original report

Clinically Integrated Sequencing Alters Therapy in Children and Young Adults With High-Risk Glial Brain Tumors

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abstract

Purpose **Brain tumors have become the leading cause of cancer-related mortality in young patients. Novel effective therapies on the basis of the unique biology of each tumor are urgently needed. The goal of this study was to evaluate the feasibility, utility, and clinical impact of integrative clinical sequencing and genetic counseling in children and young adults with high-risk brain tumors.**

Patients and Methods **Fifty-two children and young adults with brain tumors designated by the treating neuro-oncologist to be high risk (> 25% chance for treatment failure; mean age, 10.2 years; range, 0 to 39 years) were enrolled in a prospective, observational, consecutive case series, in which participants underwent integrative clinical exome (tumor and germline DNA) and transcriptome (tumor RNA) sequencing and genetic counseling. Results were discussed in a multi-institutional brain tumor precision medicine teleconference.**

Results **Sequencing revealed a potentially actionable germline or tumor alteration in 25 (63%) of 40 tumors with adequate tissue, of which 21 (53%) resulted in an impact on treatment or change of diagnosis. Platelet-derived growth factor receptor or fibroblast growth factor receptor pathway alterations were seen in nine of 20 (45%) glial tumors. Eight (20%) sequenced tumors harbored an oncogenic fusion isolated on RNA sequencing. Seventeen of 20 patients (85%) with glial tumors were found to have a potentially actionable result, which resulted in change of therapy in 14 (70%) patients. Patients with recurrent brain tumors receiving targeted therapy had a median progression-free survival (from time on therapy) of 4 months.**

Conclusion **Selection of personalized agents for children and young adults with high-risk brain tumors on the basis of integrative clinical sequencing is feasible and resulted in a change in therapy in more than two thirds of children and young adults with high-risk glial tumors.**

INTRODUCTION

Outcomes for younger patients with brain tumors remain poor, and these tumors have become the leading cause of cancer-related mortality in the young.[1] This is due, in large part, to barriers of using many of the effective oncology treatments to this patient population.[1-3] The location of many pediatric brain tumors in the thalamus and brainstem constrains the surgeon from performing a safe surgical resection or even a biopsy in some cases.[4,5] Finally, the blood-brain barrier (BBB) restricts access to the CNS to all but 5% of chemical compounds screened for drug development.[6]

At the molecular level, pediatric and adult brain tumors are dissimilar.[2,4] Recurrent mutations and gene expression profiles of pediatric brain tumors are clearly distinct from their adult counterparts.[2,7,8] Moreover, molecular characterization of pediatric brain tumors has disclosed key differences in tumors with the same pathology, among their subgroups defined by age and location.[2,4,9] Younger patients with high-risk or refractory brain tumors are in urgent need of

applicable) appear at the end of this article.

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novel effective therapies that are based, ideally, on the unique biology of each tumor.

In contrast to adult tumors, recent surveys of pediatric tumors have shown that most are driven by relatively few mutational events, many of which may be targetable with personalized clinically available agents.[2,10] Although precision medicine is an exciting potential therapeutic avenue for younger patients with brain tumors, this approach requires substantial optimization to successfully improve outcomes for children with these aggressive tumors. Our group has successfully shown the feasibility and potential clinical utility of integrative clinical sequencing in the practice of precision oncology in pediatric and adult patients with relapsed and refractory disease,[11,12] and other pediatric groups have recently reported similar findings.[13,14] Two recent studies have reported targeted DNA sequencing in pediatric patients with brain tumors.[15,16] However, to our knowledge, no studies have been primarily designed to study the role of DNA and RNA sequencing of children and adolescents with high-risk brain tumors, with a focus on their treatment and follow-up.

We therefore launched a clinical sequencing study to better understand the role of personalized genomic characterization and precision oncology in the management of these patients. We performed clinically integrated tumor (DNA/RNA) and germline (DNA) sequencing and genetic counseling for a prospective cohort of children and young adults with high-risk brain tumors. As part of this study, we discussed these results in a multidisciplinary and multi-institutional brain tumor precision medicine conference, with a special emphasis on discussion of the CNS penetration of potential targeted agents.

PATIENTS AND METHODS

Patients

We performed a single-site prospective, observational consecutive case series of patients younger than 40 years with a diagnosis of a high-risk primary brain tumor believed to have at least a 25% chance of treatment failure by the treating oncologist (including patients with tumors with unclear diagnosis or grading—if one of the diagnostic possibilities met our criteria for high risk). The Pediatric MiOncoSeq study was approved by the Institutional Review Board of the University of Michigan Medical School, and all patients or their parents or legal guardians provided informed consent (written assent if > 10 years). All patients were seen by a physician investigator and a genetic counselor. Clinically integrated sequencing was performed according to previous published methodology (Data Supplement).[11,12,17] Our study began using a whole-exome gene panel, as previously described. Partway through the study of our cohort, the MiOncoseq sequencing laboratory (Michigan Center for Translational Pathology) adjusted their panel from whole exome to a panel of 1,700 genes (OncoSeq1700) that covered all cancer consensus genes and fusion partners seen in their initially sequenced cohort (> 1,000 tumors). The Michigan Center for Translational Pathology prospectively performed paired whole exome and 1,700 gene panel on a prospective cohort of tumors and confirmed no notable difference in reporting of cancer consensus gene alterations to clinicians.

As previously described,[11] potentially actionable findings were defined as any genomic finding that could lead to a change in patient management by providing a targetable tumor molecular aberration, a change in diagnosis or risk stratification (leading to an immediate potential therapeutic application), or a change in patient or family counseling by identifying cancer-related germline findings. For medulloblastoma, sonic hedgehog (SHH) was the only subgroup designation considered actionable by itself.

