

## Prediction of Recurrence-Free Survival in Postoperative Non– Small Cell Lung Cancer Patients by Using an Integrated Model of Clinical Information and Gene Expression

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**Abstract Purpose:** One of the main challenges of lung cancer research is identifying patients at high risk for recurrence after surgical resection. Simple, accurate, and reproducible methods of evaluating individual risks of recurrence are needed.

**Experimental Design:** Based on a combined analysis of time-to-recurrence data, censoring information, and microarray data from a set of 138 patients, we selected statistically significant genes thought to be predictive of disease recurrence. The number of genes was further reduced by eliminating those whose expression levels were not reproducible by real-time quantitative PCR. Within these variables, a recurrence prediction model was constructed using Cox proportional hazard regression and validated via two independent cohorts ( $n = 56$  and  $n = 59$ ).

**Results:** After performing a log-rank test of the microarray data and successively selecting genes based on real-time quantitative PCR analysis, the most significant 18 genes had  $P$  values of  $<0.05$ . After subsequent stepwise variable selection based on gene expression information and clinical variables, the recurrence prediction model consisted of six genes (*CALB1*, *MMP7*, *SLC1A7*, *GSTA1*, *CCL19*, and *IFI44*). Two pathologic variables, pStage and cellular differentiation, were developed. Validation by two independent cohorts confirmed that the proposed model is significantly accurate ( $P = 0.0314$  and  $0.0305$ , respectively). The predicted median recurrence-free survival times for each patient correlated well with the actual data.

**Conclusions:** We have developed an accurate, technically simple, and reproducible method for predicting individual recurrence risks. This model would potentially be useful in developing customized strategies for managing lung cancer.

Non-small cell lung cancer (NSCLC) is an increasingly common and lethal disease, accounting for 25% of all cancer deaths (1). Even after surgical resection of early stage primary tumors, ~ 50% of patients die from tumor recurrence (2).

A principal challenge in lung cancer research is identifying patients at high risk for recurrence after surgical resection, as well as patients who would benefit from adjuvant treatment (3–5). The current tumor-node metastasis staging system, which is based on clinical and pathologic findings, may have reached the limit of its usefulness (3–8). Individualized risk evaluation, rather than prediction based on broad population cohorts, would be helpful in the quest to customize treatment strategies for specific patients.

Recent molecular studies, such as those involving gene expression profiling, have shown that various inherent and acquired genetic alterations influence prognosis (9). However, genetic and epigenetic factors have not been considered for use in general clinical practice, much less for use in formulating treatment modalities. Thus, there is a need for new methodologies that are applicable to clinical practice (3–5, 7, 8).

When developing clinically applicable methods that rely on genetic factors to predict prognosis, several points should be considered. First, the outcomes should be expressed as time-dependent continuous variables [e.g., time-to-recurrence (TTR) or survival duration], not as mere binary events (e.g., alive/dead or recurrence/nonrecurrence). Many epidemiologic studies

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Received 11/21/07; revised 6/16/08; accepted 6/18/08.

**Grant support:** Bio & Health Lab, Samsung Advanced Institute of Technology.

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doi:10.1158/1078-0432.CCR-07-4937

### Translational Relevance

One of the most important aspects in lung cancer research is identifying patients who are at risk for recurrence. Clinical predictors for the prognosis of patients, such as age, gender, histologic features, and current tumor-node metastasis staging system may have reached the limit of their usefulness. So, we developed a practical gene expression analysis method for the risk prediction, using time-to-recurrence for gene selection and model development, to complement the current clinical practice. By stepwise variable selection of the gene expression information obtained from microarray data with significant level of 5% and the conventional clinical variables, an integrative recurrence prediction model was formulated consisting of pathogenetic variables, such as the selected six genes, pStage, and cell differentiation. In effect, it has been shown that the sensitivity of recurrence within 2 years is 72.1% and the specificity of nonrecurrence for 5 years is 90.0%, supporting that this prediction model for the recurrence risk provides an improvement in personalized prediction compared with the currently available ones and can help to identify those at high risk of developing postresection recurrences, as well as those beneficial from adjuvant treatments. This model needs to be improved further, but lays the basic foundation of a customized lung cancer management strategy to be used in day-to-day clinical practice.

have relied on such binary events because of the difficulties inherent in processing numerous variables.

