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## Cytogenetic Prognostication Within Medulloblastoma Subgroups

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### A B S T R A C T

#### 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.<sup>1-3</sup> Unfortunately, most survivors are left with long-term disabilities secondary to the disease and treatment.<sup>4-6</sup> 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.<sup>2,7-14</sup> Mutations of specific genes such as *CTNNB* and *TP53* have shown prognostic significance.<sup>15-19</sup> Additional candidates include medulloblastoma-overexpressed genes such as *TRKC*, *ERBB2*, and *FSTL5*.<sup>20-25</sup> Several DNA copy-number aberrations have also been purported as biomarkers, although the results have often been conflicting.<sup>15,26-48</sup> 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.<sup>2,28,32,49-60</sup> According to international consensus, the principle subgroups of medulloblastoma are WNT, SHH, Group 3, and Group 4.<sup>52</sup> 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 sub-

group 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

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

NOTE. Bold font indicates significance; — indicates event not observed at sufficient frequency ( $n \leq 1$ ).

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.<sup>28</sup> An independent validation set of 453 samples with clinical follow-up on a TMA was analyzed using FISH as previously described.<sup>53</sup> Tumors were classified based on signature marker expression into molecular subgroups as previously described<sup>61</sup>; 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  $\beta$ -catenin mutation analysis were performed as previously described.<sup>28</sup>

### Prognostic Biomarker Identification

Cytogenetic events and CNAs were identified as previously described in the discovery set.<sup>28</sup> 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.

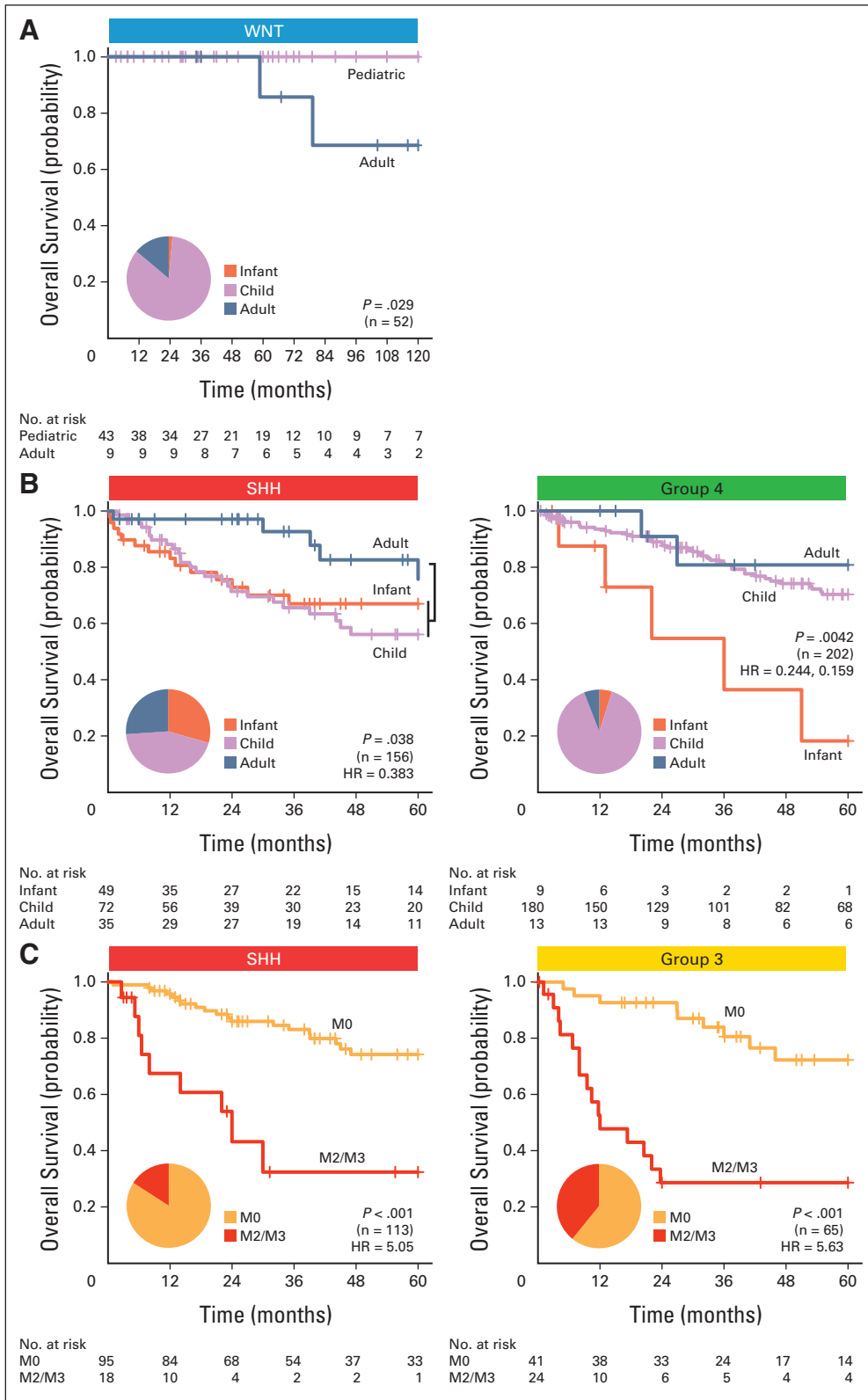
Metastatic status was not prognostic for patients with WNT tumors; however, macroscopic metastasis (M2/M3) was consistently associated with poor survival in all non-WNT subgroups, although the clinical effect was modest among patients with Group 4 disease (Fig 1C; Appendix Fig A1C, online only). Although 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

