VOLUME 28 · NUMBER 7 · MARCH 1 2010

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Gene Expression Profiling for Survival Prediction in Pediatric Rhabdomyosarcomas: A Report From the Children's Oncology Group

Elai Davicioni, James R. Anderson, Jonathan D. Buckley, William H. Meyer, and Timothy J. Triche

From the Departments of Pathology and Preventive Medicine, Keck School of Medicine, Norris Comprehensive Cancer Center, University of Southern California; Departments of Pathology and Laboratory Medicine, Saban Research Institute, Childrens Hospital Los Angeles, Los Angeles, CA; Department of Biostatistics, College of Public Health, University of Nebraska Medical Center, Omaha, NE; and Children's Medical Research Institute, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Submitted November 24, 2008; accepted October 30, 2009; published online ahead of print at www.jco.org on February 1, 2010.

Supported in part by Grants No. U01-CA-114757 from the Strategic Partnering to Evaluate Cancer Signatures (SPECS) program (T.J.T.) and No. U10 CA98543 from the Children's Oncology Group Chair, National Cancer Institute, National Institutes of Health, Bethesda, MD.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Corresponding author: Timothy J. Triche, MD, PhD, Department of Pathology, Childrens Hospital Los Angeles, 4650 Sunset Blvd, Los Angeles, CA 90027; e-mail: triche@usc.edu.

0732-183X/10/2807-1240/$20.00

DOI: 10.1200/JCO.2008.21.1268

ABSTRACT

Purpose
We investigated whether tumors from diagnostic biopsies of primary rhabdomyosarcoma (RMS) contain relevant prognostic information in the form of gene expression signatures that can be used to model and predict outcome of patients.

Patients and Methods
A 22,000-probe set microarray was used to evaluate 120 RMS specimens and correlate gene expression patterns to survival. Multivariate gene expression models or metagenes were developed using cross-validated Cox regression proportional hazards modeling and were evaluated using Kaplan-Meier analysis.

Results
A 34-metagene, based on expression patterns of 34 genes, was highly predictive of outcome. It was not highly correlated with individual clinical risk factors such as patient age, stage, tumor size, or histology. However, it was correlated with a risk classification used by the Children's Oncology Group and the biologic subsets of alveolar histology tumors.

Conclusion
These data support further evaluation of RMS metagenes to discriminate patients with good prognosis from those with poor prognosis, with the potential to direct risk-adapted therapy.

J Clin Oncol 28:1240-1246. © 2010 by American Society of Clinical Oncology

INTRODUCTION

The cure rate for patients with rhabdomyosarcoma (RMS) is more than 70% for patients with nonmetastatic disease,[1] and much of this realized gain over the past few decades can be attributed to the use of intensive multimodal therapy including surgery, radiotherapy, and chemotherapy. This cure rate is not expected to change significantly until targeted tumor-specific agents are developed. Because multimodal therapy can be associated with acute toxicities and long-term adverse effects, such as growth and developmental defects, one of the major areas for improvement relates to quality of life for young cancer survivors.[2] Recent studies have revealed that some patients can be treated effectively without radiotherapy[3] and with less intensive chemotherapy,[4] reducing acute and long-term adverse effects.[5] Moreover, there appear to be subsets of patients with metastatic disease at diagnosis with an atypically more favorable outcome.[6,7]

A crucial determinant of the overall success of such risk-adapted therapy is the effectiveness of clinical staging systems for patient prognosis and treatment assignment. Various forms of clinicopathologic staging have been used to define risk in several international clinical trial groups over the past several decades, and the latest development used in ongoing clinical trials of the Children's Oncology Group (COG; under the auspices of the COG Soft Tissue Sarcoma Committee) is a three-tier risk classification system. The COG risk classification system incorporates both of the earlier postsurgical clinical group and TNM stage schemes as well as tumor histology,[8] and it appears to be the most powerful prognostic scheme devised to date.[9]

One issue with the COG risk classification system is that many patients fall into the intermediate-risk category where survival is most heterogeneous,[10] suggesting that even the best clinical risk model has difficulty in identifying some aspects of underlying biology of tumors, in particular relating to their clinical aggressiveness.[11] Molecular staging using, for example, gene expression profiles has promise in predicting long-term patient outcome by analysis of the tumor at diagnosis.[12] An

inherent assumption of this approach, supported by recent analyses[13,14] is the hypothesis that every tumor contains informative gene expression signatures that, at the time of diagnosis, can predict the biologic behavior of the tumor over time.[15] A powerful approach for modeling patient survival data is using Cox regression proportional hazards models; recently, this has been applied to gene expression data sets[11,16-18] in efforts to generate true continuous predictors of survival that are independent of clinicopathologic variables in predicting treatment outcome.[11] In this proof-of-concept study, we describe the development of a metagene or multivariable continuous predictor of outcome using Cox regression–based modeling for 120 patients with RMS.

