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The Association of Subventricular Zone Involvement at Recurrence with Survival after Repeat Surgery in Patients with Recurrent Glioblastoma

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Abstract

Surgical resection is identified as an important prognostic factor for survival in patients undergoing initial resection of glioblastoma (GBM). However, in patients with tumor recurrence, the benefits of repeat surgery remain unclear. Recent reports have stated that the association between initial surgery for GBM and subventricular zone (SVZ) influences survival. The current study examined the relationship of SVZ involvement in recurrent GBM to survival time after reoperation. We conducted a retrospective review of 61 consecutive patients who had undergone repeat surgery for recurrent GBM at our institution between 1997 and 2010. Survival after repeat surgery were compared between patients with (n = 29) and without (n = 32) SVZ involvement at recurrence using univariate analysis with known prognostic factors, including sex, age, Karnofsky Performance Status (KPS) score at recurrence, recurrent tumor size, initial SVZ involvement, and adjuvant therapy after repeat surgery, as variables. All 26 SVZ-positive tumors at initial diagnosis recurred as SVZ-positive tumors, while 32 of 35 SVZ-negative tumors at initial diagnosis remained SVZ-negative at recurrence; the remaining three were SVZ-positive at recurrence. Survival after repeat surgery was decreased in patients with recurrent GBM involving the SVZ at recurrence (p = 0.022). No other prognostic factors for survival after repeat surgery were identified in this study. This finding may have prognostic and therapeutic significance.

Key words: glioblastoma, recurrence, repeat surgery, subventricular zone

Introduction

Glioblastoma (GBM) is the most common central nervous tumor in adults. Despite therapeutic advances, the median survival time continues to be approximately 14 months.[1] Younger age and higher Karnofsky Performance Status (KPS) scores are widely accepted independent predictors of prolonged survival.[2–4] A significant association between the extent of resection and survival has also been shown in several retrospective studies.[5–7] As several papers have reported, GBM is a histopathologically, radiographically, and genetically heterogeneous disease.[8,9] Recent studies have demonstrated that the heterogeneity of GBM may be related to the cell of origin, which has stem cell-like characteristics.[10,11]

The adult human brain harbors neural stem cells within the subventricular zone (SVZ), which is located under the ependyma of the lateral ventricle.[12] Recently, Lim et al. proposed a classification system based on the relationship of the contrast-enhanced lesion to the SVZ and the cortex on magnetic resonance imaging (MRI).[13] They found that tumors contacting the SVZ and involving the cortex more often tended to be multifocal at diagnosis as well as recurrence. In addition, lower overall survival (OS) and progression-free survival have been reported in patients with GBM involving SVZ.[14,15]

In cases of tumor recurrence, treatment options are individualized because no standard protocol has been developed. Till date, the benefits of repeat surgery for the treatment of recurrent GBM have not been fully established. Previous studies have retrospectively assessed patient outcomes after resection of recurrent GBM. The variables that were significantly associated with OS in at least one of these studies were preoperative KPS, extent of surgical resection, age, and time interval between the first and second

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surgeries.[16–19] However, whether or not location of the recurrent lesion is associated with survival after repeat surgery remains unclear. We, therefore, aimed to determine whether SVZ involvement in patients with recurrent GBM is related to decreased survival after repeat surgery.

Materials and Methods

We retrospectively reviewed the medial records of 269 adult patients who had undergone surgical resection of a supratentorial GBM at the Tohoku University Hospital from January 1, 1997 to August 31, 2010. After initial surgery, patients had received involved-field external beam radiation therapy and either nitrosourea or temozolomide chemotherapy. Repeat surgery has been considered by the following points: (1) resectable tumor without severe morbidity and (2) younger patients or older patients with high KPS. Of the 269 patients, 61 received one or more additional resective surgeries for the treatment of histologically confirmed recurrent tumor. Medical charts were reviewed for information concerning patient age at the time of initial surgery, sex, additional therapy, KPS score at recurrence, and median survival time after repeat surgery. The degree of resection was retrospectively classified as follows on the basis of MRIs obtained < 72 h after repeat surgery: gross-total resection (GTR) if no residual enhancement was noted on postoperative MRI or subtotal resection if any residual enhancement was noted on postoperative MRI.[7] Perioperative mortality was defined as death within 30 days of repeat surgery.

MRI sequences were acquired on a 1.5T scanner and typically included axial T_1-weighted, T_2-weighted fast spin-echo, and fluid-attenuated inversion-recovery sequences as well as gadopentetate dimeglumine-enhanced (Magnevist, Bayer Health Care, Leverkusen, Germany) axial and coronal T_1-weighted images. As previously reported, tumors were classified as involving the SVZ (SVZ-positive) if the contrast-enhanced lesion contacted the lining of the lateral ventricle.[2] Tumor recurrence was defined as the appearance or enlargement since prior imaging of a contrast-enhanced mass on T_1-weighted MRI. The size of the contrast-enhanced lesion was approximated using the formula for the volume of an ellipsoid (4/3 × radius × radius × radius).

Parametric data are expressed as mean ± standard deviation (SD). Nonparametric data were expressed as median [interquaritile range (IQR)]. Percentages were compared using the χ^2 test. Continuous variables were compared using Student's *t*-test or the Mann-Whitney U test where appropriate. To determine the relative impact of multiple variables on OS and survival after repeat surgery, a Cox proportional hazards model was constructed. For the univariate analysis of potential prognostic factors, time-to-event distributions of the patients were estimated using Kaplan-Meier plots, and p values were obtained using log-rank tests. Variables with significance at the 0.20 level were selected for inclusion in the multivariate model and were entered in a forward stepwise fashion. Only variables with significance at the p = 0.05 level were accepted in the final model. All statistical tests were performed using SPSS version 21 (IBM, Chicago, Illinois, USA).

Results

Among 269 patients with GBM, we obtained pre- and post operative MRI at initial surgery and follow-up MRIs from 223 patients. As shown in Fig. 1, 102

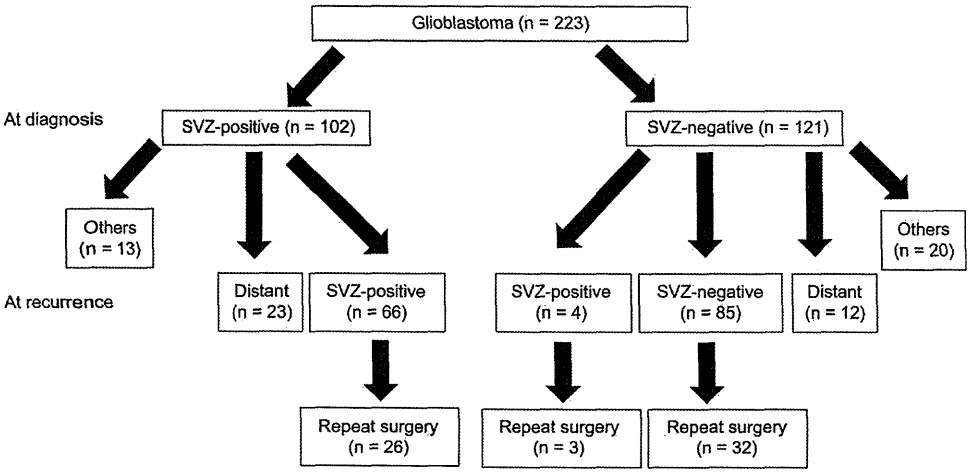

Fig. 1 Flowchart illustrating the subventricular zone (SVZ) involvement of glioblastoma at diagnosis and recurrence. Distant included the patients recurred at locations noncontiguous with the recurrent lesion [cerebrospinal fluid (CSF) dissemination or contralateral invasion]. Others included the patients survived without recurrent lesion or died from other disease.

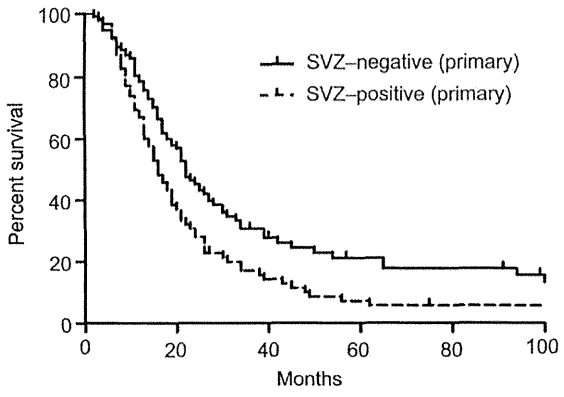

Fig. 2 Kaplan-Meier plots of OS comparing patients with SVZ-positive and SVZ-negative glioblastoma at diagnosis. Median OS was 16 months in patients with SVZ-positive lesions and 22 months in patients with SVZ-negative lesions (p = 0.005). OS: overall survival, SVZ: subventricular zone.