Second, gene expression data should be "assayable" or, in other words, measurable in the range detected by real-time quantitative PCR (QPCR). Microarray analysis has made it possible to simultaneously measure the expression of thousands of genes (3, 4, 10–12). However, analysis of cumulative microarray data is impractical in the clinical setting. Real-time QPCR is an alternative and supplementary method that consists of a simple, efficient, and effective assay (13, 14).

Third, clinical and pathologic findings remain important variables in the development of clinically applicable methods for prognosis prediction. Traditional high-risk factors, which are based on clinical and pathologic findings (e.g., advanced stage of illness, high-level involvement of lymph nodes, tumor mass size, and presence of nonsquamous cancer), certainly have prognostic value and play significant roles in treatment planning. Therefore, gene expression profiling should be evaluated in light of any possible synergies with recognized clinical prognostic factors (4, 8).

In this study, we developed a gene expression analysis method that is appropriate for the clinical setting. Data used to develop the model were gathered by analyzing microarray studies of 138 patients and selecting genes that were expressed at statistically significant levels according to TTR. We next eliminated genes for which expression levels were not reproducible by real-time QPCR. The resulting genes were used to construct a recurrence prediction model for each patient, whereby the usefulness of this model in clinical practice was shown in two independent validation cohorts.

### Materials and Methods

**Patient population and sample selection.** A total of 818 frozen tumor samples were collected from patients who underwent curative resection for NSCLC at Samsung Medical Center between January 1995 and December 2005. One or two pieces from the periphery of the tumor masses—avoiding necrotic regions—were immediately frozen at  $-80^{\circ}\text{C}$  until retrieval. Frozen tissues were selected for RNA extraction after the application of exclusion criteria (Materials and Methods in Appendix A).

**Tissue preparation and RNA extraction.** Selected frozen tissues ( $n = 406$ ) were stained with H&E to improve visualization. Necrotic tumor tissues and intervening normal tissues were removed as previously described (15). Extraction and determination of mRNA were done according to the manufacturers' protocols (easy-spin<sup>TM</sup> total RNA extraction kit, iNtRon Biotechnology; Nanodrop Technologies; Agilent RNA 6000 nano kit, Agilent Technologies, Inc.).

A total of 253 frozen tissues with acceptable RNA quality (i.e., RNA integrity number values of over 6.5) were used for the experiment. Oligonucleotide microarray experiments were done using higher quality RNA (i.e., RNA integrity number value of over 8.0,  $n = 138$ ) with sufficient follow-up duration (i.e., frozen tissues collected from 1995 to 2003, with a median of 44 months of follow-up) as the "modeling cohort." Clinical information is shown in Table 1.

To validate our proposed model, RNAs of acceptable quality (i.e., RNA integrity number values = 8.0–6.5,  $n = 56$ ) of the same duration described above were assigned to validation cohort I, whereas RNA from patients with relatively short follow-up periods (i.e., frozen tissues collected from 2004 to 2005 with a median of 33 months of follow-up,  $n = 59$ ) were assigned to validation cohort II. Clinical information from the two validation cohorts is shown in Table 1. An outline of the study is shown in Fig. 1.

**Microarray experiments and real-time QPCR.** Total RNAs from 138 patients were used to analyze mRNA expression profiles on a DNA microarray platform, according to the standard Affymetrix U133 Plus 2.0 protocol with 17 RNAs as duplicate for quality control (correlation coefficient, 0.988). Our entire microarray data set is available online<sup>8</sup> under the data series accession number GSE8894.

To ensure consistency of the mRNA expression levels observed in the microarray experiments, the same set of RNA samples ( $n = 116$ , because 22 RNAs were present in insufficient amounts) was used in the development of the real-time QPCR-based model (Materials and Methods in Appendix A).

Patient follow-up data, including recurrence information, are shown in Table 1. The routine follow-up schedule for postoperative outpatients is described in the Materials and Methods in Appendix A. The last month for follow-up was June 2007, and follow-up for patients who died was complete at the time of death. Patient participation was consistent with protocols approved by the institutional review board of Samsung Medical Center, and written informed consent was obtained (IRB 2005-12-034).