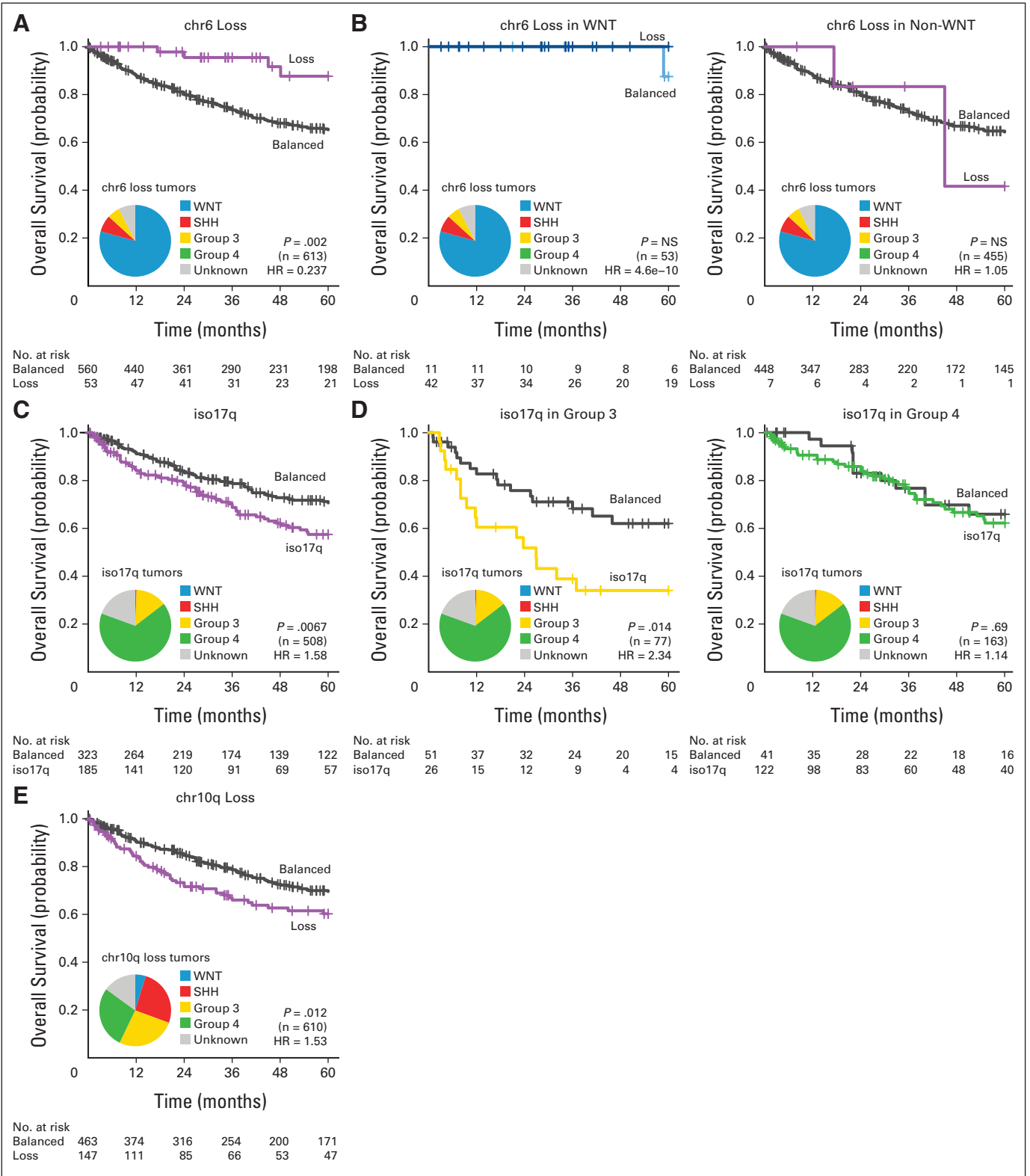
Several cytogenetic biomarkers have been associated with patient survival across medulloblastoma, but their prognostic value has seldom been assessed in the context of medulloblastoma subgroups (Table 1). Monosomy for chromosome (chr) 6 is associated with improved survival across medulloblastoma in toto (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  $\geq$  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.

