Subgroup-Specific Prognostic Implications of TP53 Mutation in Medulloblastoma

Nataliya Zhukova, University of Toronto
Vijay Ramaswamy, University of Toronto
Marc Remke, University of Toronto
Elke Pfaff, University Hospital, Heidelberg
David J.H. Shih, University of Toronto
Dianna C. Martin, University of Toronto
Pedro Castelo-Branco, University of Toronto
Berivan Baskin, University of Toronto
Peter N. Ray, University of Toronto
Eric Bouffet, University of Toronto

Only first 10 authors above; see publication for full author list.

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ABSTRACT

Purpose
Reports detailing the prognostic impact of TP53 mutations in medulloblastoma offer conflicting conclusions. We resolve this issue through the inclusion of molecular subgroup profiles.

Patients and Methods
We determined subgroup affiliation, TP53 mutation status, and clinical outcome in a discovery cohort of 397 medulloblastomas. We subsequently validated our results on an independent cohort of 156 medulloblastomas.

Results
TP53 mutations are enriched in wingless (WNT; 16%) and sonic hedgehog (SHH; 21%) medulloblastomas and are virtually absent in subgroups 3 and 4 tumors (P < .001). Patients with SHH/TP53 mutant tumors are almost exclusively between ages 5 and 18 years, dramatically different from the general SHH distribution (P < .001). Children with SHH/TP53 mutant tumors harbor 56% germline TP53 mutations, which are not observed in children with WNT/TP53 mutant tumors. Five-year overall survival (OS; ± SE) was 41% ± 9% and 81% ± 5% for patients with SHH medulloblastomas with and without TP53 mutations, respectively (P < .001). Furthermore, TP53 mutations accounted for 72% of deaths in children older than 5 years with SHH medulloblastomas. In contrast, 5-year OS rates were 90% ± 9% and 97% ± 3% for patients with WNT tumors with and without TP53 mutations (P = .21). Multivariate analysis revealed that TP53 status was the most important risk factor for SHH medulloblastoma. Survival rates in the validation cohort mimicked the discovery results, revealing that poor survival of TP53 mutations is restricted to patients with SHH medulloblastomas (P = .012) and not WNT tumors.

Conclusion
Subgroup-specific analysis reconciles prior conflicting publications and confirms that TP53 mutations are enriched among SHH medulloblastomas, in which they portend poor outcome and account for a large proportion of treatment failures in these patients.

INTRODUCTION

Medulloblastoma is a malignant small-cell embryonal neoplasm of the cerebellum. It is the most common malignant brain tumor of childhood and remains a major cause of morbidity and mortality in this age group. Currently, medulloblastomas are mostly stratified based on histologic and clinical and radiologic criteria, which include age of onset, metastatic spread, and residual tumor after surgery. Stratification and management according to clinical risk criteria and development of large cooperative clinical trials resulted in improved survival for these individuals. However, aggressive multimodal treatment protocols carry high morbidity; thus differentiation between patients with favorable and poor outcomes would be highly desirable. The difficulty to predict tumor recurrence based on clinical...
criteria only has resulted in recent extensive efforts using integrative genomics to allow for genetic and molecular stratification of the disease. As of today, none of these molecular markers are routinely used in the clinic or as part of clinical trials. We have reported previously, using the Toronto cohort, that survival is dismal for children with medulloblastoma harboring TP53 mutations. Subsequently, a similar analysis of patients from the Heidelberg cohort did not confirm the unfavorable prognosis of TP53-mutated tumors. Moreover, additional reports from English and German cohorts revealed uncertainty with regard to the role of TP53 alterations in risk stratification of medulloblastoma. Recently, several groups were able to demonstrate a unique association of certain genetic alterations in medulloblastoma with medulloblastoma harboring TP53 mutations, we hypothesized that this unique association might explain both the controversy regarding the prognostic role of TP53 mutations in medulloblastoma and the heterogeneous outcome of patients with SHH medulloblastomas. We therefore compiled both clinical and molecular data from multiple centers to generate a large discovery cohort of patient and tumors with known TP53 status. We then performed subgroup combined with outcome analysis for the first time (Appendix Table A1, online only).

We sequenced and characterized a second, large independent validation cohort from the Medulloblastoma Advanced Genomics International Consortium (MAGIC), for which high-quality clinical data and sufficient tissue were available, thereby allowing us to determine the association of TP53 mutations, molecular groups, and survival in medulloblastoma.

