

## *BRAF* Mutation and *CDKN2A* Deletion Define a Clinically Distinct Subgroup of Childhood Secondary High-Grade Glioma

Matthew Mistry, Nataliya Zhukova, Daniele Merico, Patricia Rakopoulos, Rahul Krishnatry, Mary Shago, James Stavropoulos, Noa Alon, Jason D. Pole, Peter N. Ray, Vilma Navickiene, Joshua Mangere, Marc Remke, Pawel Buczkowicz, Vijay Ramaswamy, Ana Guerreiro Stucklin, Martin Li, Edwin J. Young, Cindy Zhang, Pedro Castelo-Branco, Doua Bakry, Suzanne Laughlin, Adam Shlien, Jennifer Chan, Keith L. Ligon, James T. Rutka, Peter B. Dirks, Michael D. Taylor, Mark Greenberg, David Malkin, Annie Huang, Eric Bouffet, Cynthia E. Hawkins, and Uri Tabori

See accompanying editorial doi: 10.1200/JCO.2014.60.1823

Author affiliations appear at the end of this article.

Published online ahead of print at [www.jco.org](http://www.jco.org) on February 9, 2015.

Supported by b.r.a.i.n.child Canada, the JPA Foundation, and the Canadian Institute of Health Research Grant No.

MOP-123268. M.M. received funding from The Hospital for Sick Children RESTRA-COMP fund and Alex's Lemonade Stand Foundation. R.K. was funded in part by the Society of Neuro-Oncology (International Research Development Fellowship Program). K.L.L. received support from National Institutes of Health Grant No. P01CA142536 and the Pediatric Low-Grade Astrocytoma Foundation.

C.E.H. and U.T. contributed equally to this article.

Terms in blue are defined in the glossary, found at the end of this article and online at [www.jco.org](http://www.jco.org).

Authors' disclosures of potential conflicts of interest are found in the article online at [www.jco.org](http://www.jco.org). Author contributions are found at the end of this article.

Corresponding author: Uri Tabori, MD, Staff Neuro-Oncologist, Division of Haematology/Oncology, The Hospital for Sick Children, The University of Toronto, 555 University Ave, Toronto, Ontario, Canada, M5G 1X8; e-mail: [uri.tabori@sickkids.ca](mailto:uri.tabori@sickkids.ca).

© 2015 by American Society of Clinical Oncology

0732-183X/15/3399-1/\$20.00

DOI: 10.1200/JCO.2014.58.3922

### ABSTRACT

#### Purpose

To uncover the genetic events leading to transformation of pediatric low-grade glioma (PLGG) to secondary high-grade glioma (sHGG).

#### Patients and Methods

We retrospectively identified patients with sHGG from a population-based cohort of 886 patients with PLGG with long clinical follow-up. Exome sequencing and array CGH were performed on available samples followed by detailed genetic analysis of the entire sHGG cohort. Clinical and outcome data of genetically distinct subgroups were obtained.

#### Results

sHGG was observed in 2.9% of PLGGs (26 of 886 patients). Patients with sHGG had a high frequency of nonsilent somatic mutations compared with patients with primary pediatric high-grade glioma (HGG; median, 25 mutations per exome;  $P = .0042$ ). Alterations in chromatin-modifying genes and telomere-maintenance pathways were commonly observed, whereas no sHGG harbored the *BRAF-KIAA1549* fusion. The most recurrent alterations were *BRAF*V600E and *CDKN2A* deletion in 39% and 57% of sHGGs, respectively. Importantly, all *BRAF*V600E and 80% of *CDKN2A* alterations could be traced back to their PLGG counterparts. *BRAF* V600E distinguished sHGG from primary HGG ( $P = .0023$ ), whereas *BRAF* and *CDKN2A* alterations were less commonly observed in PLGG that did not transform ( $P < .001$  and  $P < .001$  respectively). PLGGs with *BRAF* mutations had longer latency to transformation than wild-type PLGG (median, 6.65 years [range, 3.5 to 20.3 years] v 1.59 years [range, 0.32 to 15.9 years], respectively;  $P = .0389$ ). Furthermore, 5-year overall survival was  $75\% \pm 15\%$  and  $29\% \pm 12\%$  for children with *BRAF* mutant and wild-type tumors, respectively ( $P = .024$ ).

#### Conclusion

*BRAF* V600E mutations and *CDKN2A* deletions constitute a clinically distinct subtype of sHGG. The prolonged course to transformation for *BRAF* V600E PLGGs provides an opportunity for surgical interventions, surveillance, and targeted therapies to mitigate the outcome of sHGG.

*J Clin Oncol* 33. © 2015 by American Society of Clinical Oncology

### INTRODUCTION

Gliomas are the most frequent primary CNS neoplasms in adults and children.<sup>1</sup> In contrast to adult low-grade gliomas, which invariably progress to secondary high-grade glioma (sHGG), pediatric low-grade glioma (PLGG) rarely exhibits malignant transformation.<sup>2-4</sup> Only a handful of studies have addressed the clinical and molecular parameters

leading to transformation of PLGG to sHGG. Radiation therapy is thought to play a role in malignant transformation of PLGG,<sup>5</sup> particularly in the context of cancer predisposition.<sup>6,7</sup> Before the genomic era, Broniscer et al<sup>3</sup> were the first to describe several genetic events that occur in these cancers. Recent next-generation sequencing efforts have uncovered somatic mutations in *TP53*, *ATRX*, and *IDH1/2*, among other alterations that are present in adult

low-grade gliomas and sHGGs.<sup>8</sup> In addition, *hTERT* promoter mutations with resulting re-expression of telomerase and alternative lengthening of telomeres (ALT) are major telomere maintenance mechanisms observed in adult glioblastoma and sHGG, respectively.<sup>8,9</sup> Similar efforts have characterized the genomic landscape of pediatric primary high-grade glioma (HGG) and PLGG, with the former containing somatic mutations in histone H3.3, *ATRX*, and *TP53* genes, and others,<sup>10-12</sup> and the latter almost universally exhibiting alterations causing activation of the MAPK/ERK pathway.<sup>13-15</sup>

Despite extensive characterization of primary HGG and PLGG, the incidence of childhood sHGG and the genetic determinants of PLGG transformation in children remain largely unknown. Because pediatric HGGs are invariably lethal,<sup>16</sup> there is an urgent need to identify patients with PLGGs at high risk of malignant transformation and to initiate early aggressive surgical intervention, stratify these patients for existing targeted therapies, and implement tailored surveillance.

To address these needs, we performed a population-based long-term outcome study of all patients with PLGG treated in southern Ontario, Canada, from 1986 to 2013. We then analyzed genomic and genetic alterations observed in PLGG that subsequently transformed to sHGG and correlated these findings with multiple parameters including outcome. Here, we describe a new subgroup of sHGG for which early diagnosis and intervention may improve outcome.

## PATIENTS AND METHODS

### Patient Cohort

After institutional review board approval of the study, all patients treated at the Hospital for Sick Children (SickKids) in Toronto, Ontario, Canada, for PLGG between January 1, 1986, and December 31, 2013, were reviewed for malignant transformation. In Canada, patients are almost exclusively treated at their residential location. Because SickKids is the only reference center for children in a population of 10 million people, no selection bias is expected, and this qualifies as a population-based study. Furthermore, the Ontario health system tracks clinical outcome data on all residents, enabling us to collect long-term follow-up data including transformation events and survival for more than 97% of patients, including adults, until December 2013.<sup>17,18</sup> Patients were considered as having transformation to sHGG if they exhibited one or more of the following: consecutive histologic diagnosis of low-grade gliomas and HGGs as per the WHO 2007 criteria<sup>19</sup> (n = 20); histologic diagnosis of low-grade glioma, followed by at least 1 year of stable disease, followed by clinical and radiologic progression to HGG (n = 3; Data Supplement); or clinicoradiologic evidence of low-grade glioma, at least 1 year of stable disease, and subsequent histologic diagnosis of HGG (n = 3; Data Supplement). Demographic, treatment, and outcome data were retrieved from our PLGG database, as previously described.<sup>20,21</sup> All PLGGs and corresponding sHGGs were reviewed by the study pathologist (C.E.H.).

### Genomic and Genetic Analysis of Tumors

Isolation of DNA from tumor and control tissues was carried out as previously described.<sup>22</sup> Comprehensive analysis of mutations in protein-coding genes by whole-exome sequencing (WES), using the Illumina HiSeq 2000 platform (Illumina, San Diego, CA), and of copy number alterations (CNAs) by array comparative genomic hybridization were performed on available samples. Genotyping for candidate hotspot mutations was carried out as previously described.<sup>10,15,23,24</sup> Specific CNAs were validated by real-time quantitative polymerase chain reaction. *BRAF* fusion status of tumors was obtained using interphase fluorescent in situ hybridization. Immunohistochemistry and genotyping also identified *BRAF* V600E mutations and determined p53 status. ALT was detected by the c-circle assay.<sup>25,26</sup> Further information on methods used in this study is available in the Data Supplement.

### Statistical Analysis

For all correlative studies, Fisher's exact test was used. The unpaired two-tailed *t* test was used to compare time to transformation, the number of somatic mutations, and p53 dysfunction between relevant groups. Survival analysis was performed using the Kaplan-Meier method. A log-rank test was used to compare groups, and curves were generated using Stata v12 (Stata, College Station, TX). *P* < .05 was considered significant.

## RESULTS

### Clinical Characteristics of Patients With Pediatric sHGG

Of 886 patients treated at our institution for PLGG from 1986 to 2013, 26 patients (2.9%) fulfilled the criteria for malignant transformation (Table 1; Data Supplement). Sixteen PLGGs that transformed

**Table 1.** Clinical and Pathologic Characteristics of Pediatric Secondary High-Grade Glioma

Characteristic	No. of Patients	
	PLGG	sHGG
Sex		
Male		14
Female		12
Tumor location		
Hemispheric		11
Thalamic		9
Brainstem		5
Optic pathway		1
Latency to MT, years		
Median		2.84
Range		0.32-20.3
Outcome		
Alive		3
Deceased		23
Disseminated at diagnosis	0	3
Histologic diagnosis		
Low-grade astrocytoma	9	
Pilocytic astrocytoma	7	
Pleomorphic xanthoastrocytoma	5	
Ganglioglioma	2	
Anaplastic astrocytoma		9
Glioblastoma		7
Anaplastic pleomorphic xanthoastrocytoma		5
Anaplastic ganglioglioma		2
Extent of surgical resection		
Gross total resection	1	1
Subtotal resection	15	5
Partial resection	0	3
Biopsy	5	4
Surgery done (extent unknown)	0	5
Treated with RT after diagnosis	5	14
Chemotherapy received	9	17
Age diagnosis, years		
Median	7.0	12.5
Range	0.39-15.6	0.57-33.7
OS after diagnosis, years		
Median	3.75	0.8
Range	0.52-26.1	0.02-21.7

Abbreviations: MT, malignant transformation; OS, overall survival; PLGG, pediatric low-grade glioma; RT, radiotherapy; sHGG, secondary high-grade glioma.

(69%) were diagnosed histologically as either low-grade astrocytoma or pilocytic astrocytoma, whereas the remaining were pleomorphic xanthoastrocytomas (22%) and gangliogliomas (9%). Three sHGGs were disseminated on transformation, two of which were located in hemispheric regions.

The median age at PLGG diagnosis for all patients was 7.0 years (range, 0.39 to 15.6 years). The median age at sHGG diagnosis was 12.5 years (range, 0.57 to 33.7 years), respectively. Only five (19%) of 26 patients were treated with radiotherapy at PLGG diagnosis. Three patients (11%) had cancer predisposition syndromes. These included one patient with neurofibromatosis type 1, one with Li-Fraumeni syndrome, and one with biallelic mismatch repair deficiency syndrome. None of these patients received radiation at PLGG diagnosis.

### Landscape of Point Mutations in sHGG

We performed WES on seven sHGGs and two matched PLGGs. Mean sequencing coverage of sHGG and matched control tissue ( $n = 7$  pairs) was  $94\times$  and  $114\times$ , respectively. Interestingly, the patient with Li-Fraumeni syndrome (patient 7) harbored only 21 somatic mutations in their sHGG, whereas the patient with biallelic mismatch repair deficiency (patient 16) harbored 11,953 somatic mutations, representing a hypermutator phenotype. WES analysis identified a median somatic nonsilent mutation rate of 0.45/Mb in seven sHGGs (median total mutations, 25 mutations; range, six to 11,953 mutations). sHGGs exhibited a higher somatic mutation load than primary pediatric HGGs (median, 11 mutations; range, zero to 4,628 mutations;  $P = .0042$ ; Data Supplement), available from previously published data.<sup>10</sup> Importantly, none of these sHGGs were irradiated before surgical resection.

Three sHGGs harbored heterozygous missense mutations in chromatin-modifying genes including *SETD1B*, *DOT1L*, and *DNMT1* (Data Supplement). The most recurrent mutation was *BRAF* V600E (c.1799T>A), identified in three (43%) of seven sHGGs. We then performed targeted genotyping of recurrent and common genes involved in primary HGG and adult sHGG on all childhood sHGGs. No mutations in *H3F3A* G34 or *IDH1* were identified, and only one patient with a mutation in *ATRX* was observed. Nineteen percent of sHGGs harbored *H3F3A* K27M mutations, all of which were located in the brainstem or thalamus. *BRAF* V600E was identified to be the most recurrent somatic mutation, present in seven (39%) of 18 sHGGs (Fig 1A; Data Supplement), 71% of which (five of seven sHGGs) were hemispheric.

### CNAs in sHGG

We interrogated the same seven sHGGs for recurrent CNAs using array comparative genomic hybridization. Total CNA per sHGG genome was 100, and CNA was 56 in the two patient-matched PLGGs. The only recurrent CNA observed in the sHGGs was a focal deletion of chromosome 9 (p21.3), encompassing the *CDKN2A* gene. *CDKN2A* deletions were observed in 71% of patients from our initial cohort. To determine the frequency of the *CDKN2A* gene deletion in the entire sHGG cohort, we performed real-time quantitative polymerase chain reaction on 14 tumors. Overall, 57% of sHGGs (eight of 14 sHGGs) had heterozygous or homozygous deletions of *CDKN2A* (Fig 1A; Data Supplement).

### *BRAF* V600E and *CDKN2A* Deletion Are Early Events in PLGG Undergoing Transformation

Analysis of patient-matched low- and high-grade samples revealed that *BRAF* mutations identified in the sHGG were also identified in 100% of the corresponding PLGGs and that 80% of sHGG *CDKN2A* deletions were also identified in the corresponding PLGGs (Fig 1). To determine whether these early events are unique to this subset of pediatric gliomas, we compared the frequency of these alterations to nontransformed PLGG and primary pediatric HGG. *BRAF* V600E was significantly enriched in PLGG that transformed, being present in 44% (eight of 18) of PLGGs that transformed compared with 6% (10 of 167) of PLGGs that did not transform (Fig 2A;  $P < .001$ ; Data Supplement). The mutation was also highly enriched in sHGGs compared with only one of 31 primary pediatric HGGs (Fig 2B;  $P = .0023$ ). Strikingly, this single patient had a 3-year history of headache and seizures before the diagnosis and is currently alive more than 15 years after treatment for their presumed primary HGG. Similar to *BRAF* mutations, *CDKN2A* deletions were significantly enriched in PLGG that transformed (71% v 20% of PLGGs that did not transform; Fig 2C; Data Supplement;  $P < .001$ ). Interestingly, six (75%) of eight *BRAF* mutant PLGGs harbored concomitant *CDKN2A* deletions (Fig 1). Therefore, we conclude that *BRAF* V600E mutant sHGGs are a genetically distinct group.

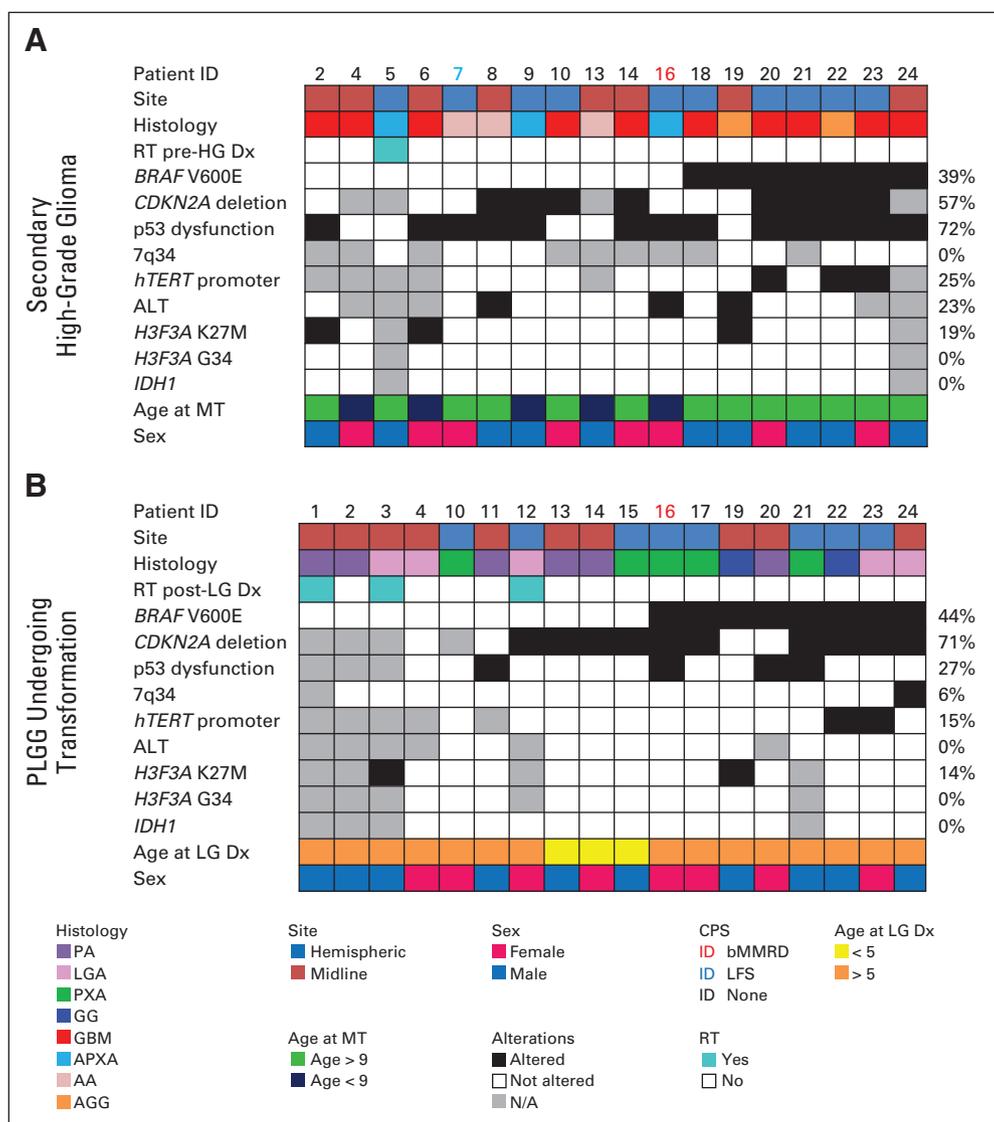
### Integrative Genetic and Molecular Analysis of Childhood sHGG

Several important biologic features emerged from our analysis of PLGGs and their sHGG counterparts. First, no sHGG harbored the 7q34 duplication, which is indicative of the oncogenic *BRAF-KIAA549* gene fusion.<sup>27,28</sup> Notably, only one of the PLGGs that later transformed harbored that aberration. For this single patient, the 7q34 duplication was identified in 10% of tumor cells examined and was not observed at all in the corresponding sHGG (Fig 1).

Second, additional evidence of clonal evolution was observed in the *TP53* data. Specifically, patient 16 harbored a deleterious *TP53* (p.R273C) missense mutation in the PLGG at 7% allele frequency. The matched sHGG had a 30% allele frequency of the same mutation. Similarly, the PLGG had less than 25% immunopositivity for p53, whereas the matched sHGG tumor had more than 90% of tumor cells staining positive for p53 (Data Supplement). Eighty percent of patients (eight of 10 patients) demonstrated increases in p53 tumor cell immunopositivity between matched low- and high-grade tumor samples. Overall, there was a higher proportion of p53 immunopositive sHGG (72%) versus PLGG (27%; Fig 1;  $P = .0149$ ; Data Supplement).

Third, telomere maintenance abnormalities were observed in 54% of sHGGs. In contrast to adult low-grade gliomas, no PLGG exhibited ALT (zero of 12 PLGGs), whereas *hTERT* promoter mutations were seen in 15% of PLGGs (two of 13 PLGGs), both of which were conserved in their high-grade counterparts. All ALT and *hTERT* promoter mutation cases were mutually exclusive (Fig 1).

Finally, alterations of the p53 and/or RB tumor suppressor pathways were identified in 14 (93%) of 15 sHGGs. Surprisingly, a high proportion of PLGGs (12 of 14 PLGGs; 86%) also harbored overexpression of p53 or *CDKN2A* deletions, suggesting early ablation of cell cycle control in PLGGs that later transform (Fig 1; Data Supplement).



**Fig 1.** Clinical, genetic and molecular characteristics in (A) secondary high-grade glioma and (B) pediatric low-grade glioma (PLGG) undergoing transformation. Frequency of genetic or molecular alterations indicated as percentage at the far right of each row. AA, anaplastic astrocytoma; AGG, anaplastic ganglioglioma; APXA, anaplastic pleomorphic xanthoastrocytoma; bMMRD, biallelic mismatch repair deficiency syndrome; CPS, cancer predisposition syndrome; Dx, diagnosis; GBM, glioblastoma; GG, ganglioglioma; HG, high grade; LFS, Li-Fraumeni syndrome; LG, low grade; LGA, low-grade astrocytoma; MT, malignant transformation; N/A, not applicable; PA, pilocytic astrocytoma; PXA, pleomorphic xanthoastrocytoma; RT, radiotherapy. p53 dysfunction is defined by more than 50% p53 immunopositive tumor cells and/or *TP53* mutation.

### ***BRAF* V600E Defines a Clinically Distinct Subset of sHGG**

To understand the clinical consequences of the identified *BRAF* V600E-driven sHGG subset, we compared onset and outcome of this subgroup to *BRAF* wild-type sHGG and other pediatric gliomas. Patients with *BRAF* mutant PLGGs that later transformed were all older than age 5 years at diagnosis (range, 5.1 to 13.4 years), and 89% of these patients were between 5 and 10 years of age, in contrast to all other patients with PLGGs that later transformed (Fig 3A).

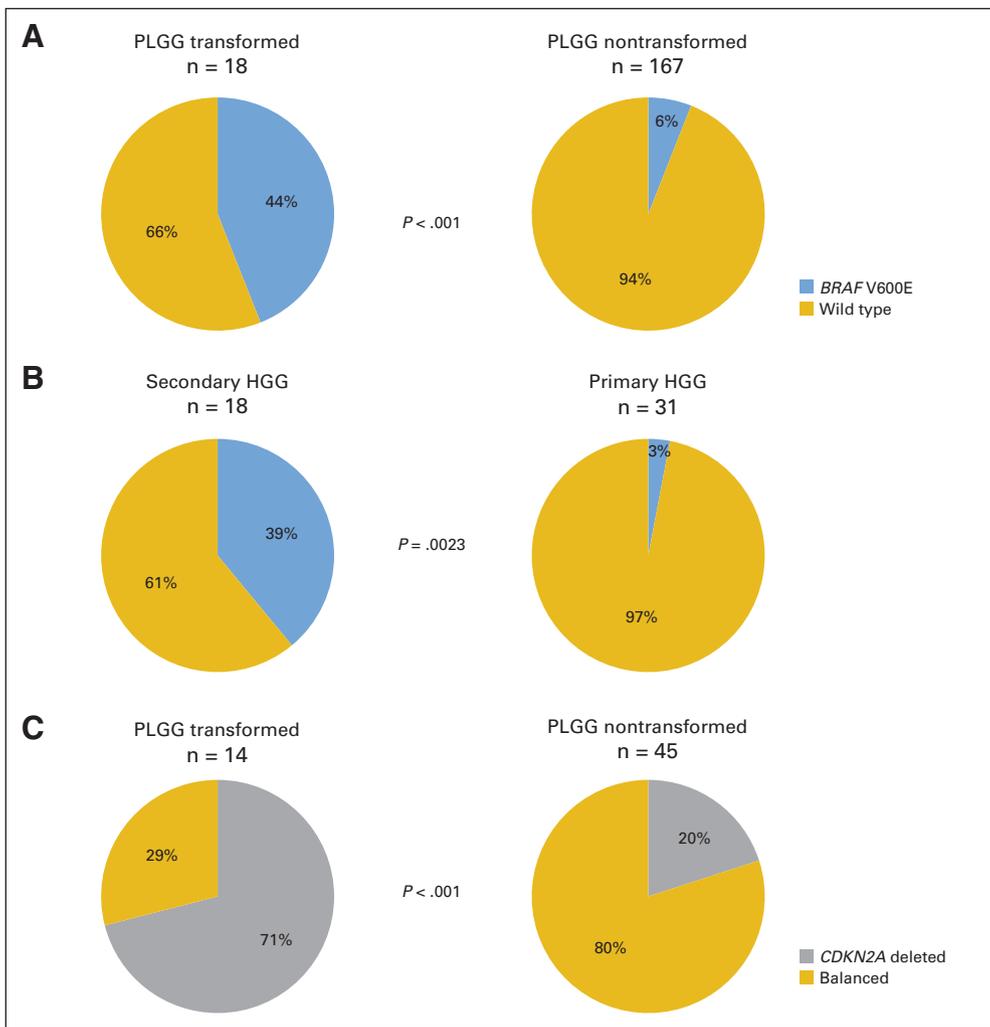
Most notably, *BRAF* V600E mutant PLGGs had significantly prolonged latency periods to transformation (median, 6.65 years; range, 3.50 to 20.3 years) compared with *BRAF* wild-type PLGGs that also experienced transformation (median, 1.59 years; range, 0.32 to 15.9 years; Fig 3B;  $P = .0389$ ). As a result, all *BRAF* mutant sHGGs were diagnosed at age greater than 9 years (Fig 1A).

Furthermore, 5-year overall survival rates of children with *BRAF* mutant and wild-type PLGGs that transformed were  $75\% \pm 15\%$  and  $29\% \pm 12\%$ , respectively ( $P = .024$ ; Fig 3C). To further confirm that these two groups of sHGG are distinct, we compared the outcomes of

these patients with those of patients with PLGG and primary HGG in our institution. Children with nontransformed PLGG had a 5-year overall survival rate of  $98\% \pm 0.5\%$ . Importantly, children with primary HGG had a 5-year overall survival rate of  $5\% \pm 4\%$ , a significantly worse outcome than any subgroup of sHGG, including *BRAF* wild-type PLGGs that transform (Fig 3D;  $P = .0163$ ). After malignant transformation, 23 of 26 patients died, and no significant difference in survival between subgroups of sHGG was observed ( $P = .49$ ; Data Supplement).

## DISCUSSION

In this population-based study, we define the risk of PLGG transformation to sHGG (2.9%) and uncover clinically applicable genetic subgroups of these cancers. The absolute risk of PLGG transformation is lower than previously estimated.<sup>3</sup> Broniscer et al<sup>3</sup> estimated that approximately 10% of PLGGs will transform to sHGG; however, that study represents a referral center experience that may be biased to



**Fig 2.** Correlative studies identifying that (A) *BRAF* V600E is enriched in pediatric low-grade gliomas (PLGGs) that later transform versus PLGGs that do not transform (*P* < .001); (B) *BRAF* V600E distinguishes secondary from primary high-grade gliomas (HGGs) in children (*P* = .0023); and (C) *CDKN2A* deletions are enriched in PLGGs that later transform versus PLGGs that do not transform (*P* < .001). Statistical significance was evaluated using Fisher's exact test.

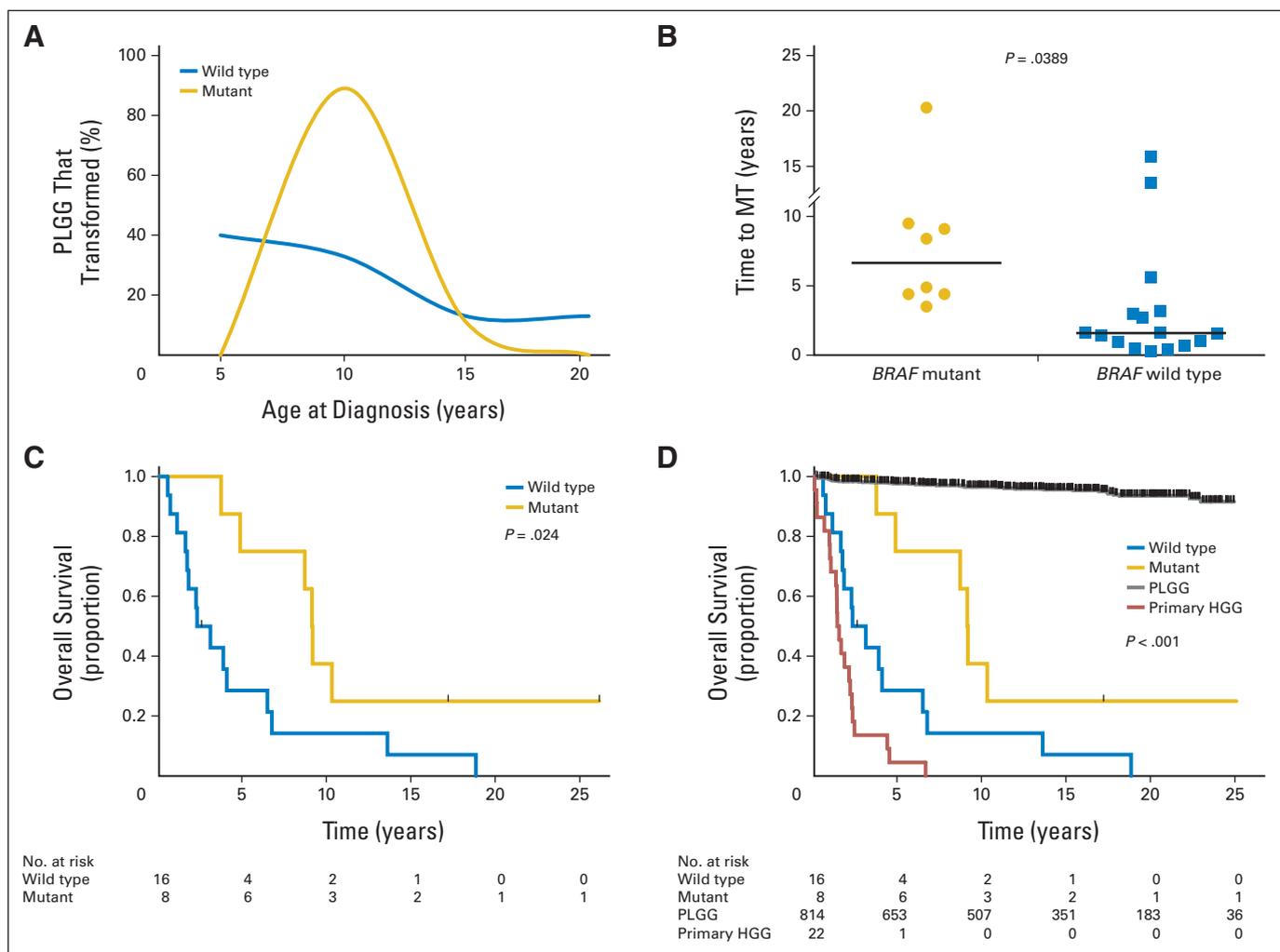
more aggressive cancers (Data Supplement). The provincial health care system in Ontario and follow-up data allowed us to track patients for more than 30 years and detect even late transformation events in survivors of PLGG. Indeed, survivors of childhood *BRAF* mutant PLGG may experience malignant transformation up to 20 years after their initial diagnosis when they are well into adulthood.

Our study reveals additional molecular and genetic differences between childhood and adult gliomas. Common alterations found in adult low-grade gliomas that transform such as *ATRX*, *IDH1*, and *ALT* were extremely rare in our cohort, whereas *BRAF* V600E, H3.3, and *hTERT* promoter mutations, which are uncommon in adult sHGG, were highly enriched in our cohort. Nevertheless, genomic analyses of both adult and pediatric sHGG reveal common aberrations in p53 and RB cancer pathways, which are key to avoiding cellular senescence and growth arrest. An important clinical implication of this observation is the high incidence of both p53-positive immunostaining and *CDKN2A* deletion observed in our PLGG cohort that subsequently experienced transformation. Because these two markers are extremely uncommon in PLGGs that do not transform, integration of these tests to the panel used at diagnosis may enable us to detect a high-risk patient population for follow-up.

The complete lack of the *BRAF-KIAA1549* gene fusion observed in sHGG represents another clinically important parameter for risk

assessment. In our PLGG database of 886 patients, only one patient with chromosome 7q34 duplication went on to experience transformation. This patient had only 10% of cells harboring this duplication, and this clone disappeared in the corresponding sHGG, suggesting that minor subclones harboring the *BRAF* duplication do not portend the same good clinical outcome. Because more than half of PLGG are expected to harbor this alteration, inclusion of this test will add important negative information on the risk of transformation.<sup>21</sup> *BRAF* V600E mutations have been described in a variety of pediatric gliomas, most frequently in gangliogliomas and pleomorphic xanthoastrocytomas.<sup>25-27</sup> The latter also manifest concomitant *CDKN2A* deletions.<sup>14</sup> Interestingly, pleomorphic xanthoastrocytomas have the tendency to transform to high-grade tumors.<sup>29,30</sup> Nevertheless, 69% of PLGGs in our cohort were initially diagnosed as grade 1 astrocytomas, demonstrating that transformation is not restricted to these pathologic subtypes. The frequency of *BRAF* mutations in pediatric HGG is controversial. Different studies reveal rates between 10% and 25%.<sup>11,31-33</sup> Our observations resolve some of these discrepancies, because the mutation may be specific to the subgroup of secondary pediatric HGG rather than primary pediatric HGG.

Perhaps the most important observation of this study is that specific alterations identified in sHGG could be traced back to their



**Fig 3.** (A) Age distribution for *BRAF* mutant and *BRAF* wild-type patients at initial diagnosis of pediatric low-grade glioma (PLGG). Eighty-nine percent of patients who were *BRAF* positive were diagnosed between 5 and 10 years of age. (B) *BRAF* mutant PLGGs have a prolonged latency to malignant transformation (MT) compared with *BRAF* wild-type PLGGs ( $P = .0389$ ). Horizontal lines indicate medians. Statistical significance was evaluated using an unpaired *t* test. (C) Kaplan-Meier survival estimates showing improved overall survival after initial diagnosis for *BRAF* mutant versus wild-type PLGGs that later transform. (D) Primary high-grade gliomas (HGGs) have a worse overall survival after initial diagnosis than *BRAF* wild-type secondary HGG ( $P = .0163$ ). Nontransformed PLGGs have a favorable overall survival compared with all other groups, at  $98\% \pm 0.5\%$ .

PLGG counterparts. Both *BRAF* V600E and *CDKN2A*-deleted tumors may be eligible for targeted therapies. If so, preventive measures, including a more aggressive surgical approach and consideration of medical therapies<sup>34</sup> even when a stable tumor is observed, may be able to mitigate the devastating transformation event. This concept is further highlighted because the *BRAF* V600E mutation and *CDKN2A* deletion were observed in 63% and 100% of tumors in peripheral locations, respectively (Fig 1B), suggesting enrichment of these alterations in tumors that are more amenable to surgical resection.

Wild-type *BRAF* sHGG could be divided into two groups. One includes the brainstem lesions that are enriched for the *H3F3A* K27M mutations. This mutation can differentiate them from other brainstem PLGGs that have excellent survival.<sup>20</sup> The second group of sHGG includes patients with cancer predisposition syndromes. These patients may also benefit from surveillance and early intervention to prevent tumor transformation.<sup>35,36</sup>

Together, we observed known alterations in 21 (88%) of 24 patients with PLGG that later transformed and no *BRAF* fusions in any secondary HGG. These genetic alterations could stratify PLGG into the following risk groups (Data Supplement): tumors that have the *BRAF* fusion but lack the rest of the previously mentioned alterations (excellent long-term outcome and extremely low risk for transformation); PLGGs harboring the *BRAF* V600E mutations and alterations in *CDKN2A* or *TP53* (higher risk for transformation and should be managed with care); PLGGs originating with cancer predisposition syndromes associated with HGG (these tumors will eventually transform); and finally, midline PLGGs harboring H3.3 K27M mutations that will behave as primary HGGs. Because all of these tests could be performed in most clinical laboratories, it may be worthwhile to add them to the current battery of tests used for PLGG.

A potential pitfall of all studies investigating transformation of PLGG to sHGG involves instances where tumors, according to the

**AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST**

Disclosures provided by the authors are available with this article at [www.jco.org](http://www.jco.org).

**AUTHOR CONTRIBUTIONS**

**Conception and design:** Matthew Mistry, Cynthia E. Hawkins, Uri Tabori

**Financial support:** Cynthia E. Hawkins, Uri Tabori

**Provision of study materials or patients:** Jennifer Chan, Keith L. Ligon, James T. Rutka, Peter B. Dirks, David Malkin, Cynthia E. Hawkins, Uri Tabori

**Collection and assembly of data:** Matthew Mistry, Nataliya Zhukova, Patricia Rakopoulos, Rahul Krishnatry, Jason D. Pole, Marc Remke, Joshua Mangerel, Ana Guerreiro Stucklin, Cindy Zhang, Doua Bakry, Suzanne Laughlin, Jennifer Chan, Keith L. Ligon, James T. Rutka, Peter B. Dirks, Michael D. Taylor, Mark Greenberg, David Malkin, Eric Bouffet, Cynthia E. Hawkins, Uri Tabori

**Data analysis and interpretation:** Matthew Mistry, Nataliya Zhukova, Daniele Merico, Patricia Rakopoulos, Rahul Krishnatry, Mary Shago, James Stavropoulos, Noa Alon, Jason D. Pole, Peter N. Ray, Vilma Navickiene, Marc Remke, Pawel Buczkowicz, Vijay Ramaswamy, Joshua Mangerel, Ana Guerreiro Stucklin, Martin Li, Edwin J. Young, Pedro Castelo-Branco, Adam Shlien, James T. Rutka, Peter B. Dirks, Michael D. Taylor, Mark Greenberg, David Malkin, Annie Huang, Eric Bouffet, Cynthia E. Hawkins, Uri Tabori

**Manuscript writing:** All authors

**Final approval of manuscript:** All authors

biopsy, are PLGGs, but nevertheless behave as aggressively as primary HGGs. This can occur in two scenarios. One is where a small biopsy may have missed malignant regions as a result of tumor heterogeneity. This is particularly the case for deep-seated tumors. Indeed, our study (three patients) and previous reports<sup>3</sup> have identified low-grade lesions on initial biopsy followed by a second biopsy revealing HGG in a short time span. The second scenario is where tumors with low-grade histology nevertheless behave as high grade. This has been demonstrated for low-grade astrocytomas of the brainstem that harbor H3.3 K27M mutations.<sup>37,38</sup> In both scenarios, the addition of molecular markers as described in this study may help to establish (eg, *BRAF-KIAA1549* fusion) or refute (eg, *H3F3A* mutation) the PLGG diagnosis. PLGGs that harbor H3.3 K27M, *TP53* mutations, or mismatch repair deficiency may in fact represent high-grade–behaving gliomas (Data Supplement), whereas cancers harboring *BRAF* V600E mutations, if they transform, will have significant lag before transformation even if they are deeply seated midline tumors. This study has the classical limitations of a retrospective analysis over several decades and should be interpreted accordingly. Although the importance of *BRAF* mutations together with *CDKN2A* as risk factors for transformation is clear, the level of risk requires further study. It will be important to test in the future how many incompletely resected tumors that harbor both alterations will not transform using long clinical follow-up.

In summary, this study defines molecular and genetic subgroups of pediatric sHGG. Most of these alterations are early events that can guide the management of patients with PLGG at risk of malignant transformation. Further population-based research is required to better define the role of *BRAF* and *CDKN2A* alterations in childhood gliomas.

**REFERENCES**

1. Ostrom QT, Gittleman H, Farah P, et al: CBRUS statistical report: Primary brain and central nervous system tumors diagnosed in the United States in 2006-2010. *Neuro Oncol* 15:iii1-ii56, 2013 (suppl 2)
2. Kleihues P, Ohgaki H: Primary and secondary glioblastomas: From concept to clinical diagnosis. *Neuro Oncol* 1:44-51, 1999
3. Broniscer A, Baker SJ, West AN, et al: Clinical and molecular characteristics of malignant transformation of low-grade glioma in children. *J Clin Oncol* 25:682-689, 2007
4. Krieger MD, Gonzalez-Gomez I, Levy ML, et al: Recurrence patterns and anaplastic change in a long-term study of pilocytic astrocytomas. *Pediatr Neurosurg* 27:1-11, 1997
5. Dirks PB, Jay V, Becker LE, et al: Development of anaplastic changes in low-grade astrocytomas of childhood. *Neurosurgery* 34:68-78, 1994
6. Sharif S, Ferner R, Birch JM, et al: Second primary tumors in neurofibromatosis 1 patients treated for optic glioma: Substantial risks after radiotherapy. *J Clin Oncol* 24:2570-2575, 2006
7. Merchant TE, Rose SR, Bosley C, et al: Growth hormone secretion after conformal radiation therapy in pediatric patients with localized brain tumors. *J Clin Oncol* 29:4776-4780, 2011
8. Kannan K, Inagaki A, Silber J, et al: Whole-exome sequencing identifies *ATRX* mutation as a key molecular determinant in lower-grade glioma. *Oncotarget* 3:1194-1203, 2012

9. Killela PJ, Reitman ZJ, Jiao Y, et al: TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. *Proc Natl Acad Sci U S A* 110:6021-6026, 2013
10. Wu G, Diaz AK, Paugh BS, et al: The genomic landscape of diffuse intrinsic pontine glioma and pediatric non-brainstem high-grade glioma. *Nat Genet* 46:444-450, 2014
11. Schwartzentruber J, Korshunov A, Liu XY, et al: Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. *Nature* 482:226-231, 2012
12. Sturm D, Witt H, Hovestadt V, et al: Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. *Cancer Cell* 22:425-437, 2012
13. Jones DT, Hutter B, Jäger N, et al: Recurrent somatic alterations of *FGFR1* and *NTRK2* in pilocytic astrocytoma. *Nat Genet* 45:927-932, 2013
14. Zhang J, Wu G, Miller CP, et al: Whole-genome sequencing identifies genetic alterations in pediatric low-grade gliomas. *Nat Genet* 45:602-612, 2013
15. Pfister S, Janzarik WG, Remke M, et al: *BRAF* gene duplication constitutes a mechanism of MAPK pathway activation in low-grade astrocytomas. *J Clin Invest* 118:1739-1749, 2008
16. Broniscer A, Gajjar A: Supratentorial high-grade astrocytoma and diffuse brainstem glioma: Two challenges for the pediatric oncologist. *Oncologist* 9:197-206, 2004
17. Cheng S, Pole JD, Sung L: Early deaths in pediatric acute leukemia: A population-based study. *Leuk Lymphoma* 55:1518-1522, 2014

18. Schechter T, Pole JD, Darmawikarta D, et al: Late mortality after hematopoietic SCT for a childhood malignancy. *Bone Marrow Transplant* 48:1291-1295, 2013
19. Louis DN, Ohgaki H, Wiestler OD, et al: The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathol* 114:97-109, 2007
20. Fried I, Hawkins C, Scheinemann K, et al: Favorable outcome with conservative treatment for children with low grade brainstem tumors. *Pediatr Blood Cancer* 58:556-560, 2012
21. Hawkins C, Walker E, Mohamed N, et al: *BRAF-KIAA1549* fusion predicts better clinical outcome in pediatric low-grade astrocytoma. *Clin Cancer Res* 17:4790-4798, 2011
22. Torchia EC, Boyd K, Rehg JE, et al: *EWS/FLI-1* induces rapid onset of myeloid/erythroid leukemia in mice. *Mol Cell Biol* 27:7918-7934, 2007
23. Parsons DW, Jones S, Zhang X, et al: An integrated genomic analysis of human glioblastoma multiforme. *Science* 321:1807-1812, 2008
24. Remke M, Ramaswamy V, Peacock J, et al: TERT promoter mutations are highly recurrent in SHH subgroup medulloblastoma. *Acta Neuropathol* 126:917-929, 2013
25. Henson JD, Cao Y, Huschtscha LI, et al: DNA C-circles are specific and quantifiable markers of alternative-lengthening-of-telomeres activity. *Nat Biotechnol* 27:1181-1185, 2009
26. Lau LM, Dagg RA, Henson JD, et al: Detection of alternative lengthening of telomeres by telomere quantitative PCR. *Nucleic Acids Res* 41:e34, 2013
27. Sievert AJ, Jackson EM, Gai X, et al: Duplication of 7q34 in pediatric low-grade astrocytomas detected

by high-density single-nucleotide polymorphism-based genotype arrays results in a novel BRAF fusion gene. *Brain Pathol* 19:449-458, 2009

28. Jones DT, Kocialkowski S, Liu L, et al: Tandem duplication producing a novel oncogenic BRAF fusion gene defines the majority of pilocytic astrocytomas. *Cancer Res* 68:8673-8677, 2008

29. Binesh F, Akhavan A, Navabii H: Pleomorphic xanthoastrocytoma with malignant transformation and multiple recurrences in an Iranian girl. *BMJ Case Rep* 20:2012, 2012

30. Rodriguez-Mena R, Joanes-Alepuz V, Barbella-Aponte R, et al: Pleomorphic xanthoastrocytoma with intraventricular extension and anaplastic transformation in an adult patient: Case report. *Neurocirugia (Astur)* 23:203-210, 2012

31. Nicolaidis TP, Li H, Solomon DA, et al: Targeted therapy for BRAFV600E malignant astrocytoma. *Clin Cancer Res* 17:7595-7604, 2011

32. Schiffman JD, Hodgson JG, Vandenberg SR, et al: Oncogenic BRAF mutation with CDKN2A inactivation is characteristic of a subset of pediatric malignant astrocytomas. *Cancer Res* 70:512-519, 2010

33. Brennan CW, Verhaak RG, McKenna A, et al: The somatic genomic landscape of glioblastoma. *Cell* 155:462-477, 2013

34. Rush S, Foreman N, Liu A: Brainstem ganglioglioma successfully treated with vemurafenib. *J Clin Oncol* 31:e159-e160, 2013

35. Bakry D, Aronson M, Durno C, et al: Genetic and clinical determinants of constitutional mismatch repair deficiency syndrome: Report from the consti-

tutional mismatch repair deficiency consortium. *Eur J Cancer* 50:987-996, 2014

36. Villani A, Malkin D, Tabori U: Syndromes predisposing to pediatric central nervous system tumors: Lessons learned and new promises. *Curr Neurol Neurosci Rep* 12:153-164, 2012

37. Khuong-Quang DA, Buczkowicz P, Rakopoulos P, et al: K27M mutation in histone H3.3 defines clinically and biologically distinct subgroups of pediatric diffuse intrinsic pontine gliomas. *Acta Neuropathol* 124:439-447, 2012

38. Buczkowicz P, Bartels U, Bouffet E, et al: Histopathological spectrum of paediatric diffuse intrinsic pontine glioma: Diagnostic and therapeutic implications. *Acta Neuropathol* 128:573-581, 2014

### Affiliations

Matthew Mistry, Nataliya Zhukova, Daniele Merico, Rahul Krishnatry, Mary Shago, James Stavropoulos, Noa Alon, Peter N. Ray, Vilma Navickiene, Joshua Mangerel, Marc Remke, Vijay Ramaswamy, Ana Guerreiro Stucklin, Martin Li, Edwin J. Young, Cindy Zhang, Pedro Castelo-Branco, Doua Bakry, Suzanne Laughlin, James T. Rutka, Peter B. Dirks, Michael D. Taylor, Mark Greenberg, David Malkin, Annie Huang, Eric Bouffet, Cynthia E. Hawkins, and Uri Tabori; The Hospital for Sick Children; Matthew Mistry, Patricia Rakopoulos, Rahul Krishnatry, Joshua Mangerel, Pawel Buczkowicz, Ana Guerreiro Stucklin, Doua Bakry, Adam Shlien, Mark Greenberg, David Malkin, Annie Huang, Eric Bouffet, Cynthia E. Hawkins, and Uri Tabori, University of Toronto; Jason D. Pole, Pediatric Oncology Group of Ontario, Toronto, Ontario; Jennifer Chan, Hotchkiss Brain Institute, University of Calgary, Calgary, Alberta, Canada; Pedro Castelo-Branco, Universidade do Algarve, Faro, Portugal; Keith L. Ligon, Dana-Farber/Boston Children's Cancer Center, Boston, MA.

### GLOSSARY TERMS

**BRAF:** an isoform of *RAF*.

**AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST**

**BRAF Mutation and CDKN2A Deletion Define a Clinically Distinct Subgroup of Childhood Secondary High-Grade Glioma**

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to [www.asco.org/rwc](http://www.asco.org/rwc) or [jco.ascopubs.org/site/ifc](http://jco.ascopubs.org/site/ifc).

**Matthew Mistry**

No relationship to disclose

**Nataliya Zhukova**

No relationship to disclose

**Daniele Merico**

No relationship to disclose

**Patricia Rakopoulos**

No relationship to disclose

**Rahul Krishnatry**

No relationship to disclose

**Mary Shago**

No relationship to disclose

**James Stavropoulos**

No relationship to disclose

**Noa Alon**

No relationship to disclose

**Jason D. Pole**

No relationship to disclose

**Peter N. Ray**

No relationship to disclose

**Vilma Navickiene**

No relationship to disclose

**Joshua Mangerel**

No relationship to disclose

**Marc Remke**

No relationship to disclose

**Pawel Buczkowicz**

No relationship to disclose

**Vijay Ramaswamy**

No relationship to disclose

**Ana Guerreiro Stucklin**

No relationship to disclose

**Martin Li**

No relationship to disclose

**Edwin J. Young**

No relationship to disclose

**Cindy Zhang**

No relationship to disclose

**Pedro Castelo-Branco**

No relationship to disclose

**Doua Bakry**

No relationship to disclose

**Suzanne Laughlin**

No relationship to disclose

**Adam Shlien**

No relationship to disclose

**Jennifer Chan**

No relationship to disclose

**Keith L. Ligon**

**Consulting or Advisory Role:** EMD Serono, Midatech

**Research Funding:** GlaxoSmithKline (Inst), Bristol-Myers Squibb (Inst)

**Patents, Royalties, Other Intellectual Property:** Patent pending on diagnostic assay (Inst)

**James T. Rutka**

No relationship to disclose

**Peter B. Dirks**

No relationship to disclose

**Michael D. Taylor**

No relationship to disclose

**Mark Greenberg**

No relationship to disclose

**David Malkin**

No relationship to disclose

**Annie Huang**

No relationship to disclose

**Eric Bouffet**

No relationship to disclose

**Cynthia E. Hawkins**

**Travel, Accommodations, Expenses:** NanoString Technologies

**Uri Tabori**

No relationship to disclose