**Statistical analyses.** Microarray data were preprocessed using GCRMA normalization with PM-MM data. Genes were selected using a log-rank test as a robust nonparametric method. The pool of genes was then reduced via real-time QPCR analysis by screening out "assayable" genes that showed ~100-fold differences, on average, between the percentiles of 0 to 3 and 97 to 100 in the microarray analysis. Among these genes, we selected 87 with unique cDNA sequences (based on a search of the public database) and with Universal Probe Library (UPL) and primers sequences that were identical to the 3'-mRNA probe used in the microarray experiments

<sup>8</sup> <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE8894>

**Table 1.** Clinical characteristics of the modeling cohort and validation cohorts I and II

		Modeling cohort (n = 138)		Validation cohort I (n = 56)		Validation cohort II (n = 59)	
Age (y)		60.68 ± 9.67	(13~82)	57.89 ± 9.68	(34~74)	62.44 ± 10.36	(42~90)
Sex	Male	103	74.6%	45	80.4%	50	84.7%
	Female	35	25.4%	11	19.6%	9	15.3%
Smoking Hx (PY)	None	38	27.5%	11	19.6%	18	30.5%
	~ 20	13	9.4%	5	8.9%	3	5.1%
	20~50	53	38.4%	22	39.3%	27	45.8%
	50~	25	18.1%	15	26.8%	4	6.8%
	Unknown	9	6.5%	3	5.4%	7	11.9%
Cell type	ADC	63	45.7%	24	42.9%	25	42.4%
	SQC	75	54.3%	32	57.1%	34	57.6%
Differentiation	WD	20	14.5%	9	16.1%	3	5.1%
	MD	80	58.0%	25	44.6%	42	71.2%
	PD	29	21.0%	14	25.0%	12	20.3%
	unknown	9	6.5%	8	14.3%	2	3.4%
Size (cm)		4.38 ± 1.95	(1.5~14)	4.82 ± 2.41	(1.3~13)	4.03 ± 1.69	(1.7~10)
Stage	I a	16	11.6%	3	5.4%	7	11.9%
	I b	72	52.2%	18	32.1%	28	47.5%
	II a	6	4.3%	1	1.8%	1	1.7%
	II b	18	13.0%	17	30.4%	9	15.3%
	III a	16	11.6%	11	19.6%	11	18.6%
	III b	10	7.2%	6	10.7%	3	5.1%
T	1	24	17.4%	5	8.9%	9	15.3%
	2	94	68.1%	32	57.1%	43	72.9%
	3	10	7.2%	13	23.2%	4	6.8%
	4	10	7.2%	6	10.7%	3	5.1%
N	0	98	71.0%	33	58.9%	39	66.1%
	1	28	20.3%	13	23.2%	9	15.3%
	2	12	8.7%	10	17.9%	11	18.6%
Median F/U (mo)		44	(3~138)	44	(3~146)	33	(3~43)
Median RFS time		49.38	(0.93~138.48)	33.93	(2.03~145.80)	.	(1.8~43.20)
Recurrence	Local	13	9.4%	9	16.1%	8	13.6%
	Distant	33	23.9%	13	23.2%	11	18.6%
	Local + Distant	15	10.9%	8	14.3%	3	5.1%
	Lung and pleura	32	45.7%	23	50.0%	8	34.8%
	Bone	20	28.6%	13	28.3%	6	26.1%
	Abdominal organ	10	14.3%	7	15.2%	4	17.4%
	Brain	6	8.6%	3	6.5%	3	13.0%
	Neck lymph node	2	2.9%	0	0.0%	2	8.7%
Nonrecurrence		77	55.8%	26	46.4%	37	62.7%

Abbreviations: PY, pack year; F/U, follow-up; RFS, recurrence-free survival. Age values are mean ± SD.

(2). Construction of models using either 10, 20, or 30 genes with the lowest  $q$  values revealed that the 20-gene model was the most accurate with less number of genes. The mRNA expression of these genes, as determined via microarray and real-time QPCR, correlated well (correlation coefficients, 0.55-0.90; Table 2). Details of the real-time QPCR analysis are described in the Materials and Methods in Appendix A.

To construct a Cox proportional hazard regression model for prognosis prediction, we used seven clinical variables (i.e., pStage, cell type, differentiation, smoking history, tumor size, gender, and age) and 18 real-time QPCR-assayable genes (Table 2). The number of combined variables was reduced to eight by stepwise selection (i.e., entry, 0.1; remove, 0.2). Subsequently, a model developed via combination of these eight clinicogenomic factors was applied to the independent validation cohorts. The calculated Cox score was transformed into survival function  $[S(t)]$  by SAS software to predict recurrence in individual patients.