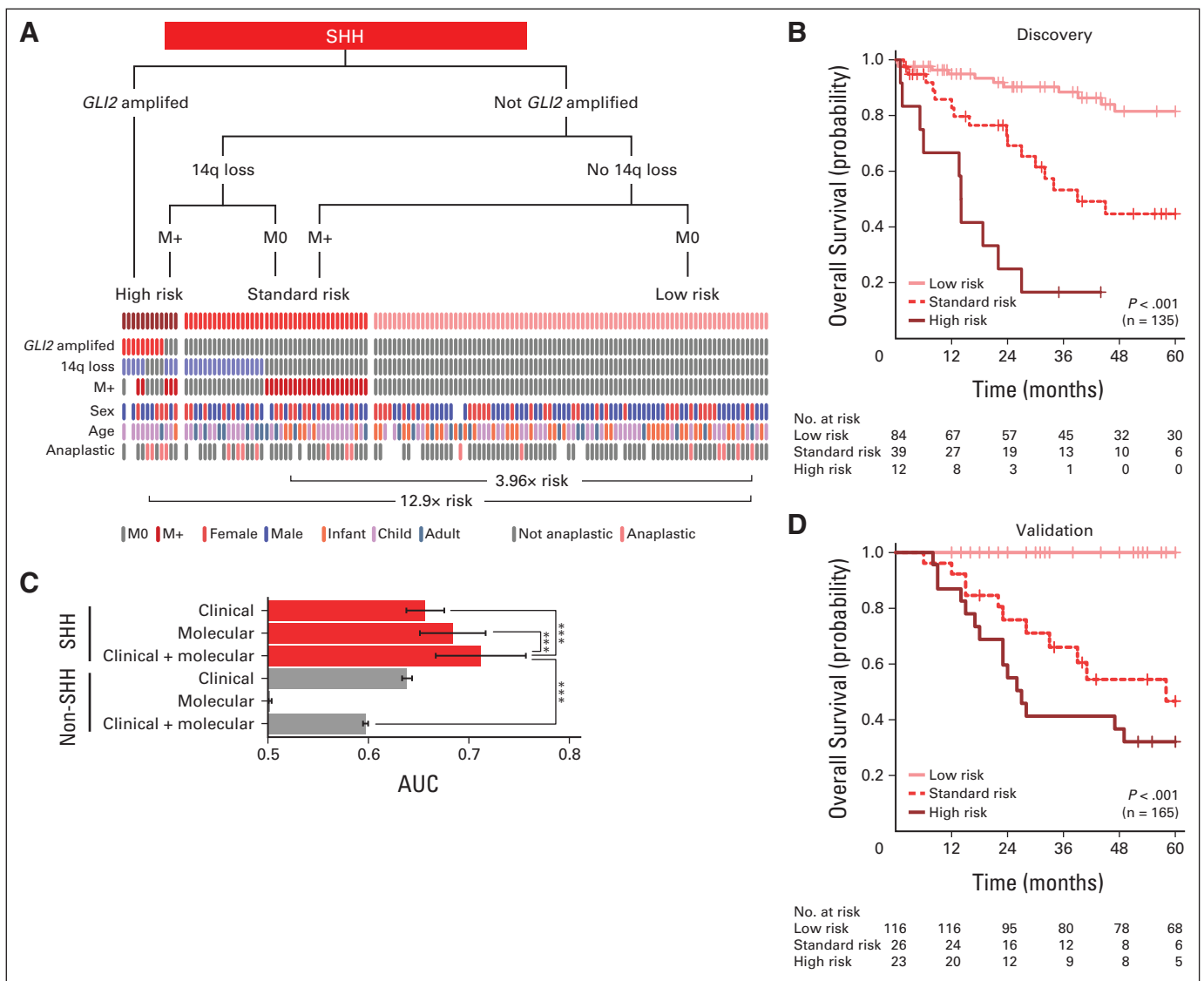


modestly significant predictor of poor outcome across medulloblastomas (Fig 2E), its prognostic power was limited to SHH tumors in a subgroup-specific analysis (Appendix Figs A2A and A2B, online only). We conclude that determination of subgroup affiliation is crucial in the evaluation and implementation 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.<sup>28,54,56,57,62</sup> The presence of chromothripsis (ie, chromosome shattering) was associated with worse survival in those with SHH tumors (Appendix Fig A3D, online only).<sup>17</sup>