PATIENTS AND METHODS

Tumor Specimens

Tumor specimens used to develop outcome prediction models were, as recently reported,[19] obtained from the Intergroup Rhabdomyosarcoma Study Group (IRSG)/Pediatric Cooperative Human Tissue Network (Columbus, OH) and Childrens Hospital Los Angeles (CHLA) institutional tumor banks from 120 patients who were enrolled in IRSG IV and V COG clinical trials. Clinical covariates were obtained from the COG Statistics and Data Center (Arcadia, CA). Two patients had mixed alveolar/embryonal histology and were considered alveolar for the purposes of this analysis. From the previously reported study, we selected only those patients with RMS histology (alveolar, embryonal, spindle-cell, and botryoid) on review diagnosis (ie, excluded all patients with non-RMS soft tissue sarcoma or undifferentiated sarcoma) and those with sufficient follow-up data (ie, alive [censored] patients with < 2 years of follow-up were omitted) for this analysis. Sample preparation and Affymetrix GeneChip Human U133A (Affymetrix, Santa Clara, CA) microarray protocols were previously described.[19,20] Complete microarray protocols can be found at the University of Southern California (USC)/CHLA Genome Core Web site at http://genomecore-chla.usc.edu/GenomeCore/GenomeCore.html.

Analysis of Gene Expression

All data management and analysis was conducted using the Genetrix suite of tools for microarray analysis (Epicenter Software, http://www.epicentersoftware.com). Probe set modeling and data preprocessing were derived using the robust multi-array algorithm implemented within the ProbeProfiler module (Corimbia, Berkeley, CA). The full data set of 22,215 probe sets was reduced to 21,718 probe sets (henceforth, genes) by eliminating genes with a standard deviation of less than 10 Affymetrix difference intensity

Table 1. Genes Used to Create the 34-Metagene Continuous Predictor of Outcome

Affymetrix ID	Gene Name	Gene Symbol
Genes correlated to good patient outcome		
214643_x_at	Bridging integrator 1	*BIN1*
219953_s_at	Chromosome 11 open reading frame 17	*C11orf17*
218314_s_at	Chromosome 11 open reading frame 57	*C11orf57*
201905_s_at	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase-like	*CTDSPL*
204643_s_at	Ecto-NOX disulfide-thiol exchanger 2	*ENOX2*
218695_at	Exosome component 4	*EXOSC4*
207688_s_at	Inhibin, beta C	*INHBC*
222250_s_at	Integrator complex subunit 7	*INTS7*
202788_at	Mitogen-activated protein kinase-activated protein kinase 3	*MAPKAPK3*
213946_s_at	Obscurin-like 1	*OBSL1*
35156_at	R3H domain and coiled-coil containing 1	*R3HCC1*
218392_x_at	Sideroflexin 1	*SFXN1*
207069_s_at	SMAD, mothers against DPP homolog 6 (*Drosophila*)	*SMAD6*
214662_at	WD repeat domain 43	*WDR43*
219548_at	Zinc finger protein 16 (KOX 9)	*ZNF16*
Genes correlated to poor patient outcome		
221588_x_at	Aldehyde dehydrogenase 6 family, member A1	*ALDH6A1*
211248_s_at	Chordin	*CHRD*
210656_at	Embryonic ectoderm development	*EED*
212546_s_at	FRY-like 1	*FRYL*
209525_at	Hepatoma-derived growth factor, related protein 3	*HDGFRP3*
220447_at	Histamine receptor H3	*HRH3*
209184_s_at	Insulin receptor substrate 2	*IRS2*
204075_s_at	KIAA0562	*KIAA0562*
204584_at	L1 cell adhesion molecule	*L1CAM*
213672_at	Methionine-tRNA synthetase	*MARS*
215921_at	Nuclear pore complex interacting protein-like 1	*NPIPL1*
209791_at	Peptidyl arginine deiminase, type II	*PADI2*
205632_s_at	Phosphatidylinositol-4-phosphate 5-kinase, type I, beta	*PIP5K1B*
211974_x_at	Recombination signal binding protein for immunoglobulin kappa J region	*RBPJ*
218394_at	Rogdi homolog (*Drosophila*)	*ROGDI*
213437_at	RUN and FYVE domain-containing 2; Run- and FYVE-domain containing protein	*RUFY3*
219196_at	Secretogranin III	*SCG3*
213434_at	Syntaxin 2	*STX2*
202342_s_at	Tripartite motif-containing 2	*TRIM2*

units of a normalized data range, and the data were log transformed. The complete tumor microarray data set (including sample covariate data) can be found on the National Cancer Institute (NCI) Cancer Array Database at https://array.nci.nih.gov/caarray/project/trich-00099.

Metagene Construction and Evaluation

Metagenes were constructed as previously described,[20] using Cox proportional hazards modeling of RMS gene expression data under cross-validation. Genes were ranked and selected using sampling statistics obtained across multiple iterations testing for significance in both training (n = 60) and testing (n = 60) randomized subsets. Weighting factors were obtained from the signed square root of the Cox χ^2 test statistic modeled on the entire cohort. The metagene score for each patient was calculated as a weighted sum of the gene expression value. Detailed descriptions of the data analysis can be found in the Data Supplement (online only).