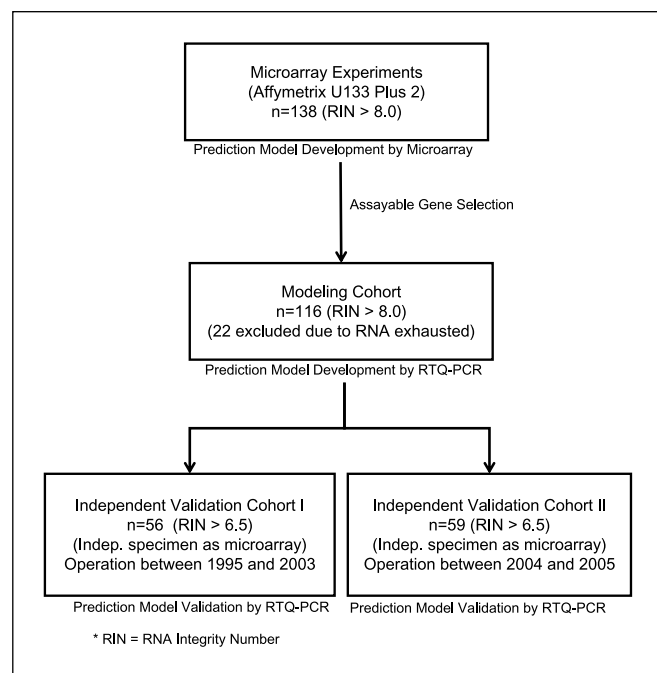
## Results

**Gene selection and modeling of recurrence prediction.** Initially, 3,112 genes with  $P$  values of  $<0.05$  were selected from a

log-rank test of microarray data from 54,675 probe sets and further subjected to multiple testing adjustments using the false discovery rate method for the rigorous selection of significant genes. Within these selected genes, we prepared two different gene sets by (a) selecting 20 genes with the smallest  $q$  values and (b) selecting 20 assayable genes whose levels of expression were measurable by real-time QPCR. Cox proportional hazard regression was then done on the two gene sets.

When samples in the first set were divided into two groups at the median Cox score (i.e., -9.28), the survival curves for the group with Cox scores higher than the median were significantly separated from curves for the group with Cox scores lower than the median ( $P < 0.0001$ ; Appendix A Fig. 2A). This result indicated that the model successfully separated the two risk groups. The selected genes and the constructed model were statistically validated using leave-one-out cross-validation.

Within the second gene set (i.e., 20 assayable genes, as defined in the Materials and Methods; Table 2), the K-M survival curve did not differ significantly from the



**Fig. 1.** Development and validation of the recurrence prediction model. The log-rank test of microarray data from 138 patients generated 3,112 genes ( $P < 5\%$ ) or 1,091 genes ( $P < 1\%$ ). Among these, 20 assayable genes with the lowest  $P$  values were used in the development of a model that was validated with two independent cohorts.

curve for the 20 genes with the smallest  $q$  values (Appendix A Fig. 1B). Time-dependent receiver operating characteristic (ROC) analysis (16) confirmed the performance of both models, with area under the curves values of  $>0.8$  (Appendix A Fig. 1C).

Thus, we defined the “gene-based model” as one that consists of assayable genes.

**Correlation between microarray and real-time QPCR data.** *LOC402110* and *C14orf78* were not included in the model development process because of insufficient RNA levels. Results for the remaining 18 genes were verified by correlating microarray and real-time QPCR data (i.e., correlation coefficients, 0.55-0.90; Table 2).

**Comparison of clinical, genomic, and clinicogenomic models for prognosis prediction.** We developed a prediction model from the modeling cohort ( $n = 116$ ) using seven selected clinical variables. This clinical model gave a  $P$  value of 0.0518 in the modeling cohort (Appendix A Fig. 2A), and  $P$  values of 0.2644 and 0.7204 in validation cohorts I and II, respectively (Fig. 2A and B). Next, we developed a gene-based prediction model from the same modeling cohort. This genomic model provided more significant results ( $P < 0.0001$ ; Appendix A Fig. 2B), and  $P$  values were 0.0710 and 0.1333 in the validation cohorts I and II, respectively (Fig. 2C and D).