Brain Tumor Precision Medicine Conference

Tumor sequencing results were reviewed in a multi-institutional brain tumor precision medicine teleconference held at the University of Michigan and attended by Seattle Children's Hospital, University of California, San Francisco, Baylor, Colorado Children's Hospital, Lurie Children's, Children's National, and Children's Hospital of Michigan. Clinical sequencing results and potential treatment options were discussed with a multidisciplinary team including clinicians from pediatric and adult neuro-oncology, pediatric neurosurgery, neuropathology, pathology/cytogenetics, pharmacology, bioinformaticians, genetic counselors, and study coordinators, to reach a consensus treatment

opinion and to generate discussion of clinical trial availability.

For discussion of targeted agents to be considered for patients, special attention was paid to likelihood of their penetration of BBB. For all agents (available on or off trial), the University of Michigan CNS Targeted Agent Prediction (CNS-TAP) rating system was developed, with seven criteria to score utility of the agent, including targeted pathway/relevance to sequencing results, preclinical data, pediatric phase I data, clinical data in CNS tumors, active clinical trials for which patient is eligible, CNS/BBB penetration, and relevant formulation (pill *v* suspension; see Data Supplement for sample checkbox evaluation of everolimus for a *PIK3CA* mutation in a patient with diffuse intrinsic pontine glioma [DIPG]). Scores are given as total number of checks for each agent. For patients with multiple targetable lesions, preference was given to lesions with higher variant allele fraction in tumor, when known.

RESULTS

Feasibility of Clinically Integrated Sequencing for Children and Young Adults With Brain Tumors

We screened 52 patients and enrolled 50 patients with high-risk primary brain tumors (mean age, 10.2 years; median age, 9.0 years; range, 1 to 39 years) between January 2014 and March 2017 (Fig 1). Two families of patients with DIPG declined participation because of the need for biopsy (offered for clinical and research purposes). Patients were referred with a wide variety of brain tumor diagnoses, as long as their treating neuro-oncologist denoted high-risk status (> 25% risk of progression or treatment failure).

Eighteen patients (36%) had relapsed or refractory tumors (of which we sequenced the original tumor in 12 cases), and 13 patients (26%) had metastatic tumors at the time of enrollment (Table 1). Thirty-two patients were enrolled at the time of their original diagnoses, when they presented with either a high-risk neoplasm or a possible diagnosis that met criteria for high-risk neoplasm after pathology review. For all patients, tissue was obtained by standard-of-care diagnostic or therapeutic surgical procedures done either at the time of enrollment or at the time of an earlier procedure. Of the 50 enrolled patients, 40 (80%) had adequate tumor specimen for DNA/RNA sequencing, including 20 (50%) glial tumors, 15 (38%) embryonal tumors, and five (13%) other tumors. Frozen samples were more often adequate than formalin-fixed paraffin-embedded (94% *v* 74%). In one case, paraffin-embedded tissue on two slides was sufficient to provide tissue for DNA, but not RNA, isolation. The 40 tumors with adequate specimen were used as a denominator for all subsequent analyses. For these tumors, results were provided to the clinician/family at a median of 78 days. A cause of delay for some cases was obtaining formalin-fixed paraffin-embedded tissue from patients at outside institutions. Patients with frozen tumor samples had a median time to results of 49 days.

Twenty-six tumors (65%) underwent DNA sequencing with a panel of 1,700 cancer-related genes (OncoSeq1700), rather than whole-exome sequencing. Rates of actionable findings were similar between both methodologies.

Clinical Utility of Integrated Sequencing for Children and Young Adults With Brain Tumors

Sequencing revealed a potentially actionable germline or tumor alteration in 25 (63%) tumors, including five germline and 22 somatic tumor alterations (two patients with both a distinct germline and tumor alteration; Table 1). For these patients, the results led to a change in clinical management/treatment in 21 patients (53%). This included 18 patients (45%) who underwent a change in therapy on the basis of sequencing results and three patients (8%) who received new genetic counseling for germline finding and future cancer risk. During the course of this study, none of these patients were found to have additional malignancies, although three of five of these patients (and the sibling of one of these patients) remain on annual surveillance programs for additional associated malignancies (Data Supplement).

We graded the actionable potential of the somatic mutations into category I to IV (Table A1) as described previously.[13] Category I included somatic mutations of established clinical utility; category II: mutations of potential utility; category III: other consensus cancer gene mutations; and category IV: other genes. On the basis of this grading system, these brain tumors harbored an average of 2.3 grade I to III potentially