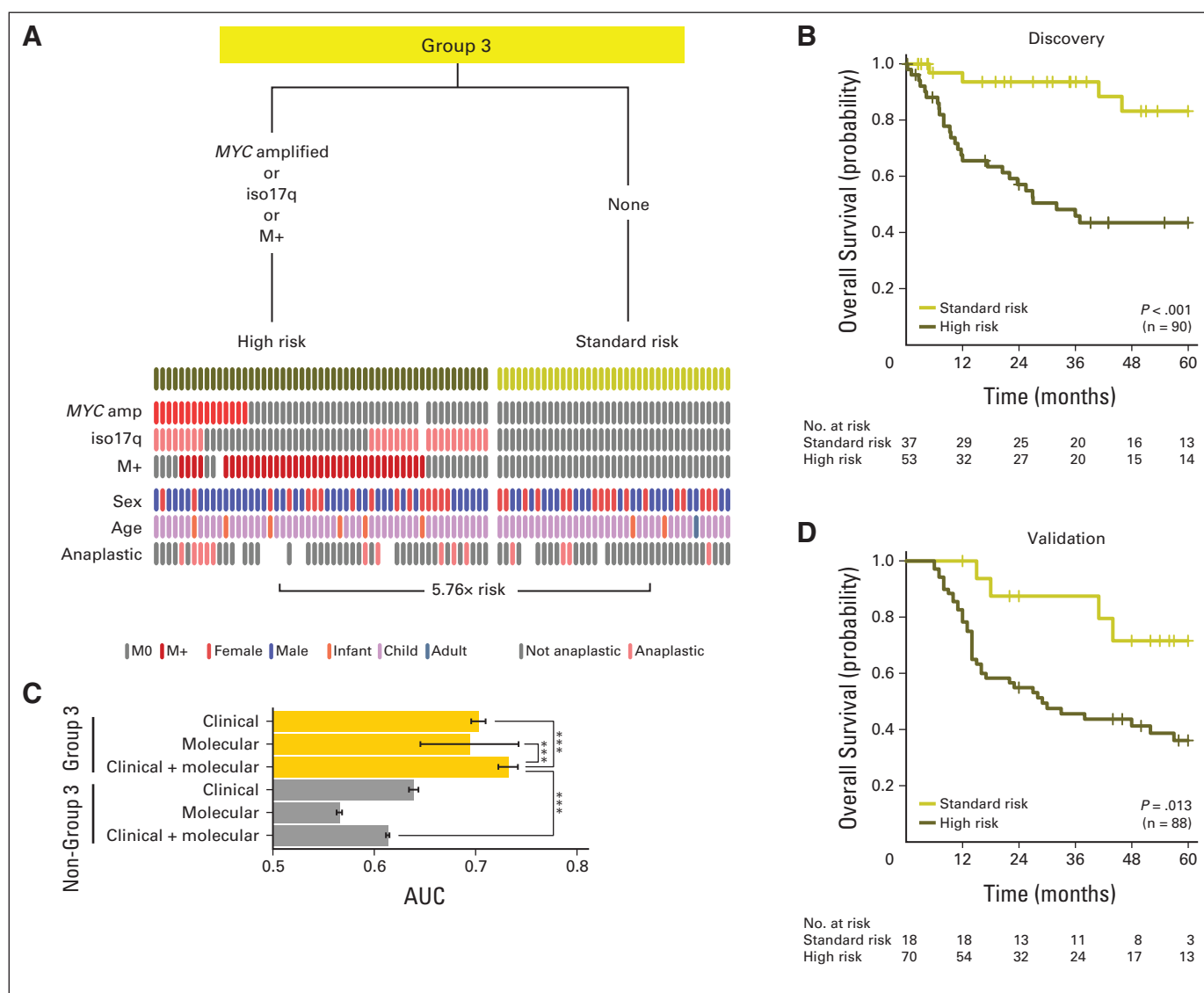


**Fig 3.** Clinical prognostication of patients with SHH medulloblastoma. (A) Risk stratification of SHH medulloblastomas by molecular and clinical prognostic markers. Decision tree, with events plot depicting status of molecular and clinical markers across risk groups below. (B) Overall survival curves 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.