Survival Analysis

Comparison of survival times was carried out using Kaplan-Meier survival plots and log-rank tests of significance. Comparisons between molecular groups and tests of association used Fisher's exact or χ^2 tests to compare the frequency distributions of patient characteristics. Multivariate tests for association of factors with survival used a Cox regression proportional hazards model.

RESULTS

Generation of Multigene Prognostic Models

A cohort of 120 pediatric RMS patient tumor samples (Supplementary Table 1, online only) with at least 2 years censored follow-up data after diagnosis (except patients who died of disease at any time point) were used to identify genes correlated to overall survival (OS) times with Cox proportional hazards regression modeling. Most of the deaths (67%) occurred within the first 2 years of diagnosis and the cause of death was attributed to the tumor in all patients except for two (one from infection on regimen and one from toxicity unrelated to the chemotherapy regimen). Of the patients who died, 24 (62%) had alveolar histology, 13 (33%) had embryonal histology, and two (5%) had mixed alveolar/embryonal histology. No reported deaths occurred in patients with spindle-cell or botryoid histology tumors.

Using Cox modeling of OS with log-transformed gene expression data (see Patients and Methods), 578 genes with significant Cox χ^2 scores over 2,500 iterations of the algorithm ($P < .01$) were identified (Supplementary Table 2, online only). Next, multigene continuous predictors of outcome were assembled and evaluated as described previously[20] (see schematic in Appendix Fig A1, online only). The maximum likelihood estimate of the χ^2 test statistics were determined for each multivariate model and showed that a 34-probe set model or 34-metagene (MG34; Table 1) had the highest significance score (blue curve, Appendix Fig A1). By permuting the gene expression data and generating new metagenes from the permuted data set, we show that permuted models do not reach statistical significance, indicating that these results are not likely due to chance alone (red curve, Appendix Fig A2, online only).

Post Hoc Analysis of MG34

To validate the performance of the MG34, RMS patients were split into three groups (tertiles, determined by the histogram bar groupings) by their computed metagene predictor scores (Fig 1A). The mean metagene predictor scores for the third tertile were five- and 17-fold greater than second and first tertile patient scores, respectively. Kaplan-Meier analysis revealed that patients in the first (n = 39; blue curve) and second (n = 41; green curve) tertiles had 5-year OS rates of 98% and 75%, respectively (Fig 1B). In contrast, patients in the third tertile (n = 40; red curve) had a 5-year OS rate of only 29% and median survival of 24 months. Of the cohort of 120 patients, there