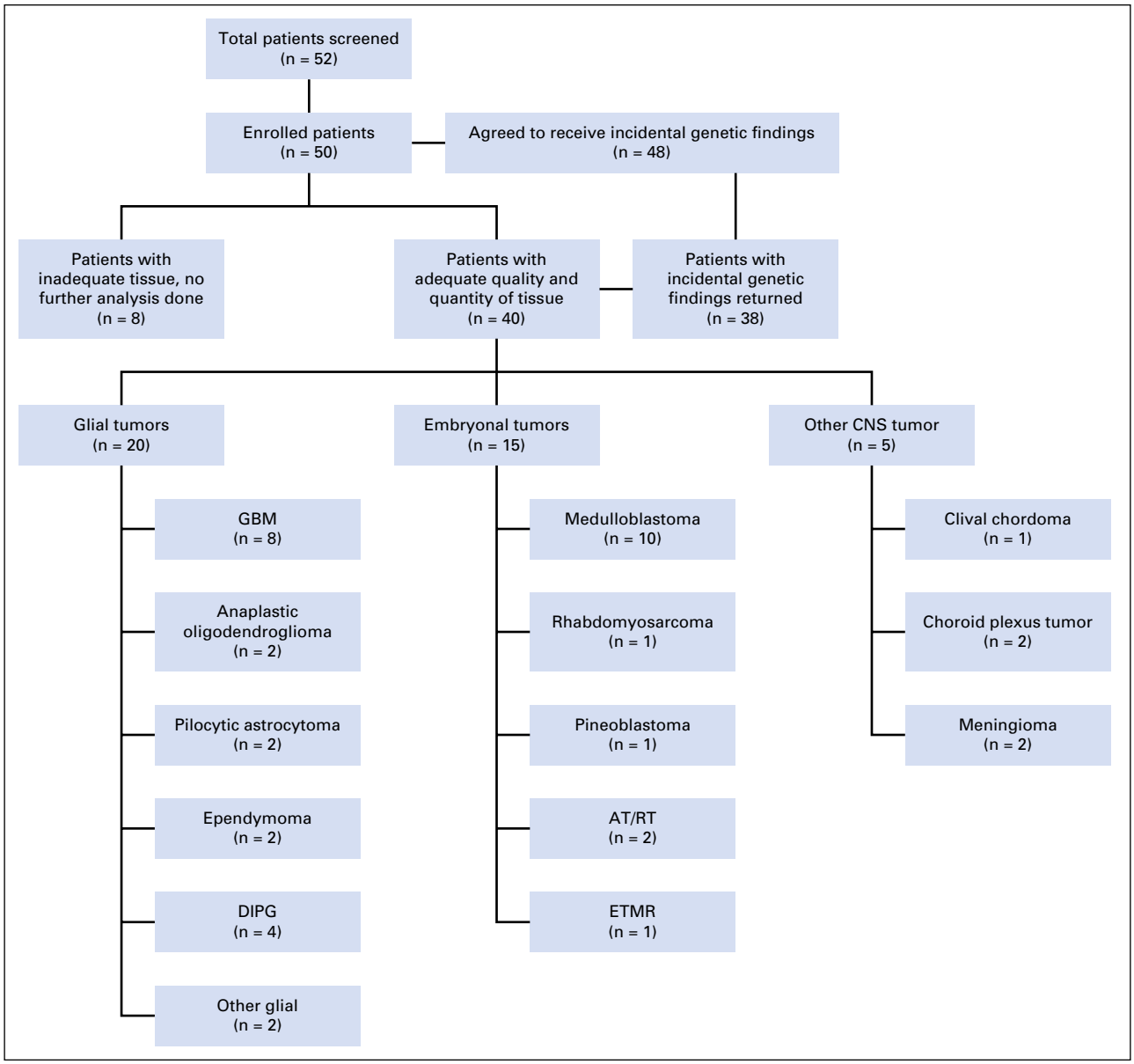


Fig 1. Study enrollment schematic. Children and their families were offered enrollment if they had a diagnosis of a primary brain tumor believed to have at least a 25% chance of treatment failure by their treating oncologist. Approximately half of tumors enrolled were glial by histology. AT/RT, atypical teratoid/rhabdoid tumor; DIPG, diffuse intrinsic pontine glioma; ETMR, embryonal tumor with multilayered rosettes; GBM, glioblastoma multiforme.

actionable mutations per tumor (range, 0 to 8; median, 2). Glial tumors showed more grade I to III mutations per tumor (average, 3.0; range, 0 to 8; median, 3) than embryonal tumors (average, 1.6; range, 0 to 5; median, 1; Data Supplement).

Most targeted therapeutic interventions included use of tyrosine kinase inhibitors, histone deacetylase inhibitors, or mechanistic target of rapamycin inhibitors. Fifteen patients (38%) underwent precision medicine–based therapy with agents selected through the CNS-TAP program, including 10 patients who also underwent radiation and one patient who underwent surgery and radiation (Data Supplement). Our group was heterogeneous in terms of tumor type, time of enrollment (diagnosis or relapse), and whether targeted therapy was given in isolation or was followed by radiation therapy, thus limiting our ability to analyze whether targeted treatments improved treatment outcomes. With the limits of our heterogeneous group, patients with recurrent/refractory brain tumors on targeted therapy had a median progression-free survival (from time on therapy) of 4 months (95% CI, 0.25 to 4 months; Data Supplement).

Despite persistent efforts and referrals to multiple centers with ongoing pediatric brain tumor clinical trials, no patient was able to enroll in a clinical trial on the basis of their sequencing results, and all targeted therapies were used on

Table 1. Feasibility and Actionability of Clinically Integrated Sequencing, by Brain Tumor Subtype