**Patients**

We assembled the clinical and biologic data of patients included in our previous studies for which subgroups have now been assigned, along with additional new samples from all centers (n = 397, Table 1). These included tumors and patient data from the Hospital for Sick Children (HSC) in Toronto, Canada, the Heidelberg database (DKFZ), and the British (Newcastle) and German cohorts. All patient samples were procured in accordance with the research ethics board of their corresponding institution. For 373 patients (92%), clinical and survival data were available for analysis. An independent validation cohort of SHH and WNT tumors with available outcome data and sufficient DNA for sequencing (n = 156) was obtained through MAGIC. Further clinical and demographic data from both cohorts are detailed in Table 1. For progression and OS analysis, time was defined as months from initial diagnosis.

Samples from all centers were either obtained as frozen tissue or formalin-fixed paraffin-embedded biopsies, and nucleic acids from the frozen tissue were extracted as previously described. Nucleic acids were extracted from frozen and formalin-fixed paraffin-embedded samples as previously described.

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**Table 1. Patient Characteristics by TP53 Mutational Status**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Discovery Cohort</th>
<th></th>
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</thead>
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<td>TP53 Mutant</td>
<td>TP53 Wild Type</td>
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<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
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<td>1.8-33</td>
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<td>16.8</td>
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<tr>
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<td>4</td>
<td>9.8</td>
<td>60</td>
<td>16.9</td>
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<tr>
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<td>213</td>
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<tr>
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<tr>
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</table>

NOTE. Histology was available for 389 cases in the discovery and 130 cases in the validation cohorts. Metastatic status at diagnosis was available in 269 cases in the discovery and 135 cases in the validation cohorts. Abbreviations: LCA, large cell/anaplastic; NA, not applicable; SHH, sonic hedgehog; WNT, wingless.
**TP53 Mutations in Medulloblastoma Subgroups**

**Subgroup Analysis**

Molecular subgrouping was performed on the HSC screening and the MAGIC validation cohort using a custom nanoString code set designed to assess the expression of 22 medulloblastoma subgroup-specific signature genes as previously described.23,24 Samples were processed at the University Health Network (UHN) Microarray Facility using an input of 100 ng of total RNA. Subgrouping of the DKFZ samples were determined by gene expression profiling or by immunohistochemistry and nanoString code set as previously described.8,21,22 Subgrouping of the Newcastle samples was determined by a limited gene expression signature as previously described.13

**TP53 Mutation Analysis**

TP53 sequencing for the HSC and validation cohorts was performed on the entire coding sequence (exons 2 through 11) with primers and methodology as previously described.8,21,22 TP53 sequencing for the Heidelberg and the Newcastle cohort was performed as previously described.6,9 For patients with TP53 mutant tumors when blood DNA was available, germline mutation status was assessed to determine the diagnosis of Li-Fraumeni syndrome (LFS).25

**Statistical Analysis**

Statistical analysis was performed in the R statistical environment (v2.15). Univariate survival analysis was performed using the log-rank test as implemented in the survival R package (v2.36). Multivariate Cox proportional hazards regression was used to adjust for additional covariates using the survival R package (v2.36). In all cases, P < .05 was considered significant. Stabilities of prognostic markers were assessed by bootstrap resampling as previously described,26 using the Akaike information criterion for variable selection by backward elimination in 1,000 bootstrap replicates. For correlative studies, the Fisher’s exact test was used.

**RESULTS**

**Characteristics of TP53 Mutated Medulloblastomas**

In the discovery cohort, we identified TP53 mutations in 41 (10%) of 397 medulloblastomas. Median age at diagnosis for patients with TP53 mutant tumors was 11.7 years (range, 1.1 to 45 years). The male to female ratio was 1:1.2. Only 10.5% of TP53 mutant tumors were metastatic at diagnosis (Table 1). Diffuse anaplasia was observed in 42.5% of mutant tumors. Only one tumor had a concomitant MYCC amplification, whereas eight tumors demonstrated simultaneous MYCN amplification. Interestingly, of these nine patients, six are alive with a mean follow-up of 4.5 years. At a median follow-up of 49 months (range, 3 to 226 months), 5-year OS for all patients in the discovery cohort was 77% ± 6% and 55% ± 8% for TP53 wild-type and mutant medulloblastomas, respectively (P < .001; Fig 1A).

