test is shown in each graph. *CI*, Confidence interval.

FIGURE E4. Evaluation of the capacity of the RNA metabolism signature to predict relapse-free survival in lung adenocarcinoma patients of the GSE31210 dataset, divided by stage: (A) Stage I; (B) Stage II; (C) Stage IA; (D) Stage IB. The P value of the log-rank test is shown in each graph. CI, Confidence interval.

FIGURE E5. Evaluation of the capacity of the RNA metabolism signature to predict overall survival in lung adenocarcinoma patients of the GSE31210 dataset, divided by stage: (A) Stage I; (B) Stage II; (C) Stage IA; (D) Stage IB. The *P* value of the log-rank test is shown in each graph. *CI*, Confidence interval.

FIGURE E6. Evaluation of the capacity of the RNA metabolism signature to predict overall survival in lung adenocarcinoma patients of the GSE13213 dataset, divided by stage: (A) Stage I; (B) Stages II/III; (C) Stage IA; (D) Stage IB. The P value of the log-rank test is shown in each graph. CI , Confidence interval.

FIGURE E7. Association between the prognostic capacity of the RNA metabolism signature and the benefit of adjuvant chemotherapy in lung adenocarcinoma patients of the DCC dataset: (A) Low-risk group; (B) Medium-risk group; (C) High-risk group. Patients without any events were censored at 60 months. Patients were divided into risk groups based on the RNA metabolism score as indicated in the Methods section. The *P* value of the log-rank test is shown in each graph. *CI*, Confidence interval.

TABLE E1. Probesets in the various array platforms used to determine the expression of the 5 genes included in the RNA metabolism prognostic score

Gene	Array platform	
	Affymetrix	Agilent
ADAR2	203865_s_at	A_23_P211207
MARS	201475_x_at	A_24_P175909
RAE1	211318_s_at	A_23_P346206
SNRPB	208821_at	A_23_P154675
SNRPE	203316_s_at	A_23_P126291

Affymetrix, Inc, and Agilent are based in Santa Clara, Calif.

TABLE E3. Kaplan-Meier estimates of survival in the 117 lung adenocarcinoma patients from the Aichi Cancer Center in Nagoya, Japan (GSE13213), separated by risk score

Time (mo)	Overall survival (ratio [95% CI])		
	Low risk	Medium risk	High risk
20	1	0.92 (0.83-0.96)	0.87 (0.69-0.95)
40	1	0.83 (0.72-0.90)	0.52 (0.33-0.67)
60	0.90 (0.47-0.99)	0.72 (0.61-0.81)	0.42 (0.25-0.58)
80	0.90 (0.47-0.99)	0.62 (0.50-0.73)	0.35 (0.19-0.52)
100	0.90 (0.47-0.99)	0.58 (0.43-0.70)	0.35 (0.19-0.52)

CI, Confidence interval.

TABLE E2. Kaplan-Meier estimates of survival in the 204 lung adenocarcinoma patients from the National Cancer Center Hospital in Tokyo, Japan (GSE31210), separated by risk score

Time (mo)	Low risk	Medium risk	High risk
Relapse-free survival			
20	1	0.93 (0.87-0.97)	0.73 (0.61-0.82)
40	0.96 (0.76-0.99)	0.85 (0.76-0.90)	0.61 (0.48-0.72)
60	0.96 (0.76-0.99)	0.78 (0.68-0.85)	0.53 (0.40-0.65)
80	0.96 (0.76-0.99)	0.74 (0.61-0.83)	0.53 (0.40-0.65)
100	0.96 (0.76-0.99)	0.74 (0.61-0.83)	0.53 (0.40-0.65)
Overall survival			
20	1	0.97 (0.92-0.99)	0.93 (0.83-0.97)
40	0.96 (0.74-0.99)	0.95 (0.89-0.98)	0.83 (0.72-0.90)
60	0.96 (0.74-0.99)	0.90 (0.81-0.94)	0.68 (0.51-0.80)
80	0.96 (0.74-0.99)	0.88 (0.79-0.93)	0.68 (0.51-0.80)
100	0.96 (0.74-0.99)	0.78 (0.52-0.91)	0.68 (0.51-0.80)

Values are ratio (95% CI).