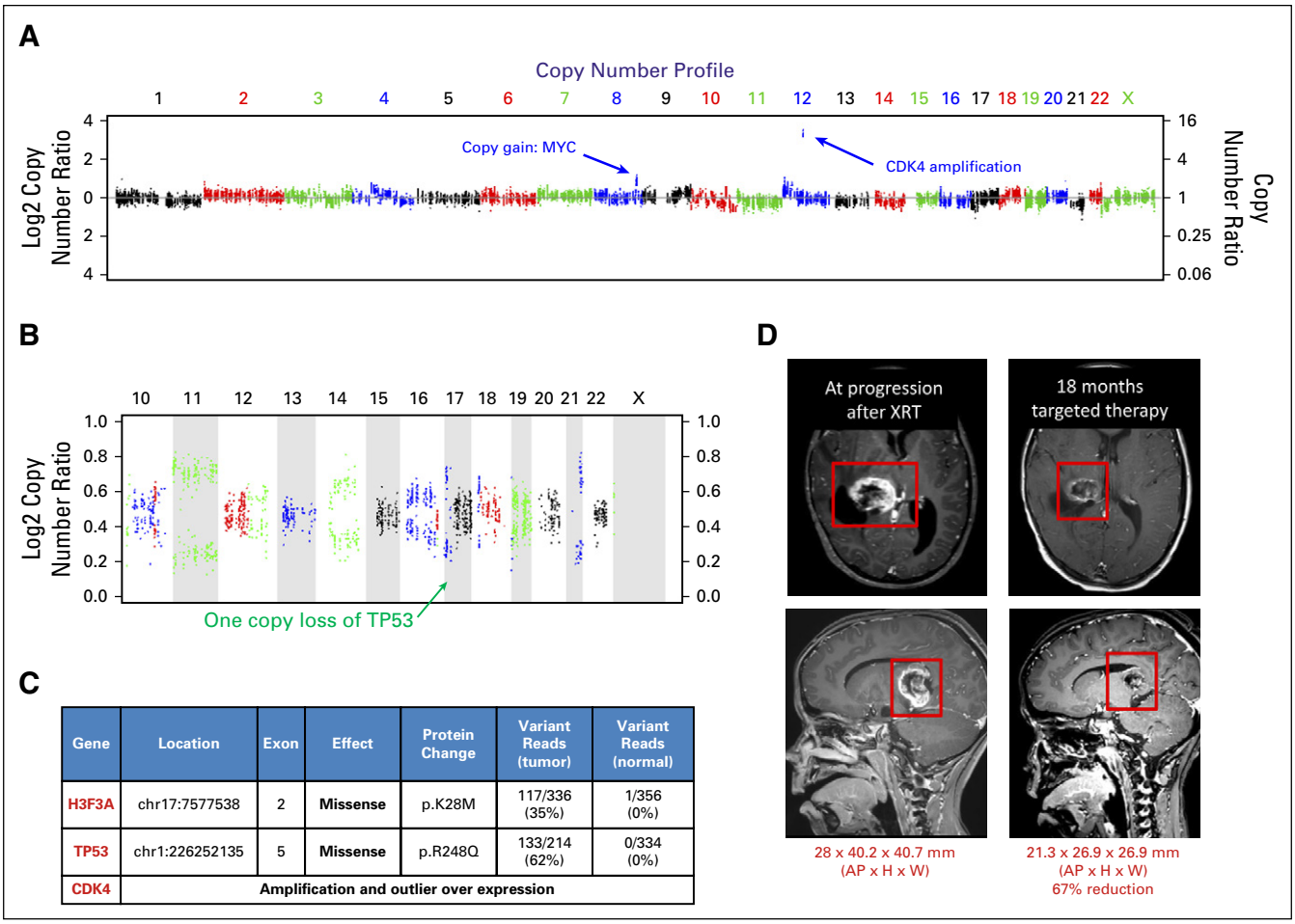
Patient Demographics and Clinical Characteristics (N = 50)	No. (%)	Actionability	No. (%)
Mean age, years	10.2	Tumors with adequate tissue, No.	40
Median age, years	9.0	Germline finding	5 (13)
Histology		Therapy/diagnostic finding	22 (55)
Glial tumors	25 (50)	Any actionable finding	25 (63)
Embyronal	18 (36)	Action taken	21 (53)
Other	7 (14)	Glial tumors, No.	20
Clinical characteristics		Germline finding	1 (5)
Metastatic	13 (26)	Therapy/diagnostic finding	17 (85)
Local	37 (74)	Any actionable finding	17 (85)
At diagnosis	32 (64)	Action taken	14 (70)
At relapse or progression	18 (36)	Embryona, No.	15
Tissue used for sequencing		Germline finding	2 (13)
Original diagnostic material	44 (88)	Therapy/diagnostic finding	3 (20)
Sample obtained at relapse	6 (12)	Any actionable finding	5 (33)
FFPE	36 (72)	Action taken	3 (20)
Frozen tissue	14 (28)	Other tumors, No.	5
Feasibility		Germline finding	2 (40)
Adequate tissue	40 of 50 (80)	Therapy/diagnostic finding	2 (40)
FFPE adequate	26 of 36 (72)	Any actionable finding	3 (60)
Frozen tissue adequate	14 of 15 (96)	Action taken	2 (40)

NOTE: Data presented as No. (%) unless otherwise noted.
Abbreviation: FFPE, formalin-fixed paraffin-embedded.

an off-trial basis. The most frequent reasons cited by clinicians or families of the five patients (of 22) for not pursuing treatment on the basis of potentially actionable findings included selecting a nontargeted salvage chemotherapy regimen (n = 3) or pursuing no therapy in the absence of clear clinical or radiologic progression (n = 1). For one patient with a recurrent medulloblastoma and SHH pathway upregulation, consideration of therapy with a targeted SHH inhibitor, such as vismodegib, was considered, but no clinical trial was available, and the provider and family decided not to pursue off-trial use of the agent.

Actionable Findings

Sequencing was of highest utility in glial tumors. Seventeen of 20 patients (85%) with glial tumors were found to have a potentially actionable result. Fourteen of these patients (70%) underwent a change in therapy on the basis of sequencing results (Table A1). As an example, patient PO-3151 is a 5-year-old with a unilateral thalamic glioblastoma who originally underwent partial resection and focal radiation. He was treated with adjuvant temozolomide and lomustine (CCNU), but magnetic resonance imaging obtained before third cycle (including magnetic resonance spectroscopy) was consistent with tumor progression before third cycle. Sequencing of his tumor revealed *H3F3A* K27M mutation, *TP53* mutation and copy loss, and *CDK4* amplification and outlier expression (all category III; Figs 2A to 2C). After discussion in our precision medicine conference, he was treated with monotherapy on the histone deactylase inhibitor panobinostat three times per week every other week, on the basis of preclinical data supporting its use for DIPG with H3.3 mutations.[18] After establishing that he tolerated this therapy, the cyclin-dependent kinase 4/6 (CDK 4/6) inhibitor palbociclib was added (three times per week, 2 weeks on/2 weeks off), which previously showed efficacy in a pediatric CNS teratoma.[19] He has had an ongoing partial response now for 18 months (25 months from diagnosis; Fig 2D). He has tolerated therapy well, with the



Gene	Location	Exon	Effect	Protein Change	Variant Reads (tumor)	Variant Reads (normal)
H3F3A	chr17:7577538	2	Missense	p.K28M	117/336 (35%)	1/356 (0%)
TP53	chr1:226252135	5	Missense	p.R248Q	133/214 (62%)	0/334 (0%)
CDK4	Amplification and outlier over expression					

Fig 2. Clinical response to panobinostat and palbociclib in H3.3-K27M mutated, cyclin-dependent kinase 4 (CDK4)-amplified glioblastoma. (A) A 5-year-old patient (PO-3151) with a history of unilateral thalamic glioblastoma underwent partial resection and focal radiation. Sequencing of his tumor revealed *CDK4* amplification. (B) Loss of heterozygosity plot shows one copy loss of TP53. (C) Somatic tumor mutations in *H3F3A* K27M mutation and *TP53* mutation and outlier expression (RNA) of CDK4 were also seen. (D) Magnetic resonance imaging of the brain (T1 with contrast) showed increase in size in enhancing mass after three cycles of adjuvant lomustine (CCNU)/temozolomide. He was treated with the oral histone deacetylase (HDAC) inhibitor panobinostat, with later addition of the CDK 4/6 inhibitor palbociclib. After 18 months of targeted therapy, magnetic resonance imaging (T1 with contrast) showed 67% reduction in size of the lesion. TP53, tumor protein 53; XRT, radiation.

exception of mild hyperbilirubinemia and count suppression.