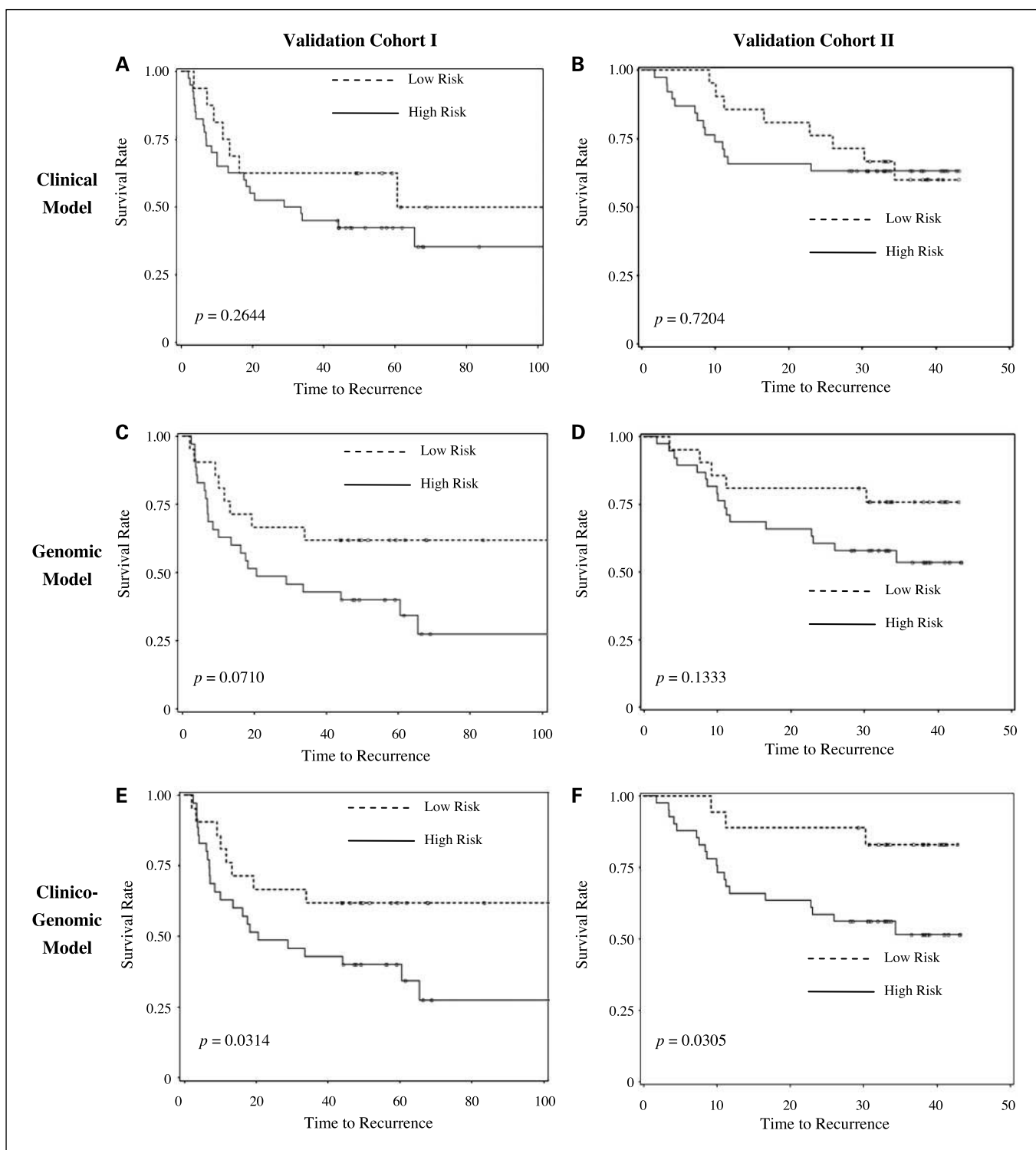
To construct a recurrence prediction model that is applicable to clinical practice, we sought to include both clinical variables and genetic information. Through the stepwise selection of variables in both classes, two clinical variables (i.e., pStage and “degree of differentiation” of the tumor cells) and six genes (i.e., *CALB1*, *MMP7*, *SLC1A7*, *GSTA1*, *CCL19*, and *IFI44*) were incorporated. This clinicogenomic model was more effective at separating the recurrence risk group from others ( $P < 0.0001$ ; Appendix A Fig. 2C) and provided more statistically significant results in validation cohorts I and II ( $P = 0.0314$  and  $P = 0.0305$ , respectively; Fig. 2E and F). Time-dependent ROC analysis further verified the superior performance of the clinicogenomic model (i.e., area under the curve,  $>0.75$ ) within the independent validation cohorts compared with the clinical