TABLE E4. Kaplan-Meier estimates of overall survival in lung adenocarcinoma from patients in the low-, medium-, and high-risk groups, combined from the separate analyses performed in the DCC, GSE31210, and GSE13213 datasets

Time (mo)	Overall survival (ratio [95% CI])		
	Low risk	Medium risk	High risk
Stage I			
20	1	0.97 (0.94-0.99)	0.93 (0.86-0.96)
40	0.98 (0.86-1.00)	0.92 (0.88-0.95)	0.78 (0.70-0.85)
60	0.95 (0.81-0.99)	0.85 (0.79-0.89)	0.68 (0.59-0.76)
80	0.95 (0.81-0.99)	0.95 (0.81-0.99)	0.62 (0.49-0.72)
100	0.95 (0.81-0.99)	0.95 (0.81-0.99)	0.62 (0.49-0.72)
Stage II/III			
20	0.93 (0.61-0.99)	0.75 (0.64-0.83)	0.67 (0.49-0.79)
40	0.80 (0.50-0.93)	0.63 (0.52-0.73)	0.40 (0.24-0.56)
60	0.72 (0.41-0.89)	0.54 (0.42-0.64)	0.33 (0.18-0.49)
80	0.72 (0.41-0.89)	0.47 (0.34-0.58)	0.27 (0.13-0.45)
100	0.72 (0.41-0.89)	0.35 (0.19-0.52)	0.27 (0.13-0.45)
Stage IA			
20	1	0.99 (0.94-1.00)	0.99 (0.90-1.00)
40	0.96 (0.73-0.99)	0.96 (0.91-0.98)	0.80 (0.68-0.88)
60	0.96 (0.73-0.99)	0.91 (0.84-0.95)	0.75 (0.62-0.84)
80	0.96 (0.73-0.99)	0.86 (0.75-0.92)	0.75 (0.62-0.84)
100	0.96 (0.73-0.99)	0.86 (0.75-0.92)	0.75 (0.62-0.84)
Stage IB			
20	1	0.96 (0.90-0.98)	0.84 (0.70-0.91)
40	1	0.90 (0.81-0.94)	0.69 (0.54-0.80)
60	0.93 (0.61-0.99)	0.78 (0.67-0.85)	0.55 (0.40-0.68)
80	0.93 (0.61-0.99)	0.67 (0.52-0.79)	0.48 (0.28-0.65)
100	0.93 (0.61-0.99)	0.67 (0.52-0.79)	0.48 (0.28-0.65)

CI, Confidence interval.

TABLE E5. Kaplan-Meier estimates of overall survival in stage-I adenocarcinoma patients from the DCC database, treated or untreated with adjuvant chemotherapy

Time (mo)	Overall survival (ratio [95% CI])	
	No adjuvant therapy	Adjuvant therapy
Low risk		
20	1	0.86 (0.33-0.98)
40	1	0.86 (0.33-0.98)
60	1	0.51 (0.12-0.81)
Medium risk		
20	0.93 (0.85-0.97)	0.91 (0.68-0.98)
40	0.86 (0.76-0.92)	0.73 (0.49-0.87)
60	0.78 (0.67-0.86)	0.59 (0.36-0.76)
High risk		
20	0.88 (0.75-0.94)	0.80 (0.41-0.95)
40	0.72 (0.57-0.82)	0.60 (0.25-0.83)
60	0.63 (0.48-0.75)	0.48 (0.16-0.74)

CI, Confidence interval.

TABLE E7. Demographics and clinical characteristics of the 204 lung adenocarcinoma patients from the National Cancer Center Hospital in Tokyo, Japan (GSE31210)

Age (y), mean ± SD	59.6 ± 7.50
Gender	
Female	109 (53)
Male	95 (47)
Smoking status	
Never-smoker	105 (51)
Ever-smoker	99 (49)
Stage	
I	162 (79)
II	42 (21)

Values are n (%), unless otherwise indicated. SD, Standard deviation.

TABLE E6. Kaplan-Meier estimates of overall survival in stage-II/III adenocarcinoma patients from the DCC database, treated or untreated with adjuvant chemotherapy

Time (mo)	Overall survival (ratio [95% CI])	
	No adjuvant therapy	Adjuvant therapy
Low risk		
20	1	1
40	0.71 (0.26-0.92)	0.50 (0.01-0.91)
60	0.71 (0.26-0.92)	—
Medium risk		
20	0.74 (0.55-0.86)	0.63 (0.45-0.76)
40	0.60 (0.40-0.75)	0.43 (0.26-0.58)
60	0.42 (0.22-0.62)	0.36 (0.21-0.52)
High risk		
20	0.31 (0.11-0.54)	0.58 (0.27-0.80)
40	0.19 (0.05-0.40)	0.42 (0.15-0.67)
60	0.09 (0.01-0.32)	0.42 (0.15-0.67)

CI, Confidence interval.

TABLE E8. Demographics and clinical characteristics of the 117 lung adenocarcinoma patients from the Aichi Cancer Center in Nagoya, Japan (GSE13213)

Age (y), mean ± SD	60.7 ± 10.17
Gender	
Female	57 (49)
Male	60 (51)
Smoking status	
Never-smoker	56 (48)
Ever-smoker	61 (52)
Stage	
I	79 (68)
II	13 (11)
III	25 (21)

Values are n (%), unless otherwise indicated. SD, Standard deviation.