As another example, a 1-year old girl presented with a recurrent posterior fossa glioblastoma multiforme. She originally underwent a gross total resection at 8 months of age, multiagent chemotherapy according to Children's Cancer Group protocol 99703,[20] and then focal radiation therapy with concomitant temozolomide (after initial relapse). The patient developed additional progression 4 months after radiation therapy. Sequencing of her initial tumor revealed significant focal amplification of *PDGFB* (19 copies; Data Supplement), outlier expression of *PDGFB* and *PDGFRA* (Data Supplement), and somatic mutations in *SETD2*, with the loss-of-function mutation in *SETD2* comprising 42% of the sequenced tumor fraction. She was treated with dasatinib for 6 months with no toxicity and resolution of her enhancing lesion on magnetic resonance imaging (Data Supplement). Unfortunately, she developed additional progression and changed to a phase I trial.

Embryonal tumors were found to have potentially actionable results in five of 15 patients (33%). Only two of these patients (13%) underwent a change in their therapy on the basis of sequencing results (Table A1) both cases involved clarification or revision of the original diagnosis.

Thirty-eight (95%) participating patients received germline sequencing results. These patients had both agreed to receive incidental genetic findings and had an adequate tumor sample to complete sequencing. Pathogenic germline findings identified in five patients (13%)

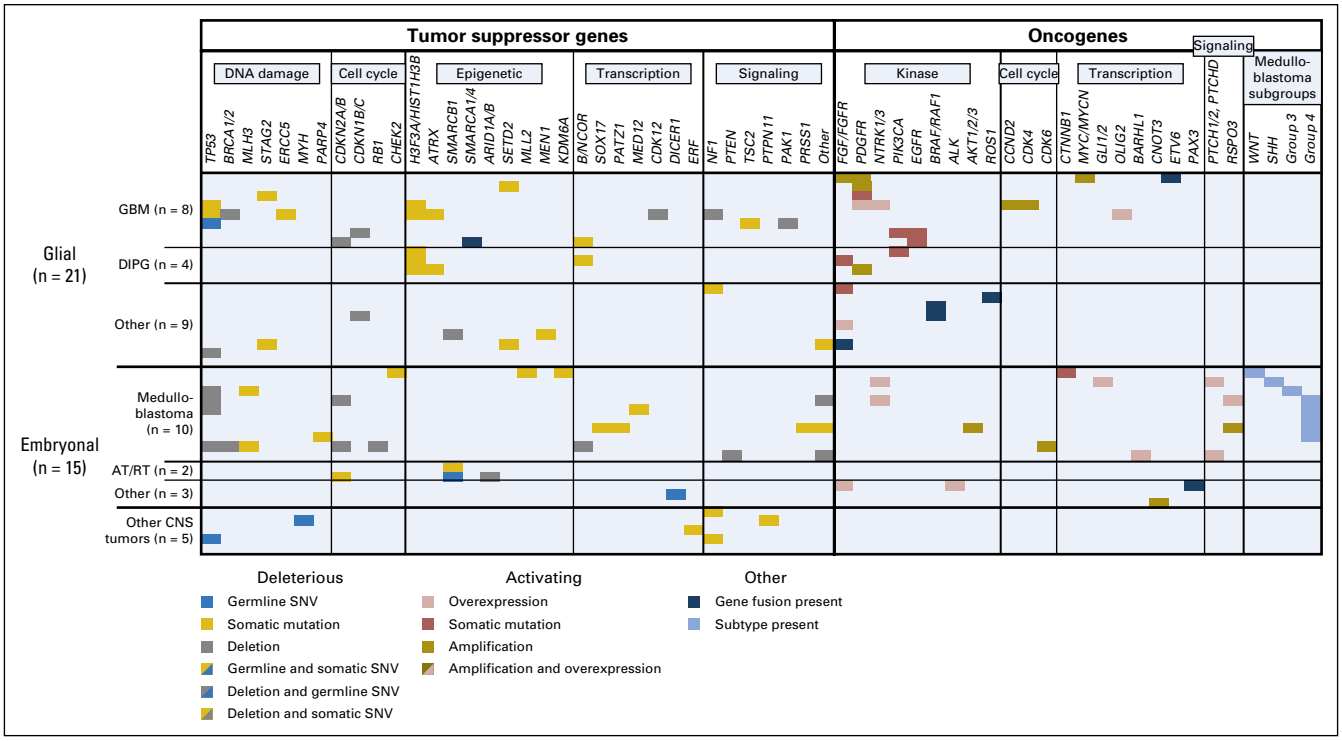


Fig 3. Genomic landscape of high-risk pediatric and young adult patients with brain tumors at diagnosis and recurrence. Activating DNA lesions (mutation, amplification, or fusion) were seen in 19 tumors (48%), most frequently in the mitogen-activated protein kinase signaling and mechanistic target of rapamycin/phosphoinositide 3-kinase pathways. Tumor suppressor mutations or deletions (germline and/or somatic) were seen in 32 tumors (80%), most frequently in DNA damage or apoptotic signaling pathways. AT/RT, atypical teratoid/rhabdoid tumor; DIPG, diffuse intrinsic pontine glioma; GBM, glioblastoma multiforme; SNV, somatic nucleotide variant.

are listed in Table A1. These included mutations associated with established syndromes (*DICER1* syndrome, Li-Fraumeni syndrome, and familial SMARCB1/INI1 deficiency). All patients with a new actionable pathogenic germline variant were seen in a cancer genetics clinic for counseling and additional testing in family members.