GBMs were SVZ-positive and other 121 GBMs were SVZ-negative. During follow-up period, 66 of 102 SVZ-positive GBMs also recurred as SVZ-positive and 23 tumors recurred at locations noncontiguous with the recurrent lesion. In other 13 patients, 5 were still alive without recurrence and 8 were dead by other disease. Finally, 26 of 66 patients with SVZ-positive recurrent GBM received repeat surgery. On the other hand, repeat surgery was performed for 3 of 4 SVZ-positive and 32 of 85 SVZ negative recurrent tumors from primary SVZ-negative tumors. Twelve tumors recurred at locations noncontiguous with the recurrent lesion. In other 20 patients, 16 were still alive without recurrence and 4 were dead by other disease. There was no significant difference for frequency of repeat surgery between SVZ-positive and negative tumors at diagnosis [22/102 (21.5%) vs. 35/121 (28.9%), p = 0.22, Fig. 1]. Median OS of SVZ-positive tumors was significantly shorter than that of SVZ-negative tumors (16 vs. 22 months, p = 0.005, Fig. 2).

The baseline demographic, clinical, and MRI characteristics of the patients evaluated and treated in this study are summarized in Table 1. The mean age (± SD) of the patients was 50.6 ± 14.6 years, and 38 patients (62%) were male. The median

Table 1 Summary of clinical, treatment, and magnetic resonance imaging characteristics of 61 patients with glioblastoma

Parameter	All patients	SVZ-positive	SVZ-negative	p
Patients				
No. (%)	61	29 (48)	32 (52)	
Sex				0.18
Male	38	21	17	
Female	23	8	15	
Age (years, mean + SD)	50.6 + 14.6	52.4 + 13.6	49.0 + 15.6	0.37
KPS at recurrence (IQR)	70 (60–80)	60 (50–70)	70 (60–90)	**0.034**
Tumor size (cm^3) (mean + SD)	15.6 + 21.4	19.4 + 21.3	12.2 + 21.1	0.19
Extent of resection (%)				0.39
Gross total	44 (72)	19 (66)	25 (78)	
Subtotal	17 (28)	10 (34)	7 (22)	
Primary lesion (%)				**< 0.0001**
SVZ-positive	26 (43)	26 (90)	0	
SVZ-negative	35 (57)	3 (10)	32 (100)	
Therapy after repeat surgery				
Chemotherapy (%)	54 (89)	25 (86)	29 (91)	0.69
SRT (%)	27 (44)	8 (28)	19 (59)	**0.019**
3rd resective surgery (%)	17 (28)	4 (14)	13 (41)	**0.024**

GBM: glioblastoma, IQR: interquatile range, KPS: Karnofsky Performance Status, MRI: magnetic resonance imaging, SD: standard deviation, SRT: stereotactic radiotherapy, SVZ: subventricular zone.

(IQR) KPS score at recurrence was 70 (60–80), while the mean tumor size (± SD) at recurrence was 15.6 ± 21.4 cm^3. GTR was performed in 44 patients (72%).

Of the 61 patients, SVZ-positive tumors were identified in 29 (48%) while SVZ-negative tumors were identified in 32 (52%). No significant difference in sex, age at recurrence, recurrent tumor size, or extent of resection at repeat surgery was observed between the two groups. However, preoperative KPS score in patients with SVZ-positive tumors was significantly lower than that in patients with SVZ-negative tumors. All 26 SVZ-positive tumors at initial diagnosis recurred as SVZ-positive tumors (Fig. 3). Only three primary SVZ-negative tumors showed SVZ involvement at recurrence; the other primary SVZ-negative tumors were still SVZ-negative at recurrence (Fig. 4).

No perioperative mortality was observed in this study. All patients underwent follow-up MRIs for postoperative evaluation. Of the 61 patients, 54 (89%) received additional chemotherapy (temozolomide, ifosfamide + cisplatin + etoposide or intrathecal methotrexate) while 27 (44%) received stereotactic radiotherapy (SRT) following repeat surgery. During the follow-up period (24–206 months), second recurrence occurred in 57 patients and a third resective surgery was done in 17 (28%) of them. Of the 29 SVZ-negative tumors with second tumor recurrence, 23 (85%) re-recurred at locations contiguous with the recurrent lesion. Therefore, a third resective surgery was possible in 13 of the 23 patients (41%).

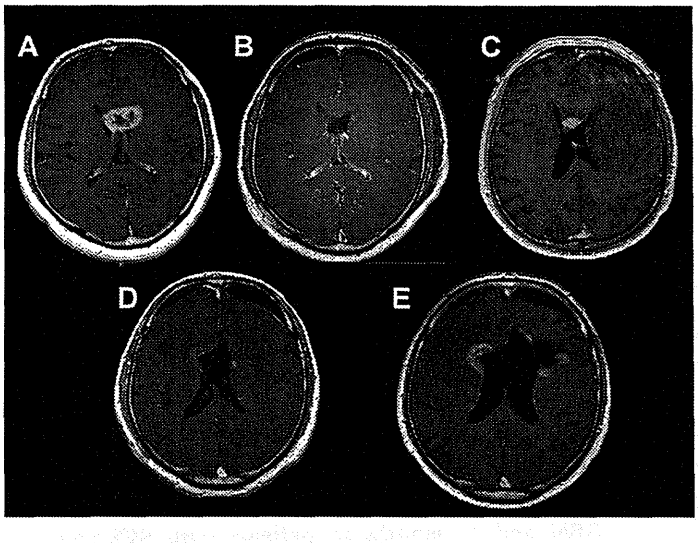

Fig. 3 A: Preoperative axial contrast T_1-weighted magnetic resonance (MR) image of a patient with primary subventricular zone (SVZ)-positive glioblastoma (GBM). The contrast-enhanced lesion contacts the anterior horn of SVZ. B: Postoperative axial contrast T_1-weighted MR image of a patient with primary SVZ-positive GBM. No residual tumor is noted on MR imaging. C: Four months after surgery, an enhanced lesion in the SVZ of the anterior horn was observed. D: Postoperative axial contrast T_1-weighted MR image of a patient with recurrent SVZ-positive GBM. Subtotal resection was performed. E: Three months after surgery, an enhanced lesion can be observed in the SVZ of the bilateral wall of the lateral ventricle.

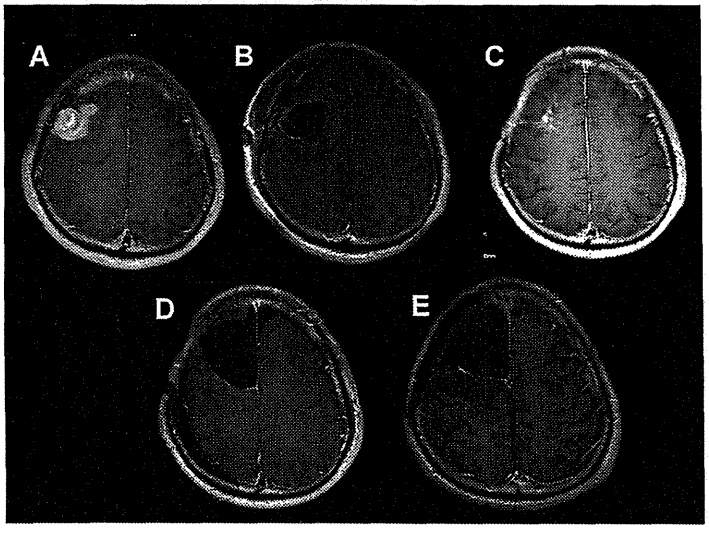

Fig. 4 A: Preoperative axial contrast T_1-weighted magnetic resonance (MR) image of a patient with primary subventricular zone (SVZ)-negative glioblastoma (GBM). The contrast-enhanced lesion does not contact the SVZ. B: Postoperative axial contrast T_1-weighted MR image of a patient with primary SVZ-negative GBM. No residual tumor is observed on MRI. C: Five months after surgery, an enhanced lesion is evident around the resection cavity. D: Postoperative axial contrast T_1-weighted MR image of a patient with recurrent SVZ-negative GBM. No residual tumor is visible on MR imaging. E: Four months after surgery, an enhanced lesion around the resection cavity can be observed.

On the other hand, in the 28 SVZ-positive GBMs with second recurrence, 21 tumors re-recurred at locations noncontiguous with the recurrent lesion [cerebrospinal fluid (CSF) dissemination in 11 and contralateral invasion in 5]. Therefore, only four patients (14%) received a third resection in this group. There was no significant difference in the number of patients who received chemotherapy after repeat surgery between the two groups. However, the number of patients who received SRT and/or underwent a third surgery was lower in the SVZ-positive group than in the SVZ-negative group (Table 1).