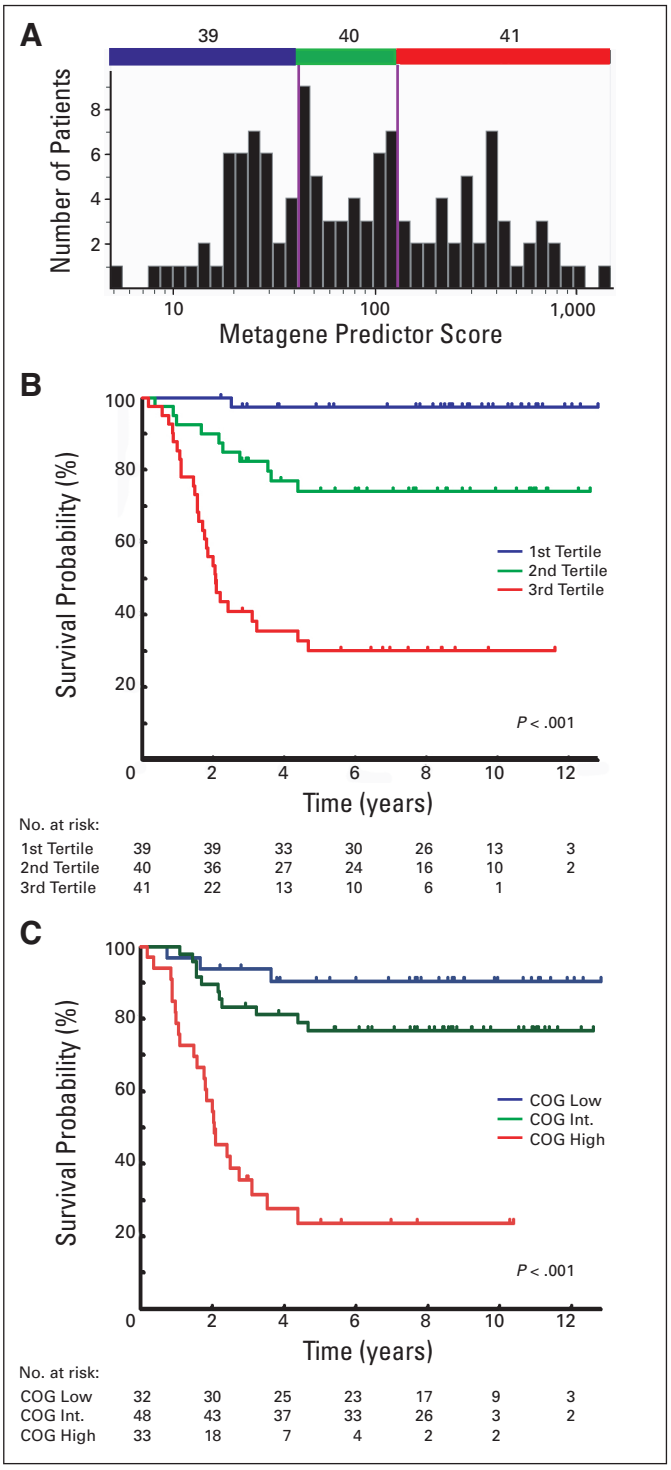

Fig 1. Metagene predictor scores determine outcome in rhabdomyosarcoma patients. (A) Histogram showing the binned distribution of the 34-metagene predictor scores for 120 patients (vertical purple lines highlight the tertile cut points). Kaplan-Meier survival analysis of all 120 rhabdomyosarcoma patients (B) using tertiles as groups and (C) for 113 RMS patients with known Children's Oncology Group (COG) risk groups (Table 2). Numbers below the curves indicate the number of patients at risk, and *P* values are from log-rank test. Int, intermediate.

Table 2. Comparison of COG Risk Groups and Metagene Tertile Groups

COG Risk Group	Risk Group Criteria	5-Year OS, (%)*	No. of Patients in MG34 Tertiles		
			1st	2nd	3rd
Low	Embryonal histology and stage 1 or stage 2/3, group I/II	90	19	11	2
Intermediate	Embryonal histology and stage 2/3, group III or alveolar histology, groups I-III	77	12	22	14
High	All patients with group IV disease	24	5	7	21
			5-Year OS (%)*		
			95	76	29

Abbreviations: COG, Children's Oncology Group; OS, overall survival; MG34, 34-metagene, based on expression patterns of 34 genes.
*Log-rank test; $P < .001$.

were seven patients for whom missing stage or clinical group data meant that we could not classify them into the risk groups now used by the COG (in current clinical trials for RMS). The remaining 113 patients were grouped according to the COG risk groups as indicated in Table 2. Kaplan-Meier analysis (Fig 1C) shows that the 5-year OS rates for patients in this cohort grouped according to the COG risk category criteria are reflective of the survival rates observed in larger cohorts from COG clinical trials.[10] We also observed that MG34 tertile groups are highly correlated to COG risk groups; for example, most high-risk patients are found in the third tertile, whereas few clinically low-risk patients are within this tertile (Table 2).

Next, we looked at the predictive value of the MG34 tertiles within the COG risk groups. COG low-risk patients (n = 32) were mostly in the MG34 first tertile (n = 19) or second tertile (n = 11) except for two embryonal histology (stage 3, group II disease) patients who were categorized in the third tertile (log-rank $P < .013$). While the log-rank test for the comparison of survival is < 0.05, it is mostly the result of early failure of one of these patients in the third tertile (Fig 2A). For intermediate-risk patients (n = 48), there appears to be clear evidence that the MG34 tertiles are predictive of survival (Fig 2B). For COG intermediate-risk group patients in the MG34 first tertile, the 5-year OS rate was 100% (n = 12), whereas second tertile (n = 22) and third tertile (n = 14) patients had 5-year OS rates of 86% and 43%, respectively (log-rank $P < .00003$). Of note, we observed that 71% (12 of 17) of COG intermediate-risk patients with tumors expressing the PAX3-FKHR fusion gene were in the MG34 third tertile, the one that is most different in terms of survival. In contrast, six of seven PAX7-FKHR and three of three fusion-negative alveolar histology tumors from the COG intermediate-risk group were in the MG34 second tertile (Supplementary Table 3, online only). Therefore, it appears that for intermediate-risk patients, higher MG34 scores (eg, third tertile) are tightly correlated to the PAX3-FKHR alveolar subtype (Supplementary Table 4, online only). For patients in the COG high-risk group, 64% (21 of 33) were in the MG34 third tertile. Five patients with group IV disease (four embryonal, one alveolar) and improved survival were categorized into the first tertile, but for the remainder of these patients, there was no appreciable difference in the survival curves between the second MG34 tertile (n = 7) and the third MG34 tertile (n = 21), except median survival was 33 months versus 22 months, respectively (Fig 2C). Comparison of the metagene predictor scores with clinical risk factors can be found in Supplementary Table 5 (online only). Appendix Figure A3 (online only) shows the distribution of MG34 scores within histologic and genetic subtypes.