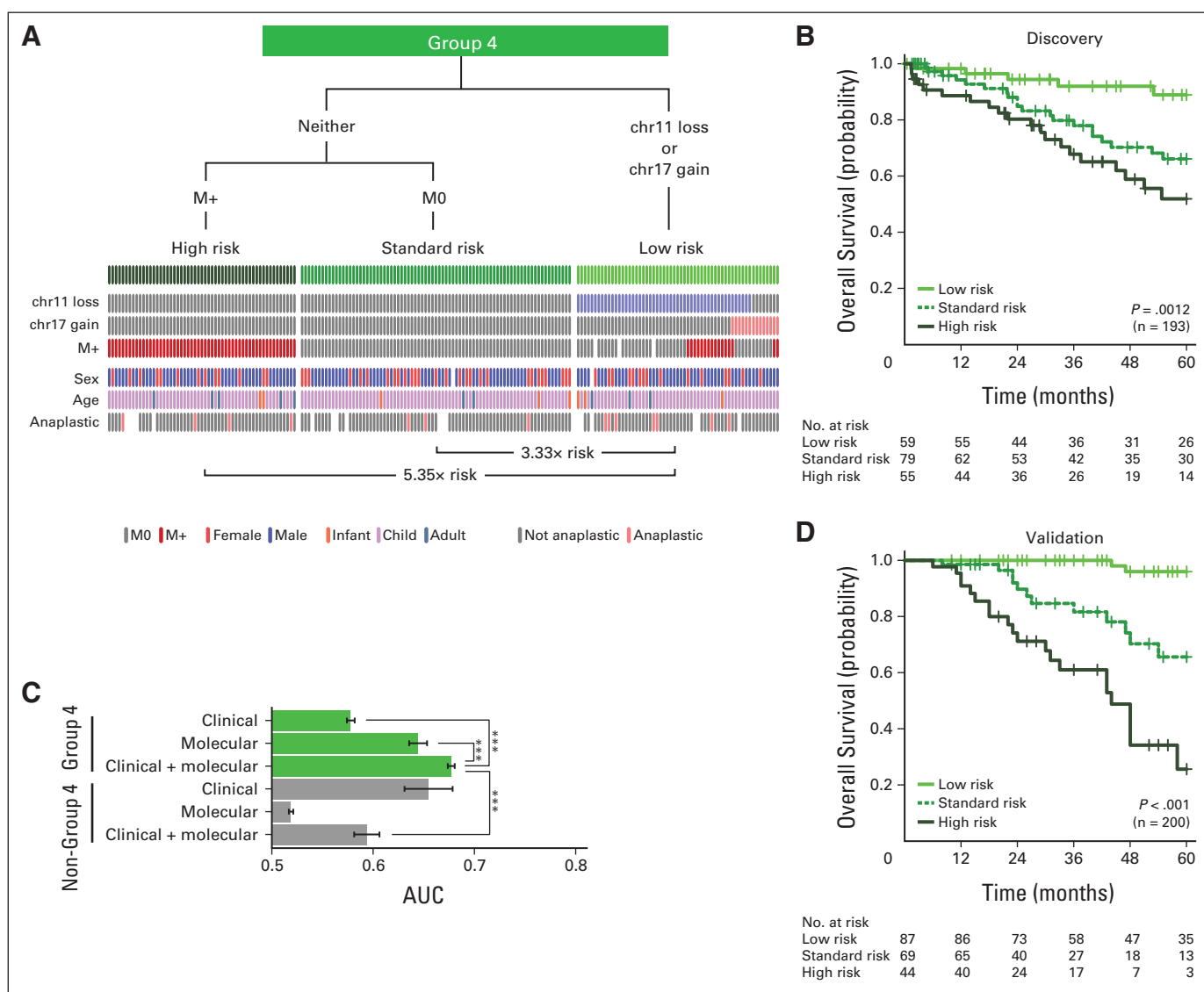
**Metastatic Status, Iso17q, and MYC Amplification Identify High-Risk Patients With Group 3 Medulloblastoma**

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.<sup>32,52</sup> However, early evidence suggests clinical heterogeneity within these core subgroups.<sup>7,30,63</sup> 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. More-

over, 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.<sup>64</sup> 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.

## AUTHOR CONTRIBUTIONS

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**Collection and assembly of data:** All authors