The median OS and survival after repeat surgery was 25 months and 11 months, respectively, in this study. Patient age, sex, KPS score at recurrence, recurrent tumor size, resection rate at recurrence, SVZ involvement at initial and repeat surgery, and therapy after repeat surgery were examined as prognostic factors for survival using univariate analysis. The results are shown in Table 2. A significant difference in median survival after repeat surgery was noted between patients with SVZ-positive recurrence and patients with SVZ-negative recurrence (Kaplan-Meier estimates: 10 months vs. 14 months; p = 0.022; Fig. 5). Median OS and survival after repeat surgery for patients with SVZ-positive recurrence of tumors that were SVZ-negative at diagnosis were 17 and 8 months, respectively. Only KPS at recurrence and SVZ involvement for survival from repeated surgery at the p = 0.20 level and were included in the multivariate model. Hazard ratios (HRs) from the multivariate results for each factor are shown

Table 2 Outcomes of 61 patients with GBM who underwent repeat surgery

Parameters	Survival from repeat surgery (months)	p
Sex		0.23
Male (n = 38)	12	
Female (n = 23)	11	
Age		0.82
< 50 (n = 27)	11	
> 50 (n = 34)	11	
KPS at recurrence		0.11
70–100 (n = 37)	9	
40–60 (n = 24)	12	
Tumor size (cm^3)		0.88
< 10 cm^3 (n = 38)	11	
> 10 cm^3 (n = 23)	11	
Resection rate		0.23
Total (n = 44)	11	
Subtotal (n = 17)	11	
Primary lesion		**0.021**
SVZ-positive (n = 26)	9.5	
SVZ-negative (n = 35)	13	
Recurrent lesion		**0.022**
SVZ-positive (n = 29)	10	
SVZ-negative (n = 32)	14	
Therapy after 2nd operation		0.87
SRT (+) (n = 19)	11	
SRT (–) (n = 38)	11	
3rd operation (+) (n = 20)	11	
3rd operation (–) (n = 37)	11	

KPS: Karnofsky Performance Scale, SRT: stereotactic radiotherapy, SVZ: subventricular zone.

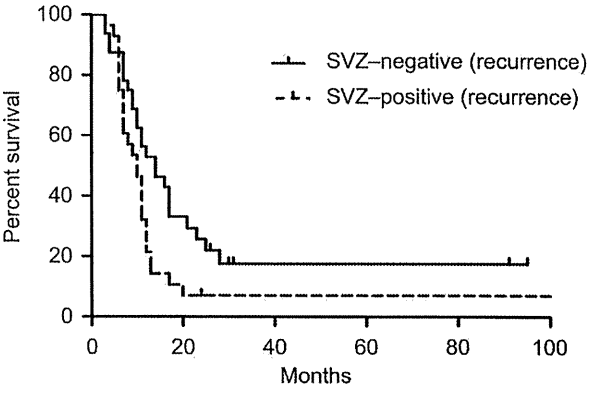

Fig. 5 Kaplan-Meier plots of survival after repeat surgery in patients with SVZ-positive and SVZ-negative GBM at recurrence. Median survival after repeat surgery was 10 months in patients with SVZ-positive recurrent GBM and 14 months in patients with SVZ-negative recurrent GBM (p = 0.022). GBM: glioblastoma, SVZ: subventricular zone.

Table 3 Multivariate analysis of survival from repeat surgery

Parameters	HR	95%CI	p
KPS at recurrence			0.13
KPS < 70	1.54	0.88–2.68	
KPS > 70	1		
SVZ (recurrent lesion)			**0.029**
SVZ-positive	1.87	1.06–3.28	
SVZ-negative	1		

CI: confidence ratio, HR: hazard ratio, KPS: Karnofsky Performance Scale, SVZ: subventricular zone.

in Table 3. When adjusting for all factors, only SVZ involvement at recurrence was a significant predictor of survival after repeat surgery (HR, 1.87; 95%CI, 1.06–3.28; p = 0.029).

Discussion

Several papers in the past decade have emphasized the importance of surgical resection for primary GBM.[5–7] However, the benefits of repeat surgery for recurrent GBM have not been completely determined. Previous papers have identified age, preoperative KPS score, and resection rate at recurrence as important prognostic factors.[16–20] However, these factors were not identified as significant prognostic factors in our study, although the results of this study are subject to the limitations of a retrospective study, only SVZ involvement at recurrence was associated with decreased survival after repeat surgery. Previous papers have reported associations between SVZ involvement, aggressive tumor behavior, and shorter OS in patients with GBM.[13–15] Lim et al. reported that contrast-enhanced lesions contacting both the cortex and SVZ were most likely to be multifocal at the time of initial diagnosis. In addition, recurrent tumors were more likely to develop at locations distant to the initial lesion in patients with SVZ involvement. In contrast, GBMs not involving the SVZ or cortex were not multifocal at initial diagnosis and always recurred within 2 cm of the resection margin.[13] Chaichana et al. reported an association between periventricular tumor location (SVZ involvement) and poor survival.[14] They proposed a classification system including periventricular involvement for the prediction of outcome in patients with primary GBM.[21] Our study confirmed that SVZ involvement at diagnosis was an important predictor of OS.

In our result, the frequency of repeat surgery in patients with SVZ-positive GBMs was lower than that in patients with SVZ-negative GBMs, however, there was no significant difference. Other factors such as invasion to eloquent lesions could be also important for indication of repeat surgery. In this study, SVZ involvement was identified at recurrence in all patients who had primary SVZ-positive GBMs. In addition, most of these tumors re-recurred at locations noncontiguous with the recurrent lesion (CSF dissemination or contralateral invasion). As a result, a third resection was possible in only four patients in this group. In contrast, except for a few cases, SVZ-negative GBMs recurred within the SVZ-negative region. In addition, the tumor location at the second recurrence was quite similar to that of the primary and first recurring lesions. Therefore, a third resection was possible in approximately half the patients with SVZ-negative recurrent GBMs. In addition, SVZ involvement was associated with survival after repeat surgery in patients with recurrent GBM. Although a third resection was not associated with survival after the second repeat surgery, more aggressive tumors with SVZ involvement may have been associated with poorer survival after repeat surgery. On the other hand, median OS and survival after repeat surgery for patients with SVZ-positive recurrence of tumors that were SVZ-negative at diagnosis were 17 and 8 months, respectively. These results suggest that OS for patients with SVZ-negative tumors at diagnosis was relatively favorable; however, if the tumors recurred with SVZ involvement, the chances of survival became low. Despite the limited availability of cases, these results may be of interest. However, what remains less well known is why SVZ involvement is associated with poorer survival. In basic science studies, Sanai et al. demonstrated that cells obtained from the lateral wall of the lateral ventricle, which is called the SVZ, harbors cells with stem cell-like features of self-renewal and multi-potentiality.[12] While some GBMs may arise from transformed SVZ stem cells, other GBMs may be initiated by neoplastic transformation of astrocyte precursor cells or dedifferentiated mature astrocytes.[22] The aggressive behavior of SVZ-positive GBMs may be related to the recruitment of neural stem cells from the SVZ that have a tendency toward invasive proliferation. However, Kappadakunnel et al. found no relationship between stem-cell gene expression and SVZ grade, but they did find an association between stem-cell gene expression and survival.[23] As these researchers noted, more research is required to clarify the relationship among SVZ, cancer stem cells, and survival.

Despite its retrospective design, this study is the first to report a possible association between recurrent GBM tumors adjacent to the SVZ and decreased survival after repeat surgery. Nonetheless, larger prospective studies may provide further relevant information. However, the findings of this study may be helpful to determine therapeutic strategies for recurrent GBM. With regard to recurrence, SVZ-negative recurrent GBMs may be good candidates for repeat surgery.

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Conflicts of Interest Disclosure

The authors report no conflict of interest. All authors who are members of The Japan Neurosurgical Society (JNS) have registered online Self-reported COI Disclosure Statement Forms through the website for JNS members.

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ORIGINAL REPORT

Cytogenetic Prognostication Within Medulloblastoma Subgroups

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ABSTRACT

Purpose
Medulloblastoma comprises four distinct molecular subgroups: WNT, SHH, Group 3, and Group 4. Current medulloblastoma protocols stratify patients based on clinical features: patient age, metastatic stage, extent of resection, and histologic variant. Stark prognostic and genetic differences among the four subgroups suggest that subgroup-specific molecular biomarkers could improve patient prognostication.

Patients and Methods
Molecular biomarkers were identified from a discovery set of 673 medulloblastomas from 43 cities around the world. Combined risk stratification models were designed based on clinical and cytogenetic biomarkers identified by multivariable Cox proportional hazards analyses. Identified biomarkers were tested using fluorescent in situ hybridization (FISH) on a nonoverlapping medulloblastoma tissue microarray (n = 453), with subsequent validation of the risk stratification models.