DISCUSSION

Previous gene expression profiling of RMS patient tumors by our group and by others[19-22] focused primarily on resolving issues of diagnosis and enhancing the understanding of tumor classification from a genome-wide perspective. While a 2006 study[20] showed that expression signatures of putative PAX-FKHR target genes may be of prognostic value in the subset of PAX-FKHR translocation-positive alveolar RMS patients and a 2009 report[19] showed differences in prognosis for molecular-based classes of RMS tumors, these findings have not yet had an impact on clinical practice for patient stratification or assignment to treatment protocols. The main reason is that they do not seem to add much more prognostic information beyond that captured by established pathologic criteria, such as favorable (ie, embryonal) versus unfavorable (ie, alveolar) tumor histology, known for nearly three decades as an independent prognostic factor. The MG34 described here appears to discriminate patient risk independent of tumor histology and, as a continuous rather than discrete variable, it reflects the spectrum of differential gene expression observed in this heterogeneous group of tumors.

Genes such as *L1CAM* that are highly expressed in poor-outcome patients, a cell adhesion molecule,[23-25] and *IRS2*[26] are associated with increased metastatic potential and invasiveness in several tumor types. Another poor-outcome gene, transcription factor *RBPSJ*, is involved in repression of differentiation in numerous cancers.[27] Conversely, a good-outcome gene, *BIN1*, is a well-characterized tumor suppressor gene that promotes muscle differentiation[28] and differentiation of tumor cells.[29] Though the functional relationship of MG34 genes in determining tumor behavior and hence outcome of RMS patients is at present unclear, these and many others (Supplementary Table 2) appear to impart independent prognostic information. In addition, we have shown that the MG34 model is one of numerous expression signatures correlated to patient prognosis (Appendix Fig A2), as has been demonstrated in other tumor systems.[30,31]

We previously reported a PAX-FKHR 33-metagene that predicted outcome in a subset of alveolar RMS patients whose tumors expressed products of PAX-FKHR fusion genes. This PAX-FKHR 33-metagene and the present pan-RMS MG34 do not show any overlap. This is not surprising given the fact that the PAX-FKHR metagene was generated from a list of putative PAX-FKHR targets derived from expression analysis of an in vitro model system (ectopic expression of