**Table 2.** List of assayable 20 genes and microarray to PCR correlation

No.	Probe set ID	Gene title	Gene symbol	q value	Correlation
1	219271_at	UDP-N-acetyl- $\alpha$ -D-galactosamine: polypeptide N-acetyl galactosaminyl transferase 14 (GalNAc-T14)	<i>GALNT14</i>	0.0003	0.7065
2	227952_at	Hypothetical LOC402110	<i>LOC402110</i>	0.0006	
3	201641_at	Bone marrow stromal cell antigen 2	<i>BST2</i>	0.0012	0.6452
4	205626_s_at	Calbindin 1, 28 kDa	<i>CALB1</i>	0.0024	0.7344
5	204259_at	Matrix metalloproteinase 7 (matrilysin, uterine)	<i>MMP7</i>	0.0042	0.8127
6	243623_at	Solute carrier family 1 (glutamate transporter), member 7	<i>SLC1A7</i>	0.0042	0.8272
7	219956_at	UDP-N-acetyl- $\alpha$ -D-galactosamine: polypeptide N-acetyl galactosaminyl transferase 6 (GalNAc-T6)	<i>GALNT6</i>	0.0043	0.5472
8	205267_at	POU domain, class 2, associating factor 1	<i>POU2AF1</i>	0.0043	0.7667
9	225342_at	Adenylate kinase 3-like 1	<i>AK3L1</i>	0.0065	0.6931
10	211748_x_at	Prostaglandin D2 synthase 21 kDa (brain)//prostaglandin D2 synthase 21 kDa (brain)	<i>PTGDS</i>	0.0071	0.7926
11	212992_at	Chromosome 14 open reading frame 78	<i>C14orf78</i>	0.0084	
12	210809_s_at	Periostin, osteoblast specific factor	<i>POSTN</i>	0.0123	0.6765
13	203924_at	Glutathione S-transferase A1	<i>GSTA1</i>	0.0125	0.8963
14	210072_at	Chemokine (C-C motif) ligand 19	<i>CCL19</i>	0.0135	0.7193
15	211668_s_at	Plasminogen activator, urokinase//plasminogen activator, urokinase	<i>PLAU</i>	0.0137	0.8087
16	205513_at	Transcobalamin I (vitamin B12 binding protein, R binder family)	<i>TCN1</i>	0.0156	0.6906
17	214453_s_at	IFN-induced protein 44	<i>IFI44</i>	0.0157	0.7171
18	213664_at	Solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1	<i>SLC1A1</i>	0.0159	0.7604
19	219869_s_at	Solute carrier family 39 (zinc transporter), member 8	<i>SLC39A8</i>	0.0162	0.6961
20	223551_at	Protein kinase (cyclic AMP-dependent, catalytic) inhibitor $\beta$	<i>PKIB</i>	0.0163	0.627

NOTE:  $q$  values were calculated by false discovery rate method using log-rank test  $P$  value.





**Fig. 2.** Comparison of clinical, genomic, and clinicogenomic model. For the clinical model with the modeling cohort (A and B) and validation cohorts I and II. For the genomic model (C and D) and for the clinicogenomic model (E and F) with the two validation cohorts, respectively.

or genomic models alone (Appendix A Fig. 3). Cox score equations for the three models are listed in Model Equations in Appendix A.

*Recurrence-free survival predictions for individual patients.* Using the Cox score calculated from the model, individualized

Kaplan-Meier recurrence-free survival graphs (K-M graphs) were plotted to predict individual survival and calculate median recurrence-free time (Fig. 3; Appendix A Table 2).

Our clinicogenomic model gave different recurrence predictions for individual patients even when patients had similar

clinical findings. Many patients with short predicted median recurrence-free survival times (PMRFS) developed recurrences soon after surgery, whereas many patients with long PMRFS survived >5 years of follow-up without recurrence. The PMRFS for each patient correlated well with the actual survival time (Fig. 3; Appendix A Table 2).

The overall accuracy of this predictive model was calculated from the relationship between the PMRFS and the actual TTR for independent validation cohorts I and II ( $n = 115$ ). The sensitivity of recurrence within 2 years and the specificity of nonrecurrence for 5 years were 72.1% and 90.0%, respectively (Appendix A Fig. 4).