Results
Subgroup information improves the predictive accuracy of a multivariable survival model compared with clinical biomarkers alone. Most previously published cytogenetic biomarkers are only prognostic within a single medulloblastoma subgroup. Profiling six FISH biomarkers (*GLI2, MYC*, chromosome 11 [chr11], chr14, 17p, and 17q) on formalin-fixed paraffin-embedded tissues, we can reliably and reproducibly identify very low-risk and very high-risk patients within SHH, Group 3, and Group 4 medulloblastomas.

Conclusion
Combining subgroup and cytogenetic biomarkers with established clinical biomarkers substantially improves patient prognostication, even in the context of heterogeneous clinical therapies. The prognostic significance of most molecular biomarkers is restricted to a specific subgroup. We have identified a small panel of cytogenetic biomarkers that reliably identifies very high-risk and very low-risk groups of patients, making it an excellent tool for selecting patients for therapy intensification and therapy de-escalation in future clinical trials.

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INTRODUCTION

Medulloblastoma, the most common malignant childhood brain tumor, is an embryonal tumor with a peak incidence in early childhood. Current therapy entails surgical resection, craniospinal irradiation, and high-dose chemotherapy. Risk stratification is based primarily on clinical variables, with high-risk patients identified as having leptomeningeal metastases at presentation and/or an incomplete resection.[1-3] Unfortunately, most survivors are left with long-term disabilities secondary to the disease and treatment.[4-6] Clinicians have hypothesized that improved patient prognostication could enable therapy intensification in high-risk patients and therapy de-escalation to maximize quality of life in lower-risk patients.

Numerous publications have attempted to identify biomarkers to either support or supplant clinical risk stratification.[2,7-14] Mutations of specific genes such as *CTNNB* and *TP53* have shown prognostic significance.[15-19] Additional candidates include medulloblastoma-overexpressed genes such as *TRKC*, *ERBB2*, and *FSTL5*.[20-25] Several DNA copy-number aberrations have also been purported as biomarkers, although the results have often been conflicting.[15,26-48] These aberrations are summarized in Table 1. Few of these putative molecular biomarkers have been validated in prospective clinical trials.

It is now recognized that medulloblastoma is a collection of heterogeneous entities with disparate demographics, transcriptomes, genetics, and clinical outcomes.[2,28,32,49-60] According to international consensus, the principle subgroups of medulloblastoma are WNT, SHH, Group 3, and Group 4.[52] Because earlier prognostic biomarker studies did not account for these subgroups, we hypothesized that some of the disparate biomarker findings could have resulted from differential subgroup representation among studies. Several previously reported biomarkers were in fact enriched within a specific subgroup of the disease (eg, monosomy 6 in WNT tumors, *MYC* amplification in Group 3 tumors). In cases where a biomarker is prognostic across all medulloblastomas, but the prognostic impact is driven by a single subgroup, we suggest that the marker be designated as subgroup driven. These surrogate markers are replaceable by subgroup status. In cases where a biomarker is variably or not effective across the spectrum of medulloblastomas but is valid only within a specific subgroup, we suggest that it be designated as subgroup specific. Such biomarkers are prognostically informative only within specific medulloblastoma subgroups.

To determine whether subgroup affiliation and cytogenetic biomarkers could support or supplant clinical variables for prognostication in patients with medulloblastoma, we assembled an international discovery cohort of 673 medulloblastomas through MAGIC (Medulloblastoma Advanced Genomics International Consortium), for which we had both clinical follow-up and whole-genome copy-number data. To begin, we identified subgroup-specific copy-number aberrations (CNAs) and integrated them with clinical variables to develop subgroup-specific risk models based on the discovery cohort. To validate our models and ensure that our technique was generalizable to routine pathology laboratories, we then studied a panel of six cytogenetic biomarkers (*GLI2*, *MYC*, chromosome 11 [chr11], chr14, 17p, and 17q) using interphase fluorescent in situ hybridization (FISH) on a formalin-fixed paraffin-embedded (FFPE) medulloblastoma tissue microarray (TMA) that included 453 medulloblastomas treated at a single center and did not overlap with the discovery cohort.

Our analysis of > 1,000 patients with medulloblastoma clearly demonstrates that subgroup affiliation can improve prognostication with clinical variables and that a majority of published molecular biomarkers are relevant only within a single subgroup. The combination of clinical variables, subgroup affiliation, and six cytogenetic markers analyzed on FFPE tissues can achieve an unprecedented level of prognostic prediction for patients that is practical, reliable, and reproducible.

PATIENTS AND METHODS

Tumor Material and Patient Characteristics

A discovery set of 673 medulloblastoma samples with clinical follow-up was acquired retrospectively from 43 cities around the world. These samples

Table 1. Previously Reported Prognostic Molecular Markers in MB

	Previous Studies		Our Study				
Marker	Cohort	Prognosis	Validated	MB (*P*)	SHH (*P*)	Group 3 (*P*)	Group 4 (*P*)
1q gain	MB[26,27]	Poor	No	.61	.59	**.018**	.33
Chr2 gain	SHH[30]	Poor	No	.16	.66	.17	.49
3q gain	MB,[32] SHH[32]	Poor	No	.14	.20	.80	—
6q gain	MB[31]	Poor	No	.61	.30	.94	.19
Chr6(q) loss	MB[15,31,34]	Good	SGD	**.002**	.90	.73	—
10q loss	MB,[32,35] SHH[30]	Poor	SGS	**.012**	**.001**	.23	.082
17p loss	MB,[26,32,36-40] SHH,[30,32] Group 4[32]	Poor	SGS	**.003**	**.011**	**.030**	.37
17q gain	MB,[31,32,35,40] SHH,[30] Group 3,[32] Group 4[32]	Poor	SGS	.095	—	**.049**	.72
Iso17q	MB[31,35,38,41]	Poor	SGS	**.005**	—	**.008**	.81
CDK6 amplification	MB[32,40]	Poor	No	.51	.36	.17	.55
GLI2 amplification	SHH[30]	Poor	SGS	**< .001**	**.001**	—	—
MYC amplification	MB[31,42-46]	Poor	SGS	**< .001**	—	**.011**	.37
MYCN amplification	MB,[31,44] SHH,[32] Group 4[32]	Poor	SGS	.92	**.002**	—	.24
OTX2 amplification	MB[47]	Poor	No	.61	—	.46	.77

NOTE. Bold font indicates significance; — indicates event not observed at sufficient frequency (n ≤ 1).
Abbreviations: chr, chromosome; iso, isochromosome; MB, medulloblastoma (across all subgroups); SGD, subgroup driven; SGS, subgroup specific.

were copy-number profiled on the Affymetrix SNP6 platform (Santa Clara, CA) to identify potential biomarkers.[28] An independent validation set of 453 samples with clinical follow-up on a TMA was analyzed using FISH as previously described.[53] Tumors were classified based on signature marker expression into molecular subgroups as previously described[61]; additional tumors were classified based on cytogenetic aberrations using standard conditional probability models. Subgroup affiliation was not available for 162 discovery samples. The validation set additionally included 50 WNT tumors not on the TMA. Details on clinical data are listed in Data Supplement 1, along with the availability of clinical and cytogenetic data. Nucleic acid isolation, TMA construction, and β-catenin mutation analysis were performed as previously described.[28]

Prognostic Biomarker Identification

Cytogenetic events and CNAs were identified as previously described in the discovery set.[28] Subsequent to biomarker discovery, cross validation was performed to estimate the reproducibility of the candidates in an independent cohort, with multiple-hypothesis correction. Additionally, sample size estimates for prospective trials of the biomarkers were calculated based on the observed hazard ratios. Additional details are available in Data Supplement 1.

Statistical Analyses

Patient survivals were analyzed using the Kaplan-Meier method. The predictive values of biomarkers were assessed through time-dependent receiver operating characteristic analyses. Details of the survival analyses and risk model selections are available in Data Supplement 1.

RESULTS

Prognostic Significance of Clinical Variables Within Medulloblastoma Subgroups

Many prior medulloblastoma biomarker studies were limited by sample size. Our study included 1,126 patients with medulloblastoma (673 discovery plus 453 validation patients; Data Supplement 1), which is more than double the sample size of any prior medulloblastoma biomarker study, and it is one of few studies to include a validation cohort (Data Supplement 2). Although the discovery cohort accumulated by MAGIC consists of medulloblastomas gathered from 43 different treating centers from around the world, the subgroup-specific outcomes mirror those previously published, with good outcomes for patients with WNT, poor outcomes for those with Group 3, and intermediate outcomes for those with SHH and Group 4 medulloblastomas (Data Supplement 2), suggesting that the discovery cohort was a representative sample (Data Supplement 1).