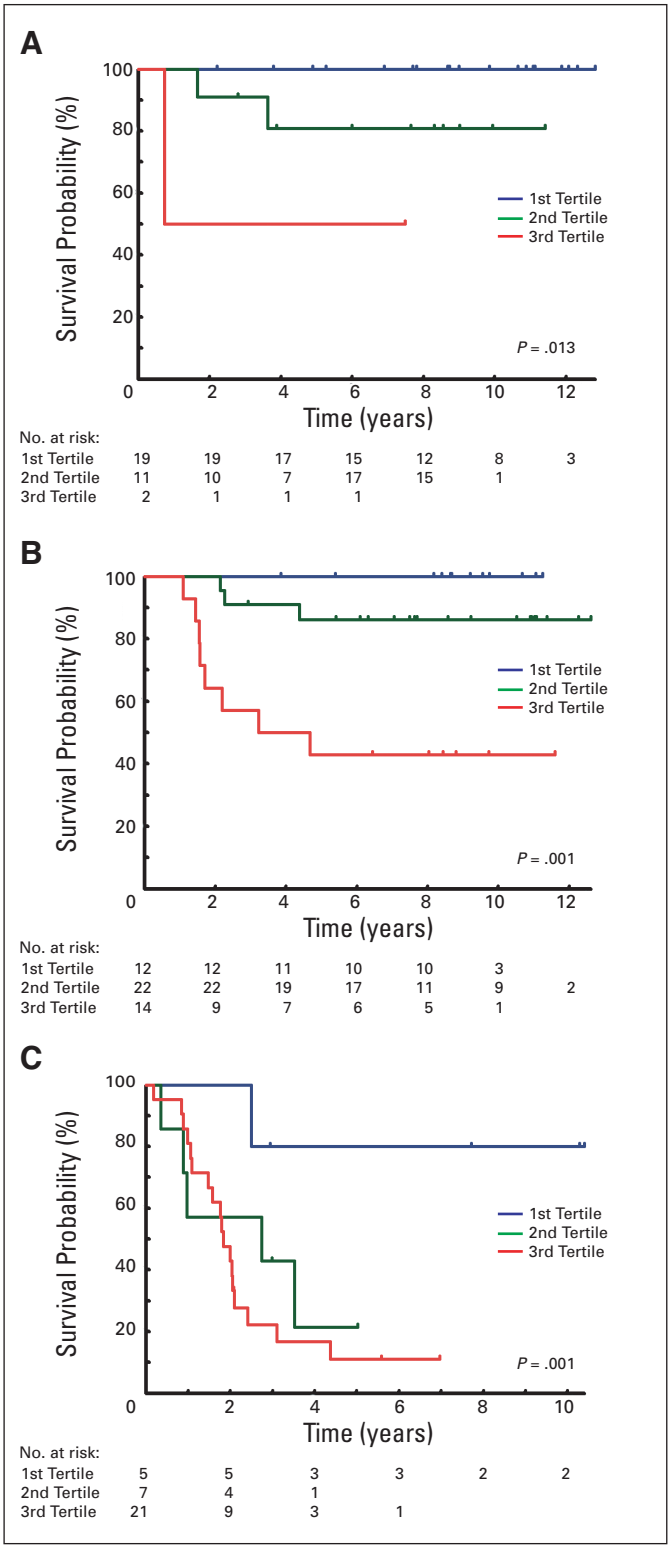

Fig 2. Evaluation of metagene predictor scores within Children's Oncology Group (COG) risk groups. Kaplan-Meier survival analysis of COG low-risk (A), intermediate-risk (B), and high-risk (C) patients using 34-metagene tertiles as groups. Numbers below the curves indicate the number of patients at risk, and *P* values are from log-rank test.

PAX-FKHR in ERMS RD cell lines) and PAX-FKHR–positive primary tumors only. However, five genes (*MYLPF, TNNC2, IL4R, NELL1,* and *BMP5*) from the cell line model system analysis were also identified in the present analysis of outcome-correlated genes in all RMS tumors (Supplementary Table 2) though they were not incorporated into the MG34 model reported here. Our working hypothesis is that PAX3 and PAX7 (and their cognate PAX-FKHR fusion genes in alveolar RMS) activate a unique transcriptional program that confers rhabdomyosarcoma-ness in general (eg, myogenic phenotype in a sarcoma). Furthermore, the PAX-FKHR fusion proteins in alveolar RMS are believed to further activate a transcriptional program that confers a more aggressive phenotype (perhaps in part characterized by some of the genes identified here and in the 2006 study). Previous work from the Barr group[32] demonstrated that levels of PAX-FKHR are also crucial where PAX-FKHR overexpressed in cell lines caused transformation at lower levels and growth suppression at higher levels. Multiple functionally significant splice forms of PAX-FKHR may have implications for tumor phenotypes such as clinical aggressiveness and the correlation between fusion gene expression; wild-type PAX3/7 expression and other factors yet to be identified likely have important roles in conferring different biologic properties to RMS cells.[32-35] In a follow-on study now underway on a larger cohort of RMS patients with higher resolution exon microarrays, we intend to address the question of whether RMS patient survival can be better modeled with separate metagenes for PAX-FKHR and fusion-negative RMS.

Kaplan-Meier analysis of the objectively derived MG34 tertiles shows patients divided into three highly disparate groups in terms of survival, which suggests that MG34 tertiles are predictive of survival. Additionally, MG34 tertiles are correlated with the COG clinicopathologic risk category used to assign patients with RMS to treatment studies. This is perhaps not surprising because both depend on features related to the biology of the tumors, although they are measured in different ways. All but two of the patients who were low risk by clinicopathologic features had tumors classified into the first or second MG34 tertiles. Most had embryonal disease, and survival did not appear to differ significantly by whether low-risk patients were in the first or second tertile. Most patients (21 of 33) who are high risk by clinicopathologic features had tumors classified into the third MG34 tertile. Patients in the COG intermediate-risk group had tumors evenly distributed across the MG34 tertiles. Poorer survival outcomes were observed for third-tertile patients within the COG intermediate-risk group, with the majority of these patients (12 of 14) having PAX3-FKHR and alveolar histology disease. The poorest prognosis MG34 third tertile appears to be associated with PAX3-FKHR alveolar histology disease; overall, 63% (25 of 40) of the MG34 third-tertile patients have PAX3-FKHR alveolar tumors, whereas the percentages are only 3% (one of 39) and 10% (four of 41) for the first and second tertiles, respectively. In contrast, 73% (eight of 11) of PAX7-FKHR alveolar histology tumors were in the second tertile, supporting previous studies that report a more favorable outcome for this subset of alveolar patients.[33,36]

While the third tertile predominately comprised the less favorable PAX3-FKHR alveolar RMS tumors, it also included eight patients with embryonal disease and two with mixed alveolar/embryonal disease. Intriguingly, the metagene tertile risk groups varied most markedly in patients who presented with metastatic disease (group IV), which is the most adverse prognostic factor for RMS patients.[6] While most of the metastatic disease patients were found in the third

tertile group, nearly a third were found in the other two tertiles and, in accordance with previous observations, these (8 of 11) had primarily group IV embryonal histology tumors.[6,37] These data suggest that a genomic-based classifier such as MG34 could be used to discern patients with high-risk disease who are most responsive to current therapeutic modalities and may provide a means to separate them from high-risk patients unlikely to respond to conventional chemotherapy regimens. This approach could therefore enable clinicians to better test experimental therapeutic agents on chemotherapy-naïve high-risk patients[9] and make testing clinical trials for these agents more efficient.