**TP53 Mutational Pattern by Subgroup**

TP53 mutations were found predominantly in the SHH and WNT groups. Specifically, TP53 mutations were observed in 11 (16%) of 66 WNT and 28 (21%) of 133 SHH tumors. In contrast, TP53 mutations were observed in only one of 122 group 4 and in 0 of 72 group 3 tumors (Fig 2A; P < .001). The age distribution observed in patients with SHH/TP53 mutant medulloblastoma revealed a Gaussian curve peaking at approximately age 15 years, whereas a bimodal age distribution was observed in SHH/TP53 wild-type tumors (Fig 2B). Most of the patients with SHH/TP53 mutant tumors (25 of 28) were between the ages of 5 and 18 years, which differs dramatically from SHH/TP53 wild-type ones (31 of 105; P < .001). None of the WNT/TP53 mutant tumors demonstrated anaplastic features, whereas 68% of SHH/TP53 mutant tumors had severe anaplasia (P < .001). Similarly, none of the WNT/TP53 mutant tumors harbored MYC or MYCN amplifications, whereas 33% of SHH/TP53 mutant tumors exhibited these genetic alterations (P = .05). TP53 mutations were observed in the DNA-binding domain (exons 4 through 8; Fig 2C). The most common mutations were in codons 248 and 175, which are the most commonly mutated residues in both somatic and germline TP53 mutation databases (International Agency for Research on Cancer).264 Of the patients for whom TP53 germline status was available (n = 20), nine patients (45%) harbored germline mutations consistent with LFS. All these individuals had SHH tumors. Although 56% of SHH/TP53 mutant tumors had a concomitant germline mutation, germline mutations were not seen in WNT/TP53 mutant tumors.

**TP53 Mutations and Survival by Subgroup**

A striking association between biologic subgroups and survival for patients with TP53 mutant tumors was observed. Specifically, patients with SHH/TP53 mutant tumors had 5-year OS of 41% ± 19%, whereas patients with WNT/TP53 mutant tumors had 5-year OS of 90% ± 9% (P = .018; Fig 1B). Five-year OS was 41% ± 9% and 81% ± 5% for patients with SHH tumors with and without TP53 mutations (P < .001; Fig 1C). However, for children between the ages of 5 and 18 years with SHH tumors, TP53 mutations accounted for 72% of deaths. Within the limitations of a small cohort, children with confirmed LFS had survival similar to that of the remaining patients with TP53 mutant SHH medulloblastomas (Appendix Fig A1, online only). In contrast, 5-year OS was 90% ± 9% and 97% ± 3% for patients with WNT tumors with and without TP53 mutations (P = .21; Fig 1D). A multivariate Cox proportional hazards regression model of 5-year survival for SHH tumors accounting for age, sex, histology, presence of metastases, and TP53 status demonstrated that TP53 mutation status is the single most important independent risk factor for this group (Table 2).

**Validation of Survival Analysis for SHH and WNT Tumors**

To validate the survival difference between WNT and SHH TP53 mutant medulloblastomas observed in our new assembled cohort, we performed additional Sanger sequencing of TP53 in a separate cohort of 156 patients with medulloblastoma from the SHH and WNT groups with adequate clinical data, who were available through MAGIC. The rate of TP53 mutations in this cohort was similar to the rate seen by our group. Most patients (81%) had nonmetastatic tumors, and the male to female and age distributions were similar to those of our initial cohort. Diffuse anaplasia was reported in 54% of SHH/TP53 mutant tumors and 12% of SHH/TP53 wild-type ones (P = .002). None of the WNT/TP53 mutant tumors were defined as anaplastic. The OS of this cohort mimicked the observed findings in our initial cohort (Figs 2E and 2F). At a median follow-up of 42 months (range, 2 to 300 months), 5-year OS for patients with SHH tumors with and without TP53 mutations was 41% ± 17% and 76% ± 4%, respectively (P = .012); in contrast, 5-year OS for patients with WNT tumors with and without TP53 mutations was 86% ± 13% and 94% ± 5%, respectively (P = .41).

**Additional Data From All Patients in the Study**

Summarizing all data from all patients from whom all clinical and molecular data were available enabled us to further clarify several observations. Combining all patients with SHH medulloblastomas...
Fig 1. Kaplan-Meier estimates of overall survival for study patients by group. (A) All discovery cohort patients by TP53 mutations. Blue line, TP53 wild type, wingless (WNT) group; gold line, TP53 mutant, sonic hedgehog (SHH) group. (B) TP53-mutant tumors stratified by subgroup analysis. Blue line, WNT group; gold line, SHH group. (C) Blue line, TP53 wild type, WNT group; gold line, TP53 mutant, SHH group. (D) WNT tumors from the discovery cohort. Blue line, TP53 wild type, WNT group; gold line, TP53 mutant, SHH group. (E) SHH tumors from the validation cohort. Blue line, TP53 wild type, WNT group; gold line, TP53 mutant, SHH group. (F) WNT tumors from the validation cohort. Blue line, TP53 wild type, WNT group; gold line, TP53 mutant, SHH group.
between the ages of 5 and 18 years (where radiation is usually given to all children, n = 84) revealed that tumors carrying TP53 mutations were associated with 21 (72%) of 29 deaths (P < .001; Appendix Fig A2, online only). The combined cohorts included 42 adults, of whom six (14%) had TP53 mutant tumors. Five of these patients died of their disease. Interestingly, two patients had WNT tumors, and none survived.

**DISCUSSION**

The genetic and genomic understanding of medulloblastoma has evolved dramatically in the past few years. International collaborations have resulted in novel classification of this brain tumor and the potential for targeted therapies for patients within specific subgroups.
Nevertheless, except for patients with WNT tumors, for whom outcome is superior to all other subgroups, survival of children with SHH, group 3, and group 4 tumors is still unsatisfactory.17

In this study, we demonstrate that characterization of TP53 mutation status can segregate individuals with SHH medulloblastoma into favorable and extremely poor survival groups. Specifically, patients with SHH/TP53 mutant medulloblastomas have profoundly worse outcome than those with SHH/TP53 wild-type tumors. The importance of this observation is further highlighted by the fact that most patients with TP53 mutant medulloblastomas have average-risk tumors, as measured by conventional nonmolecular methods, for which survival is expected to be excellent with current protocols.1,2 To validate our observations from the discovery cohort, we examined a separately ascertained cohort that included a large number of children and adults with SHH and WNT tumors. The impressive similarity in outcomes between the two cohorts argues against an unobserved variable confounding the results of our discovery cohort. Moreover, large whole-genome and exome sequencing efforts recently published by separate groups revealed an additional, albeit small number, of TP53 mutations in medulloblastoma.27-29 Interestingly, these independent groups found TP53 mutations enriched in the SHH group and associated with poor survival. In fact, of 14 SHH tumors studied by Robinson et al,29 deaths were only reported in the three TP53 mutant tumors. The observation that a significant number of deaths in the SHH cohort were associated with TP53 mutations in both our and other cohorts suggests that somatic TP53 mutations analysis should be performed on patients with SHH medulloblastomas (Fig 3).

Our study also highlights how genomic and molecular stratification could explain differences in clinical and biologic behavior of cancers with a specific genetic alteration. Indeed, although patients with SHH TP53 mutated tumors fared miserably, this aggressive genotype was completely modified in WNT tumors. Our group has recently shown that WNT activation through constitutive β-catenin activation can abrogate the radioresistance conferred by TP53-mutated medulloblastomas as a potential explanation for this difference in outcome.30 The separation of SHH subgroup patients by TP53 status would significantly improve risk stratification of this subgroup, analogous to the role of FSTL5 in stratifying group 3 and 4 patients.22

Indeed, our multivariate modeling would suggest that a simple model

<table>
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<th>Hazard Ratio</th>
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<td>.002</td>
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</table>

Abbreviations: BIF, bootstrap inclusion frequency; LCA, large cell/anaplastic; SHH, sonic hedgehog.
of subgroup along with TP53 mutation status is highly predictive of outcome.\textsuperscript{31}

SHH/TP53 mutant medulloblastomas have several biologic characteristics that can explain their clinical behavior. These tumors reveal a high rate of MYCN amplification. Furthermore, our group was recently able to demonstrate that these cancers have a high rate of single chromosomal shattering, or chromothripsis.\textsuperscript{15} This catastrophic event is associated with poor survival and a high degree of genomic instability in other cancers\textsuperscript{31-33} and therefore offers a plausible explanation for the poor survival of these patients.

The primary aim of this study was to assess somatic TP53 alterations in medulloblastoma. However, our germline data from a subgroup of these patients demonstrate a high rate of germline TP53 mutations. These findings have important implications to both the patient and other family members in terms of their subsequent cancer risk. Individuals with LFS, which is a devastating cancer predisposition syndrome, were recently shown to derive benefit from a clinical surveillance protocol.\textsuperscript{34} Therefore, genetic counseling should be offered to all patients and families with SHH TP53 mutant medulloblastomas.

Although our WNT TP53 mutant tumors had a poor outcome among adults, the data are based on a small number of tumors, albeit suggesting that adults might deserve a different approach than children regarding genetic and molecular stratification.\textsuperscript{35}

Although the effects of TP53 mutation were different in SHH and WNT subgroups, the interactions between TP53 mutation and subgroup (SHH + WNT) was not statistically significant in the discovery (\(P = .62\)) and validation cohorts (\(P = .70\)). Our data are consistent with the proposition that patients with mutant TP53 WNT tumors do not have poorer survival compared with their wild-type counterpart, although the alternative possibility cannot be ruled out because of the rarity of TP53 mutation in patients with WNT tumors. In this study and others,\textsuperscript{36} TP53 mutations were highly associated with anaplasia. In our study, anaplasia was seen in 19 (66\%) of 29 SHH TP53 mutant tumors and only in 14 (10\%) of 136 SHH TP53 wild-type ones (\(P < .001\)). This suggests that high index of suspicion for TP53 mutations should exist for SHH tumors with diffuse anaplasia.

Finally, we suggest a clinicopathologic approach to medulloblastoma based on molecular subgroups and TP53 status (Fig 3). In institutions where molecular group stratification is available, determination of molecular subgroup should be performed. Although some centers use RNA-based assays that may be difficult to reproduce by all institutions, efforts are being made to optimize antibody-based assays,\textsuperscript{14,22} which aim to define SHH tumors in a robust and simple way for most clinical laboratories.\textsuperscript{37} SHH subgroup tumors should be sent for TP53 sequencing by clinically approved laboratories. For most institutions where molecular subgroups are not available, determination of TP53 status should be performed in conjunction with nuclear \(\beta\)-catenin status. TP53 mutational analysis is preferably done by direct sequencing. However, p53 immunostaining is a very sensitive (albeit not specific) surrogate method for TP53 mutation detection in brain (and other) tumors and could be performed as an initial screening tool.\textsuperscript{2,38,39} In our previous cohort, all TP53 mutant tumors were immunopositive.\textsuperscript{7} In this cohort and in our larger pediatric brain tumor cohort, immunostaining is more than 90\% sensitive for detection of TP53 mutations, and false negatives are generally restricted to deletions and splice site mutations in which no or truncated protein is expressed.\textsuperscript{7,39,40} Tumors harboring TP53 mutations should be then sent for subgroup analysis for determination of SHH status. Because current treatment protocols for patients with average-risk medulloblastoma result in dismal survival for those with TP53 mutant SHH medulloblastomas, these patients should be considered for either enrollment in clinical trials of targeted therapy and/or other modified therapies. Furthermore, careful history of prior cancers in the family and genetic counseling should be offered to these individuals because of the high rate of LFS in this population. This practical approach can be also used in future clinical trials that are trying to stratify patients for reduced therapy.

In summary, this study serves as a proof of principle that collaborative efforts can resolve discrepancies between reports of an uncommon event in cancer. Specifically, this approach validated the prognostic role of TP53 mutations in medulloblastoma once molecular subgroup is taken into account. This study also adds another dimension to the evolving role of genomic and genetic approaches to cancer by demonstrating that SHH-driven medulloblastomas, which account for a third of medulloblastomas seen in children, should be stratified according to their TP53 status, whereas TP53 mutation status has little prognostic effect in the setting of a WNT medulloblastoma. Ongoing collaborations between centers will allow for elucidation of the role of TP53 alterations in adult medulloblastomas as well as the design of rational therapies for children with medulloblastoma in an era of precision medicine.

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

**AUTHOR CONTRIBUTIONS**

Conception and design: Nataliya Zhukova, Pedro Castelo-Branco, Eric Bouffet, Stefan Rutkowski, Cynthia Hawkins, Steven C. Cliffford, Andrey Korshunov, Stefan Pfister, Michael D. Taylor, Uri Tabori


Ellison, Cynthia Hawkins, David Malkin, Steven C. Clifford, Andrey Korshunov, Stefan Pfister, Michael D. Taylor, Uri Tabori


Manuscript writing: All authors

Final approval of manuscript: All authors

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Affiliations

Nataliya Zhukova, Vijay Ramaswamy, Marc Remke, David J.H. Shih, Dianna C. Martin, Pedro Castelo-Branco, Berivan Baskan, Peter N. Ray, Eric Bouffet, Cynthia Hawkins, David Malkin, Michael D. Taylor, and Uri Tabori, University of Toronto, Toronto; Boleslaw Lach, McMaster University, Hamilton, Ontario; Nada Jabado, McGill University, Montreal, Quebec, Canada; André O. von Bueren and Stefan Rutkowski, University Medical Center Hamburg-Eppendorf, Hamburg; André O. von Bueren, University Medical Center Goettingen, Goettingen; Elke Pfaff, David T.W. Jones, Paul A. Northcott, Marcel Kool, Dominik Sturm, Andrey Korshunov, and Stefan Pfister, University Hospital, Heidelberg; Torsten Pietsch and Marco Gessi, University of Bonn Medical Centre, Bonn; Ulrich Schüller, Ludwig-Maximilians University, Munich, Germany; Trevor J. Pugh, Broad Institute of MIT and Harvard, Cambridge; Scott L. Pomeroy, Boston Children’s Hospital and Harvard.
Medical School, Boston, MA; Yoon-Jae Cho, Stanford University, Stanford; Nalin Gupta and William A. Weiss, University of California San Francisco, San Francisco; Linda M. Liau, University of California, Los Angeles, CA; Laszlo Bogner and Almos Klekner, University of Debrecen, Debrecen; Peter Hauser, Semmelweis University, Budapest, Hungary; Byung-Kyu Cho, Seung-Ki Kim, Kyu-Chang Wang, Seoul National University Children’s Hospital; Young Shin Ra, University of Ulsan, Asan Medical Center, Seoul; Shin Jung, Université Chonnam National University Research Institute of Medical Sciences, Chonnam National University Hwasun Hospital and Medical School, Hwasun-gun, Chonnam, South Korea; Charles G. Eberhart, John Hopkins University School of Medicine, Baltimore, MD; Michelle Fevre-Montange, University of Lyon, Lyon; Anne Jouvet, Hospices Civils de Lyon, Bron, France; Maryam Fouladi, University of Cincinnati, Cincinnati, OH; Pim J. French and Max Kros, Erasmus Medical Center, Rotterdam, the Netherlands; Wieslawa A. Grajkowska, Children’s Memorial Health Institute, Warsaw, Poland; Toshihiro Kumabe, Tohoku University Graduate School of Medicine, Sendai, Japan; Jeffrey R. Leonard and Joshua B. Rubin, Washington University School of Medicine and St Louis Children’s Hospital, St Louis, MO; Luca Massimi, Catholic University Medical School, Rome, Italy; Ian F. Pollack, University of Pittsburgh School of Medicine, Pittsburgh, PA; Erwin G. Van Meir, Emory University, Atlanta, GA; Karel Zitterbart, University Hospital Brno, Masaryk University, Brno, Czech Republic; Rebecca M. Hill, Janet C. Lindsey, Ed C. Schwalbe, Simon Bailey, and Steven C. Clifford, Newcastle University, Newcastle upon Tyne, United Kingdom; and David W. Ellison, St Jude Children’s Research Hospital, Memphis, TN.

What Patients Need to Know About Managing the Cost of Care

ASCO’s Managing the Cost of Cancer Care booklet shares practical tips on financial planning before, during, and after treatment. Patients can learn about understanding the costs related to their care, find a list of questions to ask physicians about cost, and view a glossary of cost-related terms and a list of organizations offering help for people with cancer facing financial challenges. This booklet is also available in Spanish. Download the booklet at cancer.net/managingcostofcare or order free copies at asco.org/store.
Table A1. Patient Distribution According to Subgroups and TP53 Status (discovery cohort)

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<tr>
<th></th>
<th>Newcastle</th>
<th>DKFZ</th>
<th>Sick Kids</th>
<th>Boston</th>
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<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
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<tr>
<td>WNT Wild type</td>
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<td>65</td>
<td>35</td>
<td>11</td>
<td>7</td>
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<td>6</td>
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<tr>
<td>SHH Wild type</td>
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<td>5</td>
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<tr>
<td>Total</td>
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<td>100</td>
<td>314</td>
<td>100</td>
<td>60</td>
</tr>
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</table>

Abbreviations: DKFZ, Heidelberg database; SHH, sonic hedgehog; WNT, wingless.

Fig A1. Survival estimates for children with sonic hedgehog (SHH)/TP53-mutant tumors when germline TP53 status was available (n = 16).
Fig A2. Kaplan-Meier estimates of overall survival for children and infants by group. (A) Sonic hedgehog (SHH) tumors from the discovery cohort. (B) Wingless (WNT) tumors from the discovery cohort.