To assess long-term survivors, patients with WNT medulloblastoma were observed for up to 10 years, and only two deaths were observed among 53 patients, both resulting from tumor recurrence (Fig 1A; Appendix Fig A1A, online only; Data Supplement 1). Among those with SHH tumors, there were significantly better outcomes among adult patients as compared with children or infants (Fig 1B; Appendix Fig A1B, online only). Infants with Group 4 tumors had significantly worse outcomes than children or adults (Fig 1B; Appendix Fig A1B, online only), suggesting that radiation therapy is critical in the treatment of Group 4 medulloblastoma. There was no consistent association between sex and prognosis in any of the four subgroups (Data Supplement 1). Desmoplastic histology indicated a more favorable prognosis than classic histology, which was more favorable than anaplastic histology among SHH tumors (Data Supplement 1). Large-cell/anaplastic histology was prognostically significant for Group 3 medulloblastomas in the discovery cohort but not in the validation cohort.

Metastatic status was not prognostic for patients with WNT tumors; however, macroscopic metastasis (M2/M3) was consistently associated with poor survival in all non-WNT subgroups, although the clinical effect was modest among patients with Group 4 disease (Fig 1C; Appendix Fig A1C, online only). Although the prognostic significance of M0 disease as compared with M2/3 disease was convincing across SHH, Group 3, and Group 4 subgroups, the prognostic significance of isolated M1 disease (presence of tumor cells in cerebrospinal fluid) was less clear (Fig 1C; Appendix Fig A1C, online only; Data Supplement 1). Isolated M1 disease was not consistently associated with poor prognosis in the discovery or validation cohort for any subgroup, which may be the result of small sample sizes. There were no CNAs in any of the subgroups that were associated with an increased risk of leptomeningeal dissemination (Data Supplement 1). Overall, many clinical biomarkers continued to exhibit prognostic significance when medulloblastoma was analyzed in a subgroup-specific fashion (Data Supplement 1).

Subgroup and Metastatic Status Are the Most Powerful Predictive Prognostic Biomarkers

Multivariable survival analyses were conducted to examine the relative predictive value of clinical variables and subgroup affiliation. Stepwise Cox regressions revealed that subgroup affiliation significantly contributed to multivariable survival prediction, on top of a regression model already parameterized by sex, age, metastatic status, and histology (Data Supplement 2). Furthermore, Cox proportional hazards models parameterized with both clinical biomarkers and molecular subgroups achieved higher accuracy in time-dependent receiver operating characteristic analyses (Data Supplements 1 and 2). In isolation, each biomarker had modest prediction accuracy (Data Supplement 2) compared with the complete multivariable model (Data Supplement 2). In the complete model, the removal of metastatic status and subgroup led to the greatest decreases in predictive accuracy (Data Supplement 2). Taken together, these results suggest that subgroup affiliation and metastatic status are the most important predictive biomarkers and that they make nonredundant contributions to the prediction of survival. We conclude that combining both clinical and molecular biomarkers can enhance prediction of patient survival.

Subgroup Specificity of Published Molecular Biomarkers

Several cytogenetic biomarkers have been associated with patient survival across medulloblastoma, but their prognostic value has seldom been assessed in the context of medulloblastoma subgroups (Table 1). Monosomy for chromosome (chr) 6 is associated with improved survival across medulloblastoma in toto (Fig 2A; Data Supplement 1). However, the prognostic value of chr6 loss can be completely attributed to its enrichment in WNT medulloblastomas (Fig 2B; Data Supplement 1), because loss of chr6 has no prognostic value among patients with WNT or non-WNT tumors when compared with their respective controls with balanced chr6. We suggest that monosomy 6 is a subgroup-driven biomarker; its prognostic significance is driven by its enrichment in a particular subgroup, and it thus holds no further significance in subgroup-specific analysis. Furthermore, these results would add a note of caution to using monosomy 6 as the lone diagnostic criterion for WNT medulloblastoma, because it was also observed in non-WNT medulloblastomas (seven [14%] of 49

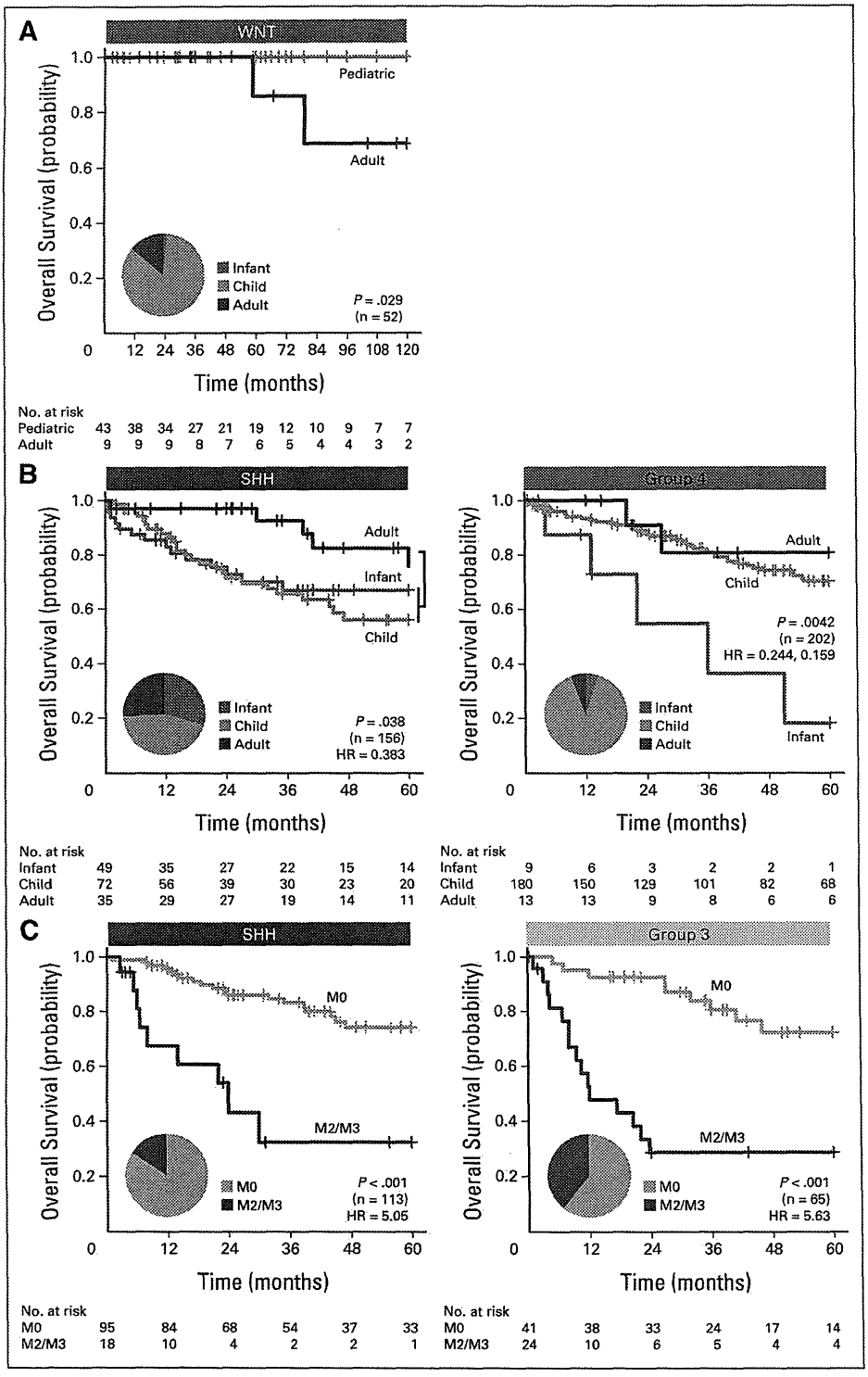

Fig 1. (A) Ten-year overall survival curves for WNT medulloblastoma by age group. (B) Overall survival curves for age groups within SHH and Group 4 subgroups (infant, age < 3 years; child, age 3 to < 16 years; adult, age ≥ 16 years). (C) Overall survival curves for metastatic status for SHH and Group 3 subgroups. Numbers below *x*-axis represent patients at risk of event; statistical significance evaluated using log-rank tests; hazard ratio (HR) estimates derived from Cox proportional hazards analyses.

monosomy 6 medulloblastomas were not in WNT subgroup), and monosomy 6 was only present in 42 (79%) of 53 WNT tumors. The prognostic role of isochromosome (iso) 17q has been controversial; for our cohort in toto, iso17q was a statistically significant predictor of poor outcome (Fig 2C). However, subgroup-specific analysis demonstrated that iso17q was highly prognostic for Group 3 but not for Group 4 medulloblastoma (Fig 2D), indicating that it is a subgroup-specific molecular biomarker. Similarly, although 10q loss was a