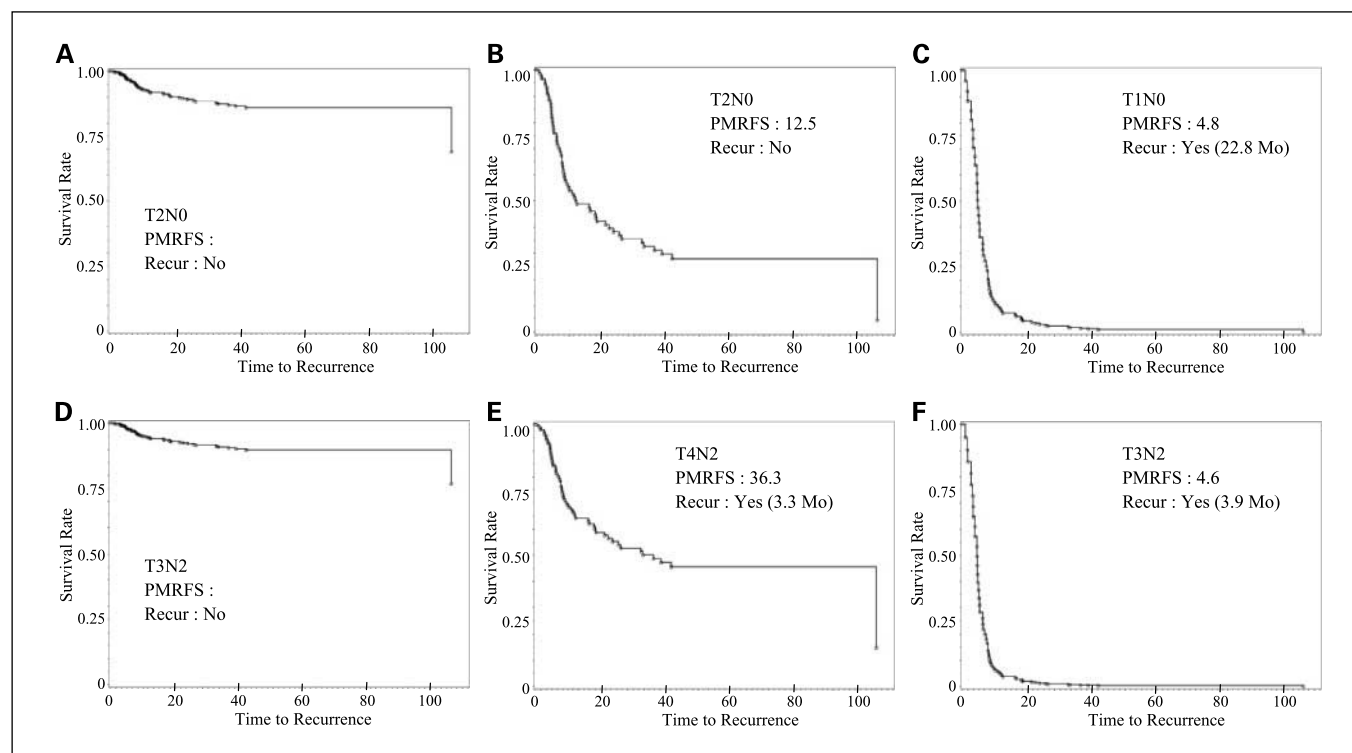
### Discussion

Fundamental knowledge of the molecular bases for and the consequent molecular classifications of NSCLC could aid in the prediction of patient outcomes. Gene expression studies that focus on cancer require a high-throughput technology, such as the microarray, to enable simultaneous analysis of tens of thousands of genes. The introduction of microarrays has inspired several approaches to the analysis of gene expression data related to patient survival (17–25). Conventional analytic methods have incorporated two to three classes of division in models for risk prediction. However, recurrence is not a fixed variable (e.g., man/woman), but rather a continuous, time-dependent variable. Such limitations prompted us to consider other ways of integrating TTR information into a gene-based risk prediction model. Several approaches have been proposed for obtaining TTR information from microarray data, such as

the Median Cut Approach (26), Supervised Principal Component Analysis (26), Lasso-Cox Approach, or CASPAR algorithm (27). Particularly, when the Median Cut Approach is used, patients with short follow-up periods are classified into high-risk groups. Such drawbacks might be overcome by using log-rank tests that select significant genes based on information regarding the continuous time-dependent property of recurrence. We used this strategy to generate 3,112 genes with  $P$  values of <0.05.

Microarray analysis is a highly promising technique, but its use in daily clinical practice is limited because of the necessary benchwork, equipment, analytic methods, and technical precision needed to generate reproducible results (3, 4, 6, 9, 28–39). Nevertheless, we used microarray analysis to (a) obtain the overall gene expression profile from which genes of interest (particularly target genes) were selected. Once we identified the target genes, we were able to (b) confirm the microarray data via real-time QPCR analysis of a finite number of genes and (c) construct a prediction model based on the results of the real-time QPCR analysis, which can be obtained within hours of mRNA preparation. Real-time QPCR analysis provides a quantitative measurement of mRNA in tumor tissues and a sensitive measurement of fold changes in the expression of individual genes. Furthermore, real-time QPCR is a simple method that can generate highly reproducible results in routine clinical settings (13, 14, 29, 40–43).