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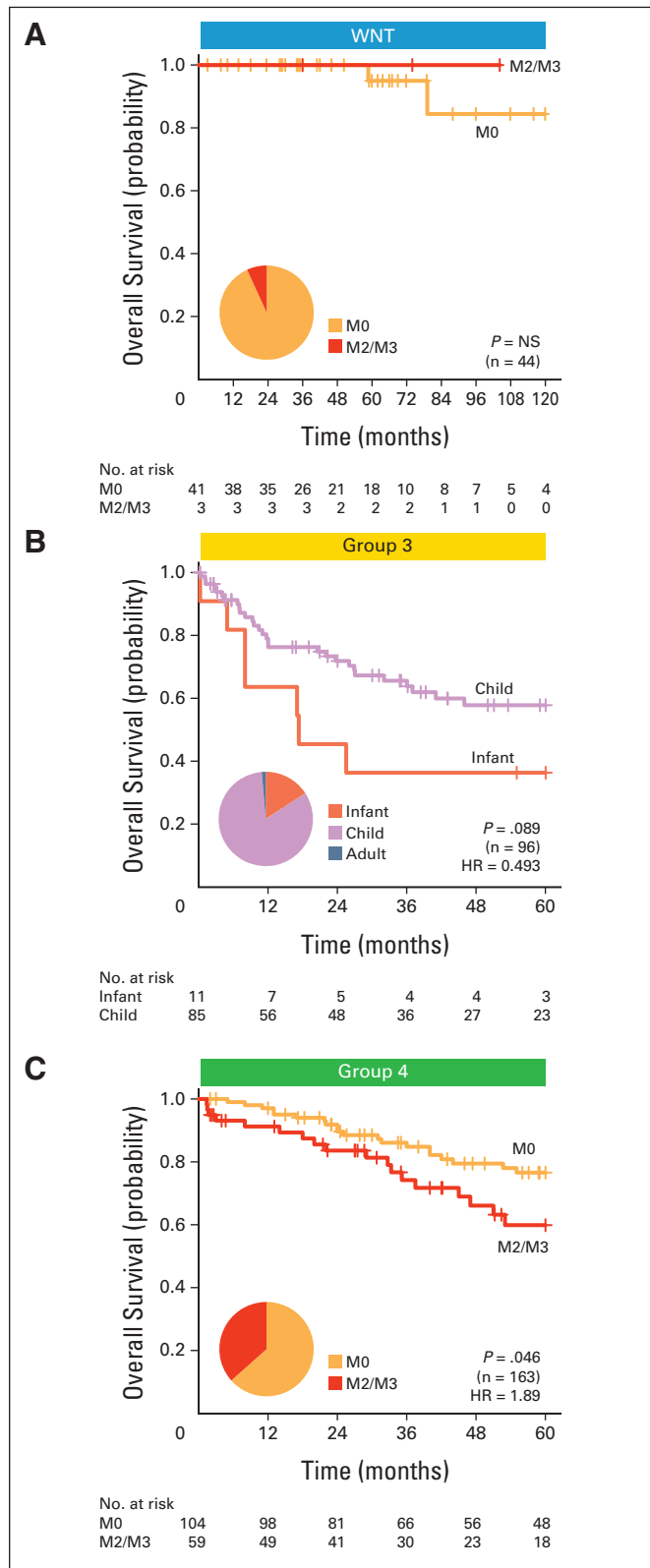
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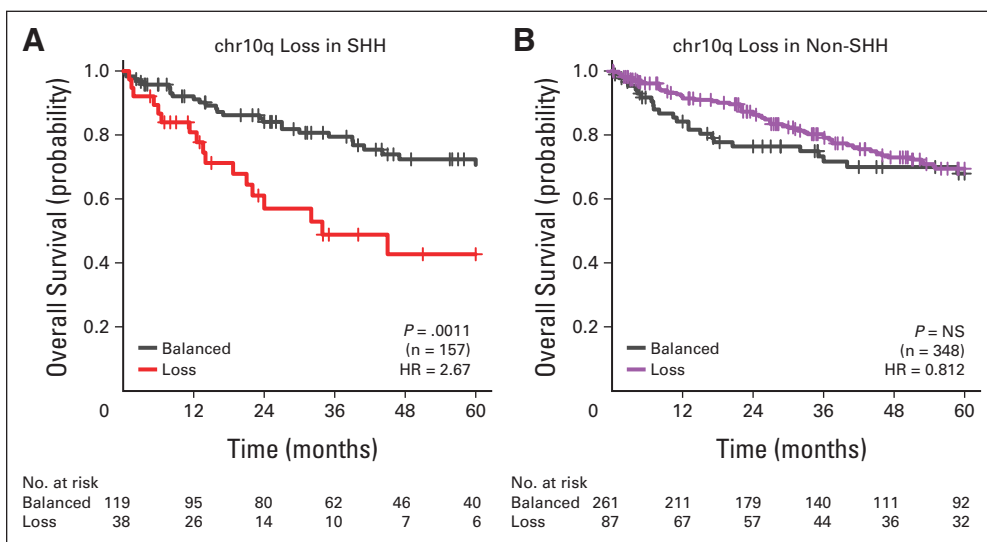