TABLE E9. Univariate Cox regression analysis for relapse-free survival in the 204 lung adenocarcinoma patients from the National Cancer Center Hospital in Tokyo, Japan (GSE31210)

Factor	n	Hazard ratio (95% CI)	P value
Age (y)			
≤60	99	1	.106
>60	105	1.57 (0.91-2.71)	
Gender			
Female	109	1	.220
Male	95	1.40 (0.82-2.38)	
Smoking status			
Never-smoker	105	1	.190
Ever-smoker	99	1.43 (0.84-2.44)	
Stage			
I	162	1	<.001
II	42	3.44 (1.98-6.00)	
EGFR			
Wild-type	88	1	.041
Mutated	116	0.57 (0.33-0.98)	
KRAS			
Wild-type	185	1	.744
Mutated	19	1.15 (0.49-2.69)	
ALK			
Wild-type	197	1	.581
Fused	7	0.57 (0.79-4.14)	
c-Myc			
Low	187	1	.611
High	16	1.26 (0.52-3.06)	
Not determined	1		
Prognostic score			
0	26	1	<.001
1-3	110	5.96 (0.80-44.13)	
4,5	68	15.63 (2.13-114.69)	

Boldface indicates statistical significance. *CI*, Confidence interval.

TABLE E10. Univariate Cox regression analysis for overall survival in the 204 lung adenocarcinoma patients from the National Cancer Center Hospital in Tokyo, Japan (GSE31210)

Factor	n	Hazard ratio (95% CI)	P value
Age (y)			
≤60	99	1	.515
>60	105	1.27 (0.62-2.62)	
Gender			
Female	109	1	.157
Male	95	1.69 (0.82-3.48)	
Smoking status			
Never-smoker	105	1	.084
Ever-smoker	99	1.91 (0.92-3.97)	
Stage			
I	162	1	<.001
II	42	4.30 (2.09-8.83)	
EGFR			
Wild-type	88	1	.038
Mutated	116	0.46 (0.22-0.96)	
KRAS			
Wild-type	185	1	.989
Mutated	19	0.99 (0.30-3.28)	
ALK			
Wild-type	197	1	.956
Fused	7	1.06 (0.14-7.78)	
c-Myc			
Low	187	1	.765
High	16	0.81 (0.21-3.17)	
Not determined	1		
Prognostic score			
0	26	1	.011
1-3	110	2.85 (0.37-21.89)	
4,5	68	7.49 (1.00-56.33)	

Boldface indicates statistical significance. *CI*, Confidence interval.

TABLE E11. Univariate Cox regression analysis for overall survival in the 117 lung adenocarcinoma patients from the Aichi Cancer Center in Nagoya, Japan (GSE13213)

Factor	n	Hazard ratio (95% CI)	P value
Age (y)			
≤60	52	1	.890
>60	65	1.04 (0.59-1.83)	
Gender			
Female	57	1	.286
Male	60	1.36 (0.77-2.39)	
Smoking status			
Never-smoker	56	1	.284
Ever-smoker	61	1.36 (0.77-2.39)	
Stage			
I	79	1	.001
II/III	38	2.53 (1.44-4.45)	
EGFR			
Wild-type	72	1	.945
Mutated	45	1.02 (0.57-1.82)	
KRAS			
Wild-type	102	1	.272
Mutated	15	1.53 (0.72-3.27)	
TP53			
Wild-type	78	1	.322
Mutated	38	1.33 (0.75-2.38)	
Not determined	1		
Prognostic score			
0	10	1	<.001
1-3	76	4.21 (0.57-30.92)	
4, 5	31	10.16 (1.36-75.83)	

Boldface indicates statistical significance. CI, Confidence interval.

TABLE E12. Demographics and clinical characteristics of the 302 lung adenocarcinoma patients from the Director’s Challenge Consortium for the Molecular Classification of Lung Adenocarcinoma dataset

Characteristic	Chemotherapy	
	No	Yes
Age (y), mean ± SD	64.6 ± 10.3	61.6 ± 9.8
Gender		
Female	116 (54)	49 (55)
Male	97 (46)	40 (45)
Smoking status		
Never-smoker	27 (13)	14 (16)
Ever-smoker	179 (87)	73 (84)
NA (n)	7	2
Stage		
I	157 (74)	39 (44)
II	35 (17)	27 (31)
III	19 (9)	22 (25)
NA (n)	2	1

Values are n (%), unless otherwise indicated. NA, Not available; SD, standard deviation.