Molecular Landscape of Brain Tumors in Cohort

Tumor DNA and RNA sequencing allowed us to explore the molecular landscape of a diverse group of high-risk brain tumors from children and young adults. Activating alterations (mutation, amplification, or fusion) in platelet-derived growth factor receptor (PDGFR) or fibroblast growth factor receptor (FGFR) pathways was seen in nine (45%) of 20 glial tumors (Fig 3), which is higher than previously published in any of these tumor subtypes.[21-23] In addition, we saw potential tumor-driving RNA fusions in eight (20%) tumors, including novel fusions in three tumors (*LRP6-ETV6* fusion in a pediatric glioblastoma [PO-3089], an *FGFR3-PHGDH* fusion in a thalamic anaplastic oligodendroglioma [PO-3124], and an *ELF4-SMARCA* fusion in a bithalamic pediatric glioblastoma [PO-3195]). All genes involved in these two fusions are expressed at approximately 99th percentile in comparison to 1,700 MI-ONCOSEQ samples. The *FGFR3-PHGDH* fusion results in the fusion of *FGFR3* exon 17 to exon 9 of *PHGDH*. The location of the *FGFR3* breakpoint in this fusion is similar to that of reported *FGFR3-TACC3* fusions in adult glioblastoma multiforme.[24] Of note, overexpression of phosphoglycerate dehydrogenase has been shown to be a negative prognostic funding in adult glioma, possibly related to increase in glioma invasion and proliferation.[25]

Sequencing allowed for medulloblastoma molecular group analysis in nine of 10 medulloblastomas with tissue suitable for RNA library formation, by comparison with established groups of highly expressed genes in each group.[26] Our population of medulloblastomas was from all four groups but was enriched for group 4 tumors (Table A1; Data Supplement). One tumor (PO-3063) had expression and copy number attributes of three subgroups (WNT, SHH, and group 3).

DISCUSSION

Younger patients with brain tumor diagnoses die as a result of their disease more commonly than those with any other tumor type.[1] In an effort to tackle this problem, we sought to study a comprehensive integration of precision medicine into the management of this patient population.

A few recent studies have assessed the utility of sequencing pediatric cancers,[11,13,14] including targeted DNA sequencing at diagnosis in pediatric patients with brain tumors.[15] To our knowledge, our study is the first to report on the feasibility of integrating DNA and RNA sequencing into the clinical management of children and young adults with high-risk brain tumors at both diagnosis and relapse, along with their treatment and response. We performed DNA and RNA sequencing and systematic selection of targeted therapies on the basis of discussions in a multi-institutional brain tumor precision medicine conference. Sequencing revealed a potentially actionable germline or somatic alteration in two thirds of brain tumors, of which one third resulted in an impact on treatment or change of diagnosis.

Our results demonstrate the utility of combining tumor exome DNA and RNA sequencing with germline DNA sequencing in pediatric brain tumors. We usually achieved more than 150× to 750× coverage of the tumor exome or 1,700 gene panel, which allowed us to detect subclonal populations < 5%. The brain tumors in our cohort harbored driving tumor mutations not previously known for their tumor type or seen only in a small number (< 5%) of tumors. This finding reinforces the need for broad (exome or > 1,000 gene panel) coverage for tumor profiling in younger patients with brain tumors. In addition, this series supports the use of RNA-seq, which identified fusions in 20% of tumors, allowed us study gene expression levels, and also helped us with identification of medulloblastoma subgroups. Germline sequencing revealed germline cancer predisposition alterations in 13% of tumors, which is consistent with previous studies of pediatric malignancies that have included germline sequencing.[11,13] Our median turnaround time was suboptimal. Future efforts for molecular profiling will be clinically more relevant if available within 1 to 2 weeks, and novel platforms have been able to bring the time down to days.[27]

The brain tumors in our younger patient cohort were found to have relatively few somatic mutational events, as compared with mutational surveys of adult brain tumors, which are more heavily mutated.[28,29] As seen in previous surveys of pediatric cancer,[2,12] the somatic mutations that were present in our tumors, especially in our gliomas, were frequently targetable with personalized clinically available agents. Of note, 14 of 17 (83%) of our patients who underwent therapy changes on the basis of sequencing had a high-grade glial tumor, whereas only one of 23 (4%) of our patients who did not undergo therapy change had a high-grade glial tumor. The lack of efficacious treatments or available clinical trials for many of our patients with brain tumors influenced clinicians' interest in pursuit of targeted therapies in this patient population. The multidisciplinary and multi-institutional conference also provided a consensus opinion that improved clinician and patient/family comfort with nonstandard therapy. Although no formal survey was performed on this topic, multiple families expressed comfort with pursuing a targeted therapy without a formal second opinion because of the attendance and input from clinicians from multiple other children's hospitals around the country.

Pediatric and young adult patients with GBM and DIPG highlight the unique opportunities and drawbacks of precision medicine in this patient population. In our 12 patients with these diagnoses (and adequate tissue), all harbored potentially actionable mutations by our criteria, for which therapy was altered in 11. However, all of these tumors harbored multiple consensus cancer gene alterations (category I to III), with an average of 3.8 per tumor, and one with eight consensus cancer gene alterations. The selected targeted therapy may have led to the evolution of resistant subclonal tumor cell populations in tumors that progressed.[30] Recent studies have shown a variable degree of heterogeneity within pediatric and adult high-grade gliomas, which could account for treatment failure with the use of a single agent.[31,32] Current and future clinical trials will approach some of these issues by starting with multiple agents targeting nonredundant pathways.[30]

The timing of the biopsy is also likely important, because gliomas have also been shown to develop numerous mutations in response to cytotoxic chemotherapy and radiation.[9] As a limitation of our study, a minority of our patients with progressive tumors underwent biopsy or resection at progression. Nevertheless, targeted therapies chosen for some of these patients in our study contributed to potential clinical benefit (although response attribution is uncertain because of previous irradiation in many cases). Therapies were generally administered orally and on an outpatient basis. Targeted agents were monitored for toxicity by standard-of-care guidelines (off study),

with reduction or holding dose for grade 3 or 4 adverse events. None of the patients receiving targeted therapy had grade 3 or 4 adverse events, cytopenia requiring transfusion, or admission for fever and neutropenia.