Appendix

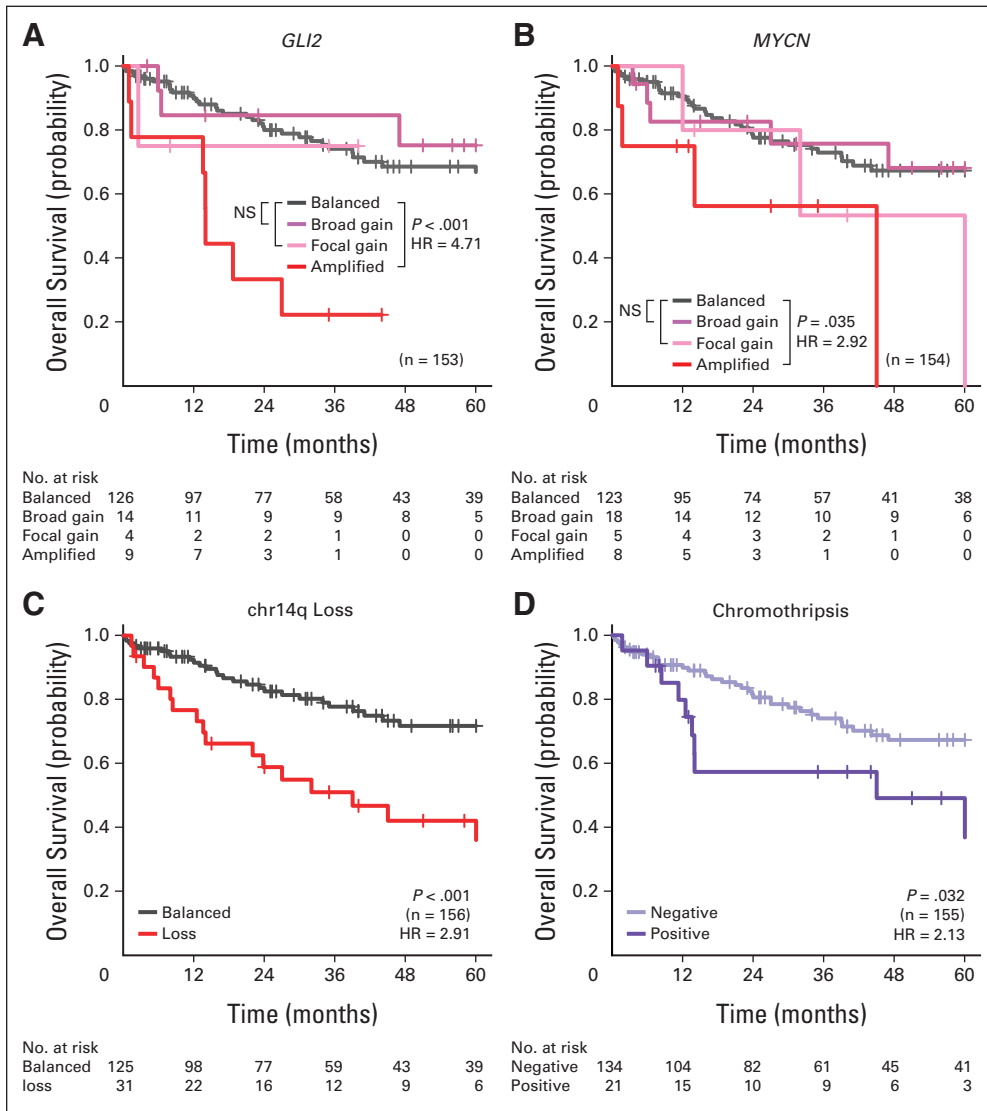


**Fig A1.** (A) Ten-year overall survival curves for WNT medulloblastoma by metastatic status. (B) Overall survival curves for age groups within Group 3 subgroup (infant, age < 3 years; child, age 3 to < 16 years). (C) Overall survival curves for metastatic status for Group 4 subgroup. Numbers below x-axis represent patients at risk of event; statistical significance evaluated by log-rank tests; hazard ratio (HR) estimates derived from Cox proportional hazards analyses. NS, not significant.