Current methods for RMS staging have evolved to direct risk-adapted therapy[9] using complex clinical risk models.[38] This is important not only for patient management but also for evaluation of the effects of different treatment regimens in clinical trials. However, it appears that clinicopathologic-based staging systems do not identify many of the fundamental differences in underlying tumor biology.[11] The MG34, a continuous predictor of patient outcome when split into tertile groups, performed similarly to the COG risk groups in a training cohort. This is notable since the MG34 risk groups were derived from statistical cut points (ie, tertile groups) and were not optimized by post hoc analysis. Further work is required to expand genome-wide analyses to further training and independent validation cohorts, efforts that will likely require hundreds of patient samples as has been done previously for analysis of other prognostic factors on routine clinical material (eg, formalin-fixed paraffin embedded tumor sections).[7,22] The present work suggests that the focus should be on the intermediate-risk patients, the most prevalent type of RMS clinical trial patients. In this subgroup, the MG34 model appears to add prognostic information and separates out significant numbers of patients with more favorable or worse prognosis than the OS trends in this heterogeneous risk category. On the basis of these initial results, genomic classifiers for prognosis and the substratification of patients at the time of diagnosis have great promise as clinical tools to better the treatment, management, and outcomes for RMS patients.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: Elai Davicioni, GenomeDx Biosciences (C); Jonathan D. Buckley, Epicenter Software (C)
Consultant or Advisory Role: None **Stock Ownership:** Elai Davicioni, GenomeDx Biosciences; Jonathan D. Buckley, Epicenter Software
Honoraria: None **Research Funding:** None **Expert Testimony:** None
Other Remuneration: None

AUTHOR CONTRIBUTIONS

Conception and design: Elai Davicioni, Jonathan D. Buckley, Timothy J. Triche
Financial support: Timothy J. Triche
Administrative support: William H. Meyer, Timothy J. Triche
Provision of study materials or patients: James R. Anderson, William H. Meyer
Collection and assembly of data: Elai Davicioni, James R. Anderson, Timothy J. Triche
Data analysis and interpretation: Elai Davicioni, James R. Anderson, Jonathan D. Buckley, Timothy J. Triche
Manuscript writing: Elai Davicioni, James R. Anderson, William H. Meyer, Timothy J. Triche
Final approval of manuscript: Elai Davicioni, James R. Anderson, Jonathan D. Buckley, William H. Meyer, Timothy J. Triche

REFERENCES

1. McDowell HP: Update on childhood rhabdomyosarcoma. Arch Dis Child 88:354-357, 2003
2. Stevens MC: Treatment for childhood rhabdomyosarcoma: The cost of cure. Lancet Oncol 6:77-84, 2005
3. Schuck A, Mattke AC, Schmidt B, et al: Group II rhabdomyosarcoma and rhabdomyosarcomalike tumors: Is radiotherapy necessary? J Clin Oncol 22:143-149, 2004
4. Crist WM, Anderson JR, Meza JL, et al: Intergroup rhabdomyosarcoma study-IV: Results for patients with nonmetastatic disease. J Clin Oncol 19:3091-3102, 2001
5. Meza JL, Anderson J, Pappo AS, et al: Analysis of prognostic factors in patients with nonmetastatic rhabdomyosarcoma treated on intergroup rhabdomyosarcoma studies III and IV: The Children's Oncology Group. J Clin Oncol 24:3844-3851, 2006
6. Breneman JC, Lyden E, Pappo AS, et al: Prognostic factors and clinical outcomes in children and adolescents with metastatic rhabdomyosarcoma: A report from the Intergroup Rhabdomyosarcoma Study IV. J Clin Oncol 21:78-84, 2003
7. Oberlin O, Rey A, Lyden E, et al: Prognostic factors in metastatic rhabdomyosarcomas: Results of a pooled analysis from United States and European cooperative groups. J Clin Oncol 26:2384-2389, 2008
8. Raney RB, Anderson JR, Barr FG, et al: Rhabdomyosarcoma and undifferentiated sarcoma in the first two decades of life: A selective review of intergroup rhabdomyosarcoma study group experience and rationale for Intergroup Rhabdomyosarcoma Study V. J Pediatr Hematol Oncol 23:215-220, 2001
9. Breitfeld PP, Meyer WH: Rhabdomyosarcoma: New windows of opportunity. Oncologist 10:518-527, 2005
10. Meyer WH, Spunt SL: Soft tissue sarcomas of childhood. Cancer Treat Rev 30:269-280, 2004
11. Bair E, Tibshirani R: Semi-supervised methods to predict patient survival from gene expression data. PLoS Biol 2:E108, 2004
12. Simon R, Radmacher MD, Dobbin K, et al: Pitfalls in the use of DNA microarray data for diagnostic and prognostic classification. J Natl Cancer Inst 95:14-18, 2003
13. Ramaswamy S, Ross KN, Lander ES, et al: A molecular signature of metastasis in primary solid tumors. Nat Genet 33:49-54, 2003
14. Weigelt B, Hu Z, He X, et al: Molecular portraits and 70-gene prognosis signature are preserved throughout the metastatic process of breast cancer. Cancer Res 65:9155-9158, 2005
15. Simon R: Roadmap for developing and validating therapeutically relevant genomic classifiers. J Clin Oncol 23:7332-7341, 2005
16. Li H, Gui J: Partial Cox regression analysis for high-dimensional microarray gene expression data. Bioinformatics 20:i208–i215, 2004 (suppl 1)
17. van Houwelingen HC, Bruinsma T, Hart AA, et al: Cross-validated Cox regression on microarray gene expression data. Stat Med 25:3201-3216, 2006
18. Pawitan Y, Bjohle J, Wedren S, et al: Gene expression profiling for prognosis using Cox regression. Stat Med 23:1767-1780, 2004
19. Davicioni E, Anderson MJ, Finckenstein FG, et al: Molecular classification of rhabdomyosarcoma: Genotypic and phenotypic determinants of diagnosis—A report from the Children's Oncology Group. Am J Pathol 174:550-564, 2009
20. Davicioni E, Finckenstein FG, Shahbazian V, et al: Identification of a PAX-FKHR gene expression signature that defines molecular classes and determines the prognosis of alveolar rhabdomyosarcomas. Cancer Res 66:6936-6946, 2006
21. Wachtel M, Dettling M, Koscielniak E, et al: Gene expression signatures identify rhabdomyosarcoma subtypes and detect a novel t(2;2)(q35;p23) translocation fusing PAX3 to NCOA1. Cancer Res 64:5539-5545, 2004