The CNS-TAP tool provided a useful framework for selection of targeted agents on the basis of previously published data and the likelihood of BBB penetration. Despite this, the molecular tumor board was often conflicted after discussion of multiple promising agents, and the reproducibility of the board's recommendations was not always fully consistent. Our group is currently optimizing the tool to provide a numerical score for all relevant agents that will be available to clinical researchers as a Web-based tool. We are also developing a prospective trial, which will compare CNS-TAP–selected therapy to clinician choice, as well as measuring outcomes for a more homogenous cohort prospectively compared with historical controls. These studies will be crucial to our understanding of the true value of targeted therapy for children with brain tumors.

In summary, selection of personalized agents on the basis of integrative clinical sequencing of high-risk brain tumors of children and young adults is feasible and frequently results in change of management. Our study highlights the utility of this approach, particularly in glial tumors. Going forward, continuing efforts to match therapies to the molecular profile of individual tumors should lead to improved outcomes for younger patients with brain tumors. Our results demonstrate the promise of this approach.

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Table A1. Clinically Integrated Sequencing Results for Patients

Patient ID	Diagnosis	Age at Enrollment (years)	Informative and Actionable Genomic Findings	Incidental Germline Findings	Standard Therapy Without Sequencing	Potential Actions Based on Sequencing Results	Actionable Finding Grade[13]	Action Taken	Clinical Actions and Outcome
Glial tumors									
PO-3089	GBM, progressive	1	**PDGFRA**, MYC, PVT1, CHIC2, RBPJ, FGF2, ING4, ZNF384 **amplification**; LRP6-ETV6 fusion; PDGFRA, MYC, PVT1, CHIC2, RBPJ, FGF2, ING4, ZNF384 overexpression	None	Clinical trial for relapsed brain tumors *v* palliative radiation	PDGFR inhibitors	**PDGRFA = 3** MYC = 3 PVT1 = 4 CHIC2 = 3 RBPJ = 4 FGF2 = 4 ING42 = 4 ZNF384 = 3 LRP6 = 4 ETV6 = 3	Yes	Patient died of progressive disease within 2 weeks of starting dasatinib
PO-3118	Pilocytic astrocytoma, progressive	4	**KIAA1549-BRAF fusion**	None	Clinical trial for relapsed brain tumors *v* salvage chemotherapy	BRAF inhibitor or MEK inhibitor targeting MAP kinase pathway	**BRAF (not V600E) = 3**	No	Patient pursuing salvage chemotherapy (nontargeted)
PO-3119	Ependymoma, at diagnosis	6	Chr17p copy loss (includes TP53), chr17q copy gain (includes ERBB2—not overexpressed)	None	Radiation with or without chemotherapy	Not actionable	TP53 = 3 ERBB2 = 2/3 17p and 17q = 4	N/A	Patient in clinical remission after chemotherapy and radiation

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Table A1. Clinically Integrated Sequencing Results for Patients (Continued)

Patient ID	Diagnosis	Age at Enrollment (years)	Informative and Actionable Genomic Findings	Incidental Germline Findings	Standard Therapy Without Sequencing	Potential Actions Based on Sequencing Results	Actionable Finding Grade[13]	Action Taken	Clinical Actions and Outcome
PO-3126	Pilocytic astrocytoma, progressive	9	Neuropathology determined tissue inadequate	N/A	Clinical trials for relapsed brain tumors *v* salvage chemotherapy	N/A	N/A	N/A	Patient pursuing salvage chemotherapy (nontargeted)
PO-3127	Anaplastic astrocytoma, at diagnosis	5	Neuropathology determined tissue inadequate	N/A	Chemotherapy *v* focal radiation	N/A	N/A	N/A	Patient in clinical remission for 2 years after therapy with temozolomide and bevacizumab
PO-3129	Glioneuronal tumor with astrocytic features, at recurrence	13	Neuropathology determined tissue inadequate	None	Resection; consideration of focal radiation	N/A	N/A	No	Patient in clinical remission for 18 months after resection and focal radiation
PO-3143	Ependymoma, recurrent	13	Chr 1q, 3, 6, 9, 10q, 12, 15, 17, 19, 20, 22 copy gain	None	Resection; consideration of focal radiation	Not actionable	{1q, 3, 6, 9…} = 4	N/A	Patient in clinical remission after resection and focal radiation

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Table A1. Clinically Integrated Sequencing Results for Patients (Continued)

Patient ID	Diagnosis	Age at Enrollment (years)	Informative and Actionable Genomic Findings	Incidental Germline Findings	Standard Therapy Without Sequencing	Potential Actions Based on Sequencing Results	Actionable Finding Grade[13]	Action Taken	Clinical Actions and Outcome
PO-3151	GBM, at diagnosis	6	**CDK4 amplification**, CCND2 copy gain, TP53 one copy loss; TP53 (p.R248Q) mutation, **H3F3A (p.K28M, K27M)** point mutation copy neutral LOH; CDK4, CCND2, PDGFRA, NTRK2 overexpression	None	Radiation, followed by consideration of maintenance temozolomide	CDK inhibitors, epigenetic inhibitors	**CDK4 = 3** CCND2 = 3 TP53 = 3 **H3F3A = 3** PDGFRA= 3 NTRK2 = 2	Yes	Patient with stable disease post XRT; adjuvant therapy for 18 months with panobinostat and palbociclib with partial response, 25 months from diagnosis
PO-3152	DIPG, at diagnosis	5	H3F3A copy gain (6 copies); **HIST1H3B (p.K28M) hotspot mutation, PIK3CA (p.E545K) hotspot activating mutation** and (p.C378R) recurrent	None	Radiation, followed by clinical trial for DIPG *v* surveillance	PI3K inhibitors (including mTOR inhibitors), epigenetic inhibitors	H3F3A = 3 **HIST1H3B = 4** **PIK3CA = 2/3**	Yes	Patient completed radiation; adjuvant everolimus for 2 months; progression, followed by repeat radiation; adjuvant therapy with everolimus/ panobinostat; partial response after 9 months, 18 months from diagnosis, then progressive disease