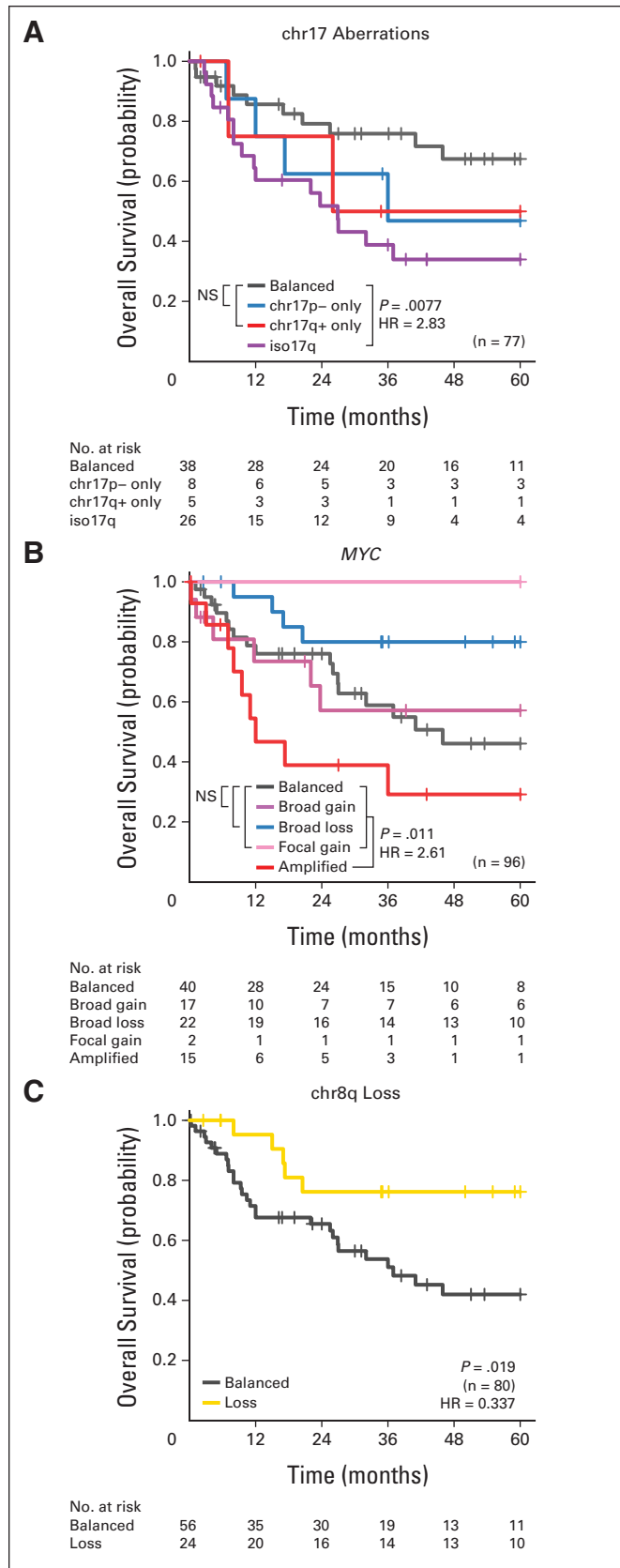




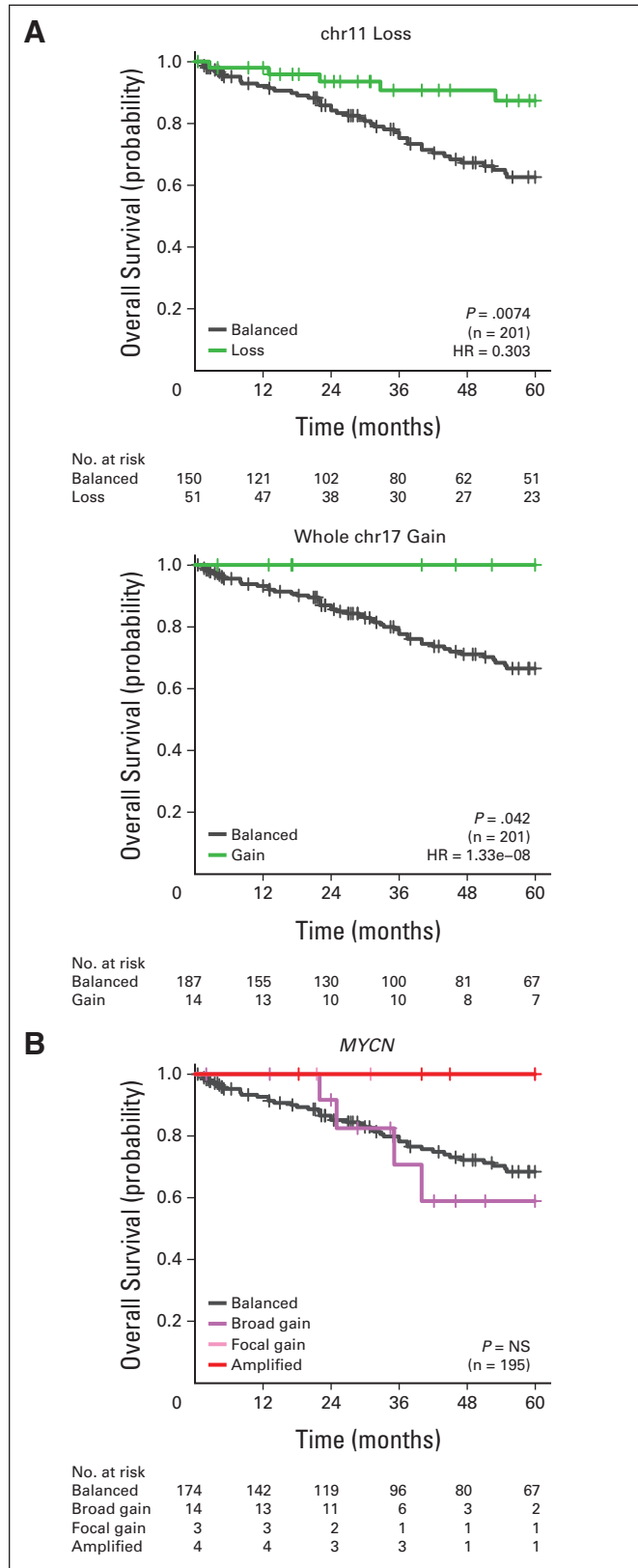
**Fig A2.** Overall survival curves for chromosome 10q (chr10q) status in (A) SHH and (B) non-SHH medulloblastomas; survival differences evaluated by log-rank tests; hazard ratio (HR) estimates derived from Cox proportional hazards analyses. NS, not significant.



**Fig A3.** Clinical prognostication of patients with SHH medulloblastoma. Overall survival curves for (A) *GLI2* copy-number status, (B) *MYCN* copy-number status, (C) chromosome 14q (chr14q) status, and (D) chromothripsis status. HR, hazard ratio; NS, not significant.



**Fig A4.** Clinical prognostication of patients with Group 3 medulloblastoma. Overall survival curves for (A) chromosome 17 (chr17) copy-number aberrations, (B) MYC copy-number status, and (C) chr8q status. HR, hazard ratio; iso, isochromosome; NS, not significant.



**Fig A5.** Clinical prognostication of patients with Group 4 medulloblastoma. Overall survival curves for (A) chromosome 11 (chr11) status and whole chr17 status and (B) *MYCN* copy-number status. HR, hazard ratio; NS, not significant.