22. Wachtel M, Runge T, Leuschner I, et al: Subtype and prognostic classification of rhabdomyosarcoma by immunohistochemistry. J Clin Oncol 24:816-822, 2006

23. Allory Y, Matsuoka Y, Bazille C, et al: The L1 cell adhesion molecule is induced in renal cancer cells and correlates with metastasis in clear cell carcinomas. Clin Cancer Res 11:1190-1197, 2005

24. Gavert N, Conacci-Sorrell M, Gast D, et al: L1, a novel target of beta-catenin signaling, transforms cells and is expressed at the invasive front of colon cancers. J Cell Biol 168:633-642, 2005

25. Thies A, Schachner M, Moll I, et al: Overexpression of the cell adhesion molecule L1 is associated with metastasis in cutaneous malignant melanoma. Eur J Cancer 38:1708-1716, 2002

26. Nagle JA, Ma Z, Byrne MA, et al: Involvement of insulin receptor substrate 2 in mammary tumor metastasis. Mol Cell Biol 24:9726-9735, 2004

27. Pursglove SE, Mackay JP: CSL: A notch above the rest. Int J Biochem Cell Biol 37:2472-2477, 2005

28. Wechsler-Reya RJ, Elliott KJ, Prendergast GC: A role for the putative tumor suppressor Bin1 in muscle cell differentiation. Mol Cell Biol 18:566-575, 1998

29. Sakamuro D, Prendergast GC: New Myc-interacting proteins: A second Myc network emerges. Oncogene 18:2942-2954, 1999

30. Fan C, Oh DS, Wessels L, et al: Concordance among gene-expression-based predictors for breast cancer. N Engl J Med 355:560-569, 2006

31. Nevins JR, Potti A: Mining gene expression profiles: Expression signatures as cancer phenotypes. Nat Rev Genet 8:601-609, 2007

32. Xia SJ, Barr FG: Analysis of the transforming and growth suppressive activities of the PAX3-FKHR oncoprotein. Oncogene 23:6864-6871, 2004

33. Sorensen PH, Lynch JC, Qualman SJ, et al: PAX3-FKHR and PAX7-FKHR gene fusions are prognostic indicators in alveolar rhabdomyosarcoma: A report from the Children's Oncology Group. J Clin Oncol 20:2672-2679, 2002

34. Bennicelli JL, Advani S, Schafer BW, et al: PAX3 and PAX7 exhibit conserved cis-acting transcription repression domains and utilize a common gain of function mechanism in alveolar rhabdomyosarcoma. Oncogene 18:4348-4356, 1999

35. Du S, Lawrence EJ, Strzelecki D, et al: Co-expression of alternatively spliced forms of PAX3, PAX7, PAX3-FKHR and PAX7-FKHR with distinct DNA binding and transactivation properties in rhabdomyosarcoma. Int J Cancer 115:85-92, 2005

36. Kelly KM, Womer RB, Sorensen PH, et al: Common and variant gene fusions predict distinct clinical phenotypes in rhabdomyosarcoma. J Clin Oncol 15:1831-1836, 1997

37. Pappo AS, Anderson JR, Crist WM, et al: Survival after relapse in children and adolescents with rhabdomyosarcoma: A report from the Intergroup Rhabdomyosarcoma Study Group. J Clin Oncol 17:3487-3493, 1999

38. Qualman SJ, Bowen J, Parham DM, et al: Protocol for the examination of specimens from patients (children and young adults) with rhabdomyosarcoma. Arch Pathol Lab Med 127:1290-1297, 2003