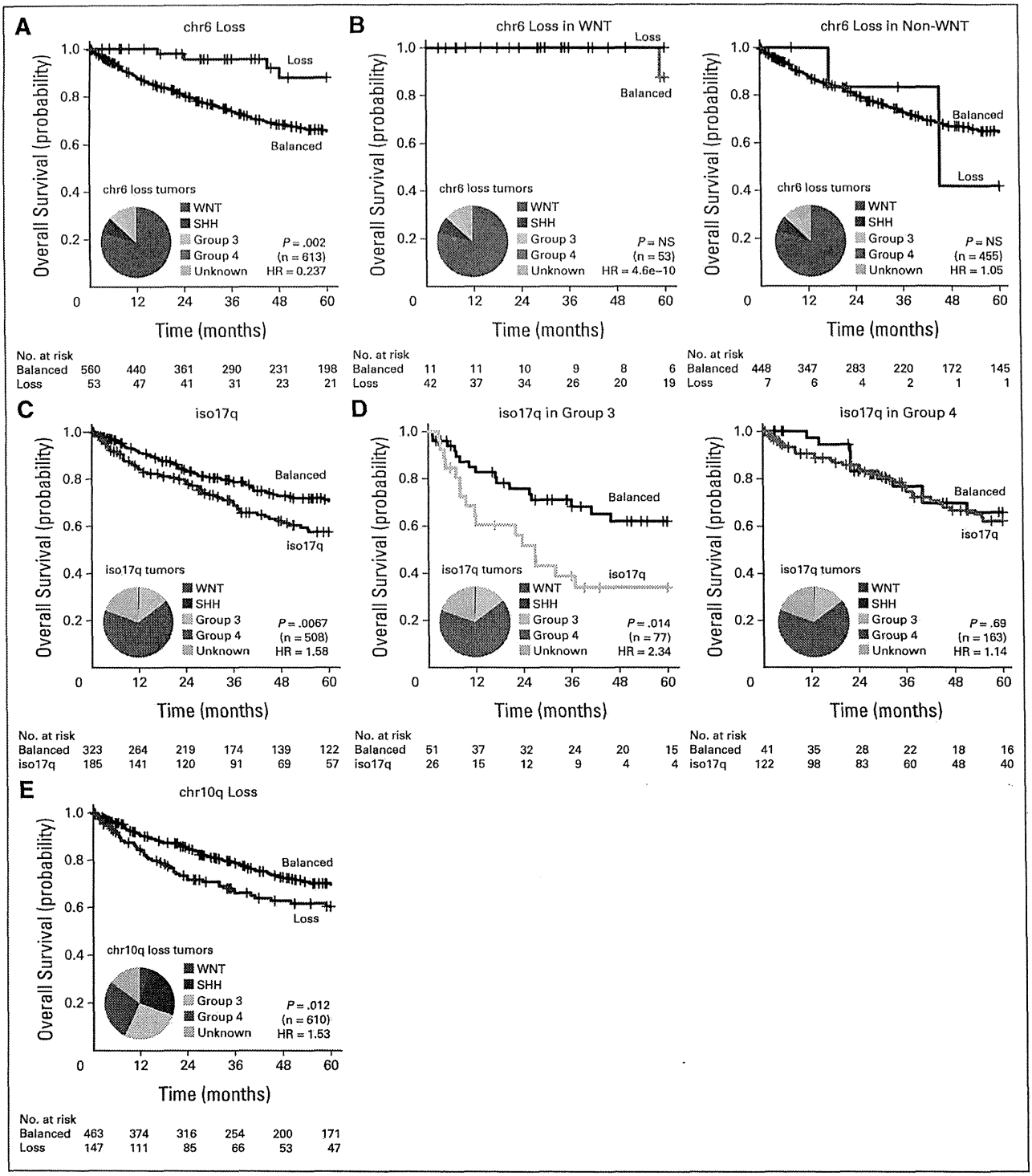

Fig 2. (A) Overall survival curves and frequency distribution of chromosome 6 (chr6) status across entire cohort. (B) Overall survival curves for chr6 status in WNT and non-WNT medulloblastomas. (C) Overall survival curves and frequency distribution of isochromosome 17q (iso17q) across entire cohort. Patients with broad gain or loss of chr17 excluded. (D) Overall survival curves for iso17q status in Group 3 and Group 4 subgroups. (E) Overall survival curves for chr10q status across entire cohort. HR, hazard ratio; NS, not significant.

modestly significant predictor of poor outcome across medulloblastomas (Fig 2E), its prognostic power was limited to SHH tumors in a subgroup-specific analysis (Appendix Figs A2A and A2B, online only). We conclude that determination of subgroup affiliation is crucial in the evaluation and implementation of molecular biomarkers for patients with medulloblastoma (Table 1; Data Supplement 1), because some putative biomarkers are merely enriching for a specific subgroup (ie, subgroup driven), whereas most others are significant only within a specific subgroup (ie, subgroup specific).

Patients With SHH Tumors Can Be Stratified Into Three Distinct Risk Groups

We identified 11 CNAs that were prognostically significant in our SHH medulloblastoma discovery set (Appendix Figs A3A to A3D, online only; Data Supplement 1) in univariable survival analyses. Given the considerable number of candidates, the reproducibility of the identified biomarkers was assessed through cross validation to facilitate candidate prioritization, and the sample sizes required for prospective trials were estimated for future studies (Data Supplement 1). Specific amplifications but not broad gains encompassing *GLI2* or *MYCN* were associated with poor prognosis (Appendix Figs A3A and A3B, online only; Data Supplement 1). Loss of chr14q conferred significantly inferior survival (Appendix Fig A3C, online only). There was no minimal region of deletion on chr14 in patients with SHH tumors (Data Supplement 1), and recent medulloblastoma resequencing efforts have not identified any recurrent single-nucleotide variants on chr14 in SHH medulloblastoma.[28,54,56,57,62] The presence of chromothripsis (ie, chromosome shattering) was associated with worse survival in those with SHH tumors (Appendix Fig A3D, online only).[17]

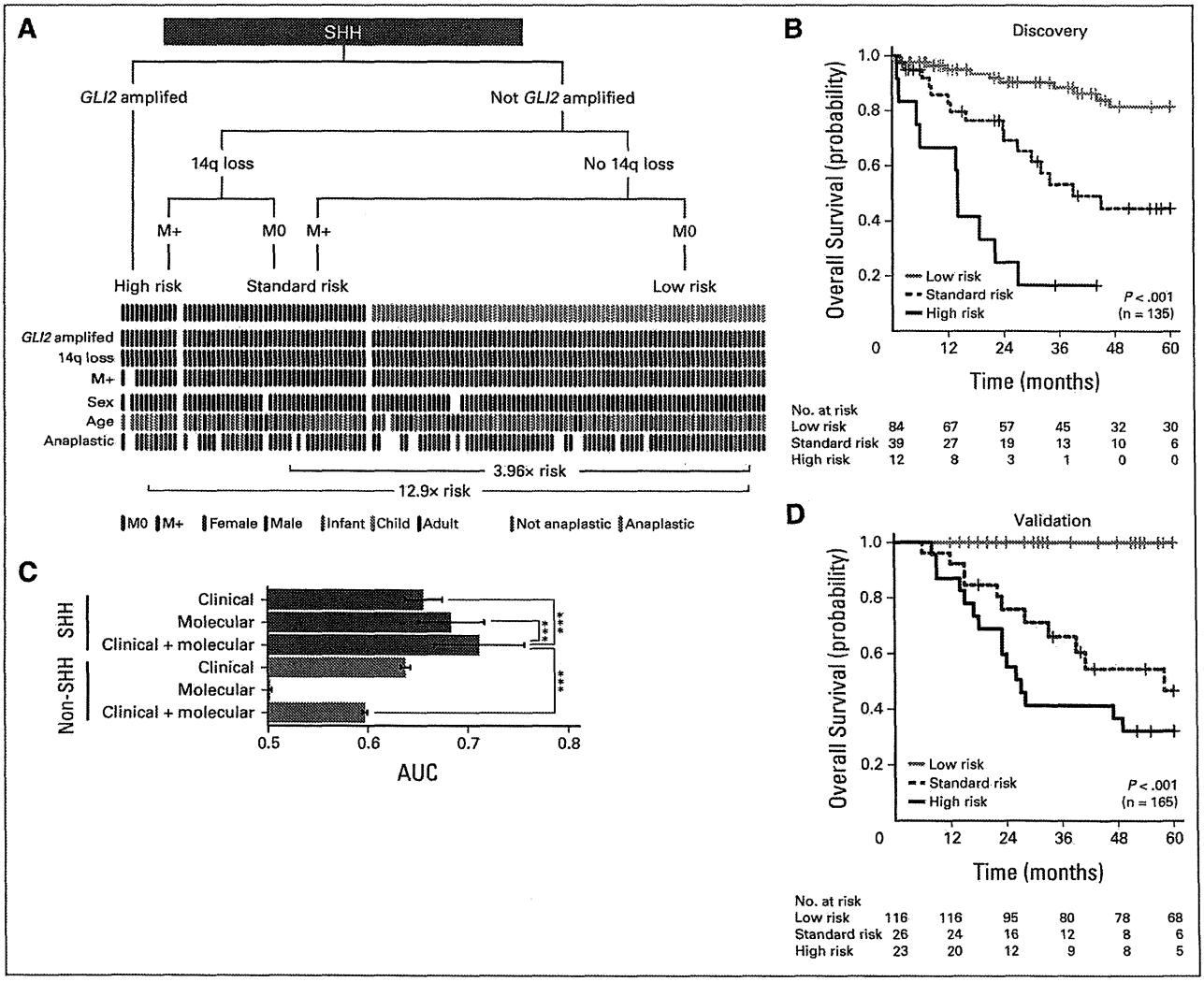

Fig 3. Clinical prognostication of patients with SHH medulloblastoma. (A) Risk stratification of SHH medulloblastomas by molecular and clinical prognostic markers. Decision tree, with events plot depicting status of molecular and clinical markers across risk groups below. (B) Overall survival curves for SHH risk groups. (C) Average time-dependent areas under the curve (AUCs) for risk groups stratified using only clinical or molecular markers or both. Risk stratification regimens applied to SHH and non-SHH medulloblastomas. ***$P < .001$ by Friedman rank sum tests. (D) Survival curves for SHH risk groups in validation cohort. Survival differences evaluated by log-rank tests; hazard ratio estimates derived from Cox proportional hazards analyses.