Real-time QPCR-based models have been described in the literature; however, high degrees of correlation between microarray data and real-time QPCR data have not been shown (22, 25). Gene selection is the most important step in the



**Fig. 3.** Kaplan-Meier recurrence-free survival curves of individualized prediction. Exemplary six cases at two different pathologic stage: pStage I (patients a, b, and c) and pStage III (patients d, e, and f). Patients classified into the same pStage gave different recurrence predictions according to our recurrence prediction model. MRFS, median recurrence-free survival time. In cases when Kaplan-Meier curve does not reach below 50%, “-” was used for MRFS.

development of a real-time QPCR-based model. Based on our experience and that of others (44), mRNA levels of a common housekeeping gene can vary between patients by as much as 10-fold. Construction of a model using real-time QPCR-based data requires that differences in the expression of component genes are large enough to overcome background noise from differences in diagnostic technology and variation in the expression of housekeeping genes. We therefore chose assayable genes from the pool of microarray data that showed a minimum of a 100-fold difference in gene expression between recurrence and nonrecurrence patients. This step enhanced subsequent detection of gene expression changes via real-time QPCR.

In this study, we measured gene expression levels in 138 tumor specimens using the Affymetrix U133 Plus 2.0 microarray with 54,675 probes. We then constructed a model and validated it using two independent cohorts ( $n = 56$  and  $n = 59$ ) to overcome issues concerning sample size and number of probes (3, 4, 10–12, 38, 45, 36).

Recently, gene expression profiles have been compared with conventional clinical predictors, such as age, gender, tumor stage, and histologic features, to determine the accuracy of predicting prognoses of patients with NSCLC (17–25). It was not clear whether a gene expression profile might be an independent specific prognostic factor in itself or whether such a profile might be influenced by other prognostic factors (4). In 2006, Potti et al. showed that the genomic model adds prognostic value to the widely accepted clinical predictors of outcome (e.g., age, gender, tumor size, stage of disease, histologic subtype, and smoking history; ref. 23). In the current study, we constructed a model using both clinical and genomic prognostic factors that fundamentally differed in metagene construction and binary prediction tree analysis (23). Future studies should explore the biological significance of each gene used in our predictive model.

Recent advances in genetics, molecular medicine, and biotechnology foretell that increasingly complex biomedical and molecular information will soon underlie a coherent system of personalized medicine. Cancer diagnosis, treatment planning, and customized adjuvant therapy will focus on stratified or individual patients rather than on broader

population cohorts. Many risk prediction studies divide a test group into two subgroups of poor or good prognosis and evaluate the outcomes of each subgroup. However, many patients might prefer individualized clinical decisions, because the random assignment of a patient into one or the other subgroup may not reflect a true prognosis.

To address this issue, we constructed recurrence-free survival curves for individual patients, which were validated by the predicted PMRFS and by the actual TTR of independent validation cohorts (Fig. 3; Appendix A Table 2). Of 115 validation cohorts, three patients with each of two types of pathologic stage were chosen: pStage I (i.e., patients a, b, c) or pStage III (i.e., patients d, e, f). Although patients in the same pStage had similar clinical findings, our clinicogenomic model gave different PMRFS (Fig. 3), so that the PMRFSs of the patients with pStage I disease were over 120, 12.5, and 4.8 months, respectively, and those of the patients at pStage III were over 120, 36.3, and 4.6 months, respectively. Patients c and f, who had short PMRFS, suffered recurrences soon after surgery. However, patients a and d, whose survival did not fall under 50% on the K-M graph, survived >5 years of follow-up without recurrence. Relationships between the PMRFS and the actual TTR of cohorts I and II ( $n = 115$ ) are shown in Appendix A Fig. 4. The sensitivity of recurrence within 2 years and the specificity of nonrecurrence for 5 years were found to be 72.1% and 90.0%, respectively. Thus, the PMRFS for each patient correlated well with the actual data.

Our results suggest that the proposed model enhances the current system of personalized recurrence risk prediction. Although further improvement is needed, our model allows a customized lung cancer management strategy that can be used in day-to-day clinical practice.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

### Acknowledgments

We thank Matthew Meyerson, Roel Verhaak, Sanghyuk Lee, and Won Ki Kang for useful comments suggestions, and discussions.

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