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Table A1. Clinically Integrated Sequencing Results for Patients (Continued)

Patient ID	Diagnosis	Age at Enrollment (years)	Informative and Actionable Genomic Findings	Incidental Germline Findings	Standard Therapy Without Sequencing	Potential Actions Based on Sequencing Results	Actionable Finding Grade[13]	Action Taken	Clinical Actions and Outcome
PO-3159	GBM, recurrent	1	**PDGFB amplification**; SETD2 (p.S396*) mutation; **PDGFB**, **PDGFRA overexpression**	None	Consideration of clinical trial for relapsed brain tumors	PDGF inhibitors	**PDGFB = 3** SETD2 = 3 **PDGFRA = 3**	Yes	Patient progressed after radiation; started on adjuvant therapy with dasatinib; partial response for 6 months; progressive disease; currently enrolled on phase I trial (nontargeted)
PO-3163	Anaplastic oligodendroglioma (spinal), recurrent	8	**FGFR1 (p.K655I and p.K656E) activating mutations**, NF1 (p.S1391*) mutation; NF1 fs deletions (p.T1541fs, p.1155_1161del, p.1575_1594del), likely biallelic inactivation	None	Clinical trial for relapsed brain tumors *v* palliative radiation	FGF inhibitors, NF1-tareted therapy (including mTOR inhibitor)	**FGFR1 = 2/3** NF1 = 3	Yes	Patient completed radiation; then progressive disease at 6 months; then treated with everolimus with stable disease for 2 months, 5 months from diagnosis of progression

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Table A1. Clinically Integrated Sequencing Results for Patients (Continued)

Patient ID	Diagnosis	Age at Enrollment (years)	Informative and Actionable Genomic Findings	Incidental Germline Findings	Standard Therapy Without Sequencing	Potential Actions Based on Sequencing Results	Actionable Finding Grade[13]	Action Taken	Clinical Actions and Outcome
PO-3164	DIPG, at diagnosis	13	H3F3A (p.K28M) hotspot activating, **FGFR3 (p.K650E) hotspot activating mutations**; BCOR fs insertion (p.Ser1676fs) and fs deletion (p.Gly421fs)	None	Radiation, followed by clinical trial for DIPG *v* surveillance	FGF inhibitors, epigenetic inhibitors	H3F3A = 3 **FGFR3 = 2/3** BCOR = 3	Yes	Patient completed radiation; adjuvant therapy with ponatinib; progressive disease after 6 months, 10 months from diagnosis
PO-3176	GBM, at diagnosis	15	Chr 1, 7, 9, 18, X copy gain, Chr 19, 22 copy loss, homozygous deletion of PAK1; **TSC2 inactivating mutation**	**TP53 exon 1 and 2 deletion**	Radiation, followed by consideration of maintenance temozolomide	mTOR inhibitor; family referral to genetic counseling	{1, 7, 9, 18…} = 4 PAK1 = 4 **TSC2 = 3** **TP53 = 3**	Yes	Family seen in genetics clinic for genetic counseling for Li-Fraumeni syndrome. Patient completed radiation; adjuvant therapy with Novocure and everolimus with stable disease/NED for 8 months, 16 months from diagnosis

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Table A1. Clinically Integrated Sequencing Results for Patients (Continued)

Patient ID	Diagnosis	Age at Enrollment (years)	Informative and Actionable Genomic Findings	Incidental Germline Findings	Standard Therapy Without Sequencing	Potential Actions Based on Sequencing Results	Actionable Finding Grade[13]	Action Taken	Clinical Actions and Outcome
PO-3184	GBM, at diagnosis	17	**H3F3A (p.K28M) hotspot mutation**, TP53 (p.H179R) copy neutral LOH mutation; ATRX (p.D1313fs) recurrent loss, ERCC5 (p.K917fs) subclonal; NF1 homozygous loss, BRCA1, CDK12 copy loss; OLIG2 overexpression	None	Radiation, followed by consideration of maintenance temozolomide	Epigenetic inhibitors, NF1-tareted therapy (including MEK inhibitor)	**H3F3A = 3** TP53 = 3 ATRX = 3 ERCC5 = 3 **NF1 = 3** BRCA1 = 3 CDK12 = 3 OLIG2 = 3	Yes	Patient completed radiation; adjuvant therapy with vorinostat and trametinib, with partial response for 5 months, then developed progressive disease

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Table A1. Clinically Integrated Sequencing Results for Patients (Continued)

Patient ID	Diagnosis	Age at Enrollment (years)	Informative and Actionable Genomic Findings	Incidental Germline Findings	Standard Therapy Without Sequencing	Potential Actions Based on Sequencing Results	Actionable Finding Grade[13]	Action Taken	Clinical Actions and Outcome
PO-3195	GBM (bithalamic), at progression	11	**EGFR: in-frame deletion, p.T483_G485del; BCOR (on chr X): frameshift deletion, p.T269fs; CDKN2C: homozygous deletion**; ELF4: Homozygous deletion; ELF4-SMARCA1 fusion; EGFR: p.V292L FUBP1: splice donor, p.Q40	None	Radiation, followed by clinical trial for relapsed brain tumors *v* salvage chemotherapy	EGFR inhibitors, CDK4/6 inhibitors	**EGFR = 3** **BCOR = 3** **CDKN2C = 3** ELF4 = 3 SMARCA1 = 3 FUBP1 = 3	Yes	Patient completed radiation; adjuvant therapy with osimertinib with partial response for 3 months, now 6 months from diagnosis
TP-2128	Pilocytic astrocytoma, recurrent	33	CDKN2A/2B homozygous deletion, Chr8p, 9p, 14