To integrate the individual biomarkers into a risk stratification model, multivariable Cox analyses were performed on all significant biomarkers. Through multiple stepwise regression procedures, a consensus set of biomarkers was selected for inclusion in the model in an unbiased manner. The proposed risk stratification scheme represents the model that was most consistent with available data in the discovery cohort, from among many possible alternatives (Figs 3A and 3B; Data Supplement 1). *GLI2* amplification, 14q loss, and leptomeningeal dissemination identified high- and standard-risk patients. Specifically, *GLI2* amplification alone identified patients with poor prognosis (Figs 3A and 3B; Data Supplement 1). Absence of these markers defined a low-risk group of patients who exhibited survivorship reminiscent of patients with WNT tumors. Importantly, none of the covariates, particularly age and anaplastic histology, could explain the survival differences observed among risk groups (Figs 3A and 3B; Data Supplement 1). Direct application of the proposed risk stratification scheme on the independent validation cohort yielded distinct survivorship rates for the three risk groups, thereby validating the model (Fig 3D).

Two additional stratification schemes were constructed using only clinical biomarkers or only cytogenetic markers; however, the proposed model, which combines both types of biomarkers, yielded the highest accuracy (Fig 3C; Data Supplement 1). Furthermore, the accuracy of the combined risk model was drastically reduced when applied across patients with non-SHH tumors, further underscoring the importance of taking subgroup into consideration during risk stratification. We conclude that by using two molecular biomarkers (*GLI2* and 14q FISH) and metastatic status, we can practically and reliably predict prognosis for patients with SHH medulloblastoma.

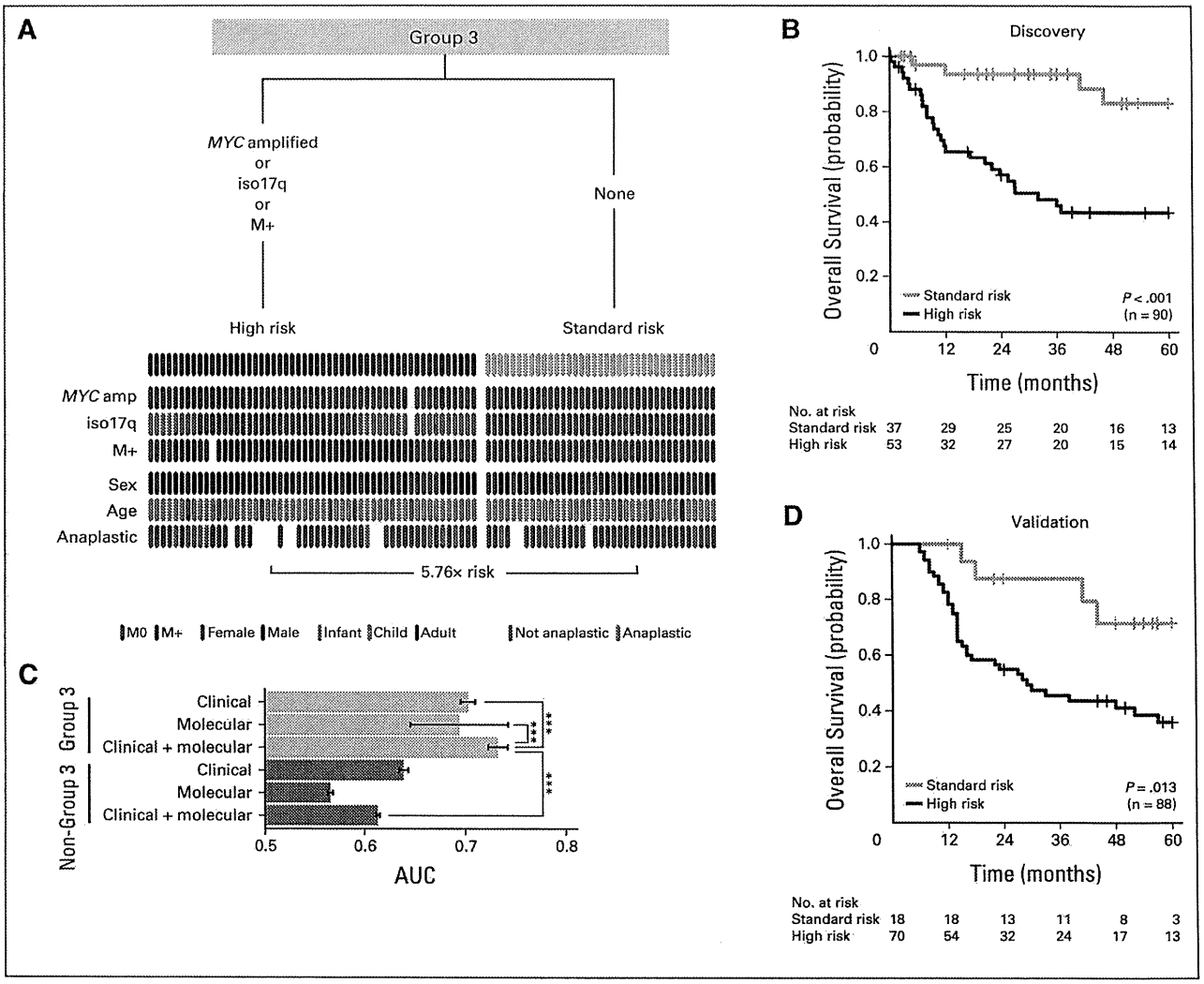

Fig 4. Clinical prognostication of patients with Group 3 medulloblastoma. (A) Risk stratification of Group 3 medulloblastomas by molecular and clinical prognostic markers. Decision tree, with events plot depicting status of molecular and clinical markers across risk groups below. (B) Overall survival curves for Group 3 risk groups. (C) Average time-dependent areas under the curve (AUCs) for risk groups stratified using only clinical or molecular markers or both. Risk stratification regimens applied to Group 3 and non–Group 3 medulloblastomas. ***$P < .001$ by Friedman rank sum tests. (D) Survival curves for Group 3 risk groups in validation cohort. Survival differences evaluated by log-rank tests; hazard ratio estimates derived from Cox proportional hazards analyses. Iso, isochromosome.

Metastatic Status, Iso17q, and MYC *Amplification Identify High-Risk Patients With Group 3 Medulloblastoma*

In patients with Group 3 tumors, iso17q and *MYC* amplification remained the only cytogenetic markers associated with poor survival (Appendix Figs A4A and A4B, online only), whereas chr8q loss and chr1q gain were the only good prognosis markers (Appendix Fig A4C, online only; Data Supplement 1). In multivariable survival analyses, patients with metastasis, iso17q, or *MYC* amplification represented the high-risk group (Figs 4A and 4B). Critically, absence of these markers identified a population of patients with Group 3 tumors with favorable prognosis. The risk groups were not associated with any clinical covariates, including age (Figs 4A and 4B; Data Supplement 1). Consistent with the findings in patients with SHH tumors, optimal risk stratification of those with Group 3 tumors required the use of both clinical and molecular prognostic markers, which have little prognostic value outside of Group 3 (Fig 4C; Data Supplement 1). Our proposed risk stratification scheme was validated on the nonoverlapping validation cohort using three molecular biomarkers (*MYC*, 17p, and 17q FISH) and metastatic status (Fig 4D).

Identification of a Low-Risk Group of Patients With Metastatic Group 4 Medulloblastoma

Patients with Group 4 tumors with whole-chromosome loss of chr11 or gain of chr17, in addition to 10p loss, exhibited better survival under univariable Cox models (Appendix Fig A5A, online only; Data Supplement 1). There was no cytogenetic marker associated with poor prognosis (Data Supplement 1). Specifically, neither *MYCN* gain nor amplification was associated with poorer survival in those with Group 4 tumors, in stark contrast to patients with SHH tumors, reinforcing

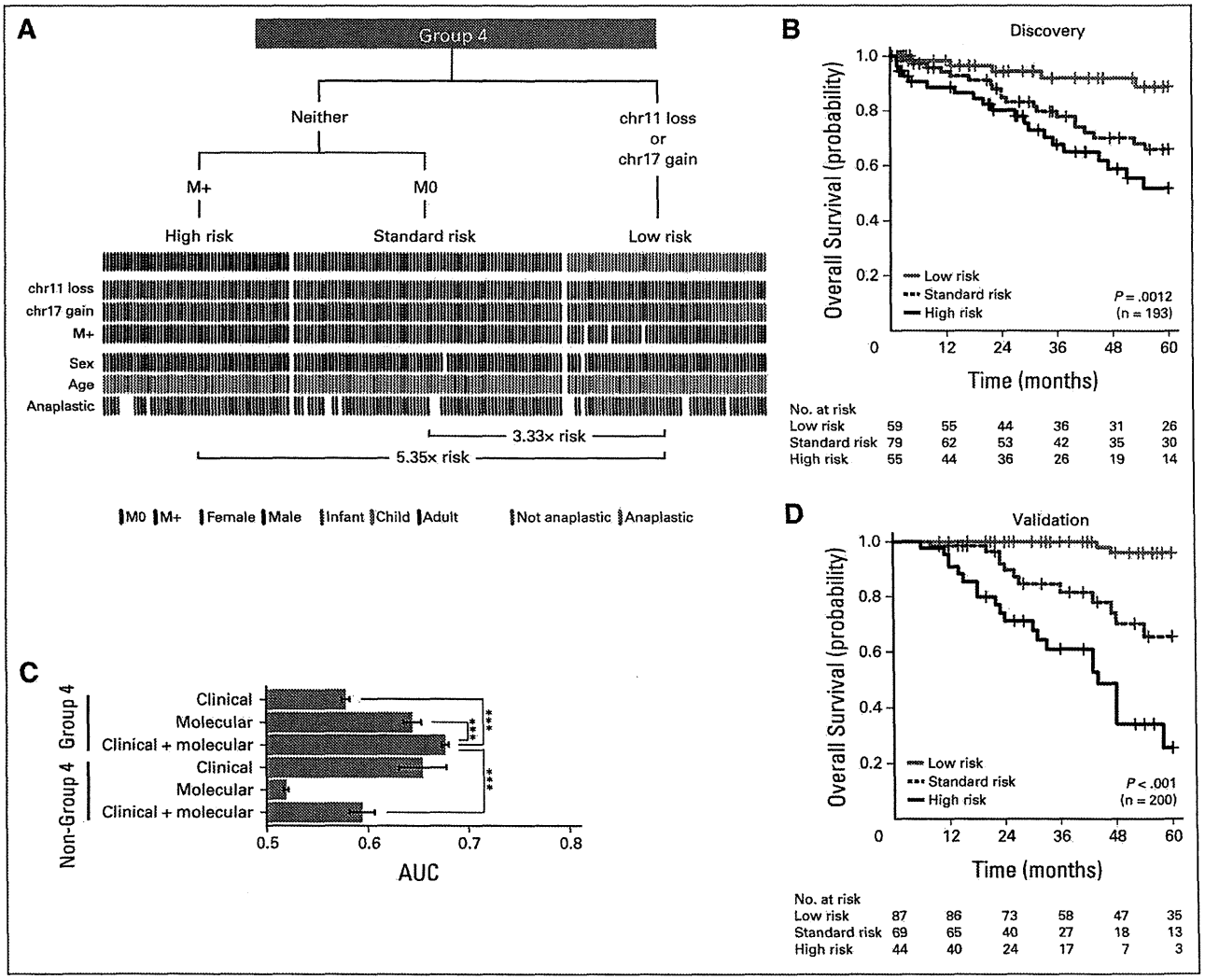

Fig 5. Clinical prognostication of patients with Group 4 medulloblastoma. (A) Risk stratification of Group 4 medulloblastomas by molecular and clinical prognostic markers. Decision tree, with events plot depicting status of molecular and clinical markers across risk groups below. (B) Overall survival curves for Group 4 risk groups. (C) Average time-dependent areas under the curve (AUCs) for risk groups stratified using only clinical or molecular markers or both. Risk stratification regimens applied to Group 4 and non–Group 4 medulloblastomas. ***$P < .001$ by Friedman rank sum tests. (D) Survival curves for Group 4 risk groups in validation cohort. Survival differences evaluated by log-rank tests; hazard ratio estimates derived from Cox proportional hazards analyses. Chr, chromosome.

the distinction in their underlying biology (Appendix Fig A5B, online only; Data Supplement 1). Similarly, none of the cytogenetic biomarkers identified for patients with Group 3 tumors (eg, iso17q) had any prognostic value for those with Group 4 tumors (Data Supplement 1). After unbiased model selection, the consensus set of biomarkers resulted in a risk stratification scheme in which leptomeningeal dissemination identified high-risk patients with Group 4 tumors, except in the context of chr11 loss or chr17 gain (Figs 5A and 5B). The biology underlying chr11 loss was not apparent, because there was no obvious minimal common region of deletion (Data Supplement 1), nor were there any recurrent single-nucleotide variants on chr11 reported. Patients with Group 4 tumors with either chr17 gain or chr11 loss, irrespective of metastatic status, exhibited excellent survivorship in both the discovery and validation cohorts (Figs 5B and 5D), and the survival differences were not explainable by covariates (Data Supplement 1). Consistent with other subgroups, the risk stratification model using both clinical and molecular biomarkers achieved the highest accuracy (Fig 5C). Critically, the cytogenetic biomarkers identified low-risk patients with Group 4 tumors who would be otherwise designated as high risk by evidence of metastasis and/or anaplastic histology; this finding could not be extrapolated to patients with SHH or Group 3 medulloblastoma (Figs 5A to 5C; Data Supplement 1). We conclude that through the use of three molecular biomarkers (chr11, 17p, and 17q FISH) and metastatic status, we can reliably predict the prognosis of patients with Group 4 medulloblastoma.

DISCUSSION

Current consensus identifies the existence of four major subgroups of medulloblastoma, with excellent prognosis for those with WNT tumors, intermediate prognosis for those with SHH and Group 4 tumors, and poor prognosis for those with Group 3 tumors.[32,52] However, early evidence suggests clinical heterogeneity within these core subgroups.[7,30,63] Practical and reliable prognostication of risk could allow for therapy intensification in high-risk children to improve survival and de-escalation of therapy in low-risk children so as to avoid the significant complications of therapy. However, the majority of published medulloblastoma biomarker studies included only small cohorts of patients, were not validated on nonoverlapping cohorts, and were performed in the presubgrouping era. Our prognostic study of 1,123 medulloblastomas, using techniques (eg, FISH) compatible with FFPE tissues, has identified clinically applicable risk stratification for SHH, Group 3, and Group 4 medulloblastomas.

We have demonstrated that medulloblastoma subgroup affiliation is significantly more informative for predicting patient outcome than existing clinical variables and that by incorporating subgroup status with conventional clinical parameters for risk stratification, the accuracy of survival prediction can be dramatically improved. Moreover, we have proposed, tested, and validated novel subgroup-specific risk stratification models incorporating both clinical and molecular variables. These models performed robustly both in the discovery cohort consisting of heterogeneously treated groups of patients and in a nonoverlapping validation cohort of patients treated at a single institution according to standardized treatment protocols. Because we do not have detailed treatment information for patients in the discovery cohort, it is possible that treatment protocols (type, duration, or intensity) could have affected our results. We suggest that this possibility can only be eliminated through examination of our stratification model in a sufficiently large prospective cohort. Although our study used single-nucleotide polymorphism arrays or interphase FISH on FFPE sections, it is possible that other approaches such as array comparative genomic hybridization could also be used to determine the copy-number status of the six markers.[64] Through the incorporation of current clinical variables, subgroup affiliation, and our six copy-number prognostic markers, as detailed in Data Supplement 1, rapid prognostication is feasible in the setting of a regular hospital neuropathology laboratory, making it a clinically utile technique. Because both subgrouping assays and prognostic FISH markers will need to be performed in a Clinical Laboratory Improvement Amendments–approved laboratory, we suggest that these assays be adopted and optimized in most major neuro-oncology centers, whereas smaller centers may consider sending tissues for analysis at larger centers. Our findings demonstrate the utility of incorporating tumor biology into clinical decision making and offer a novel perspective on risk stratification using FISH applicable on paraffin sections; thus, they could be translated immediately into routine clinical practice.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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Manuscript writing: All authors
Final approval of manuscript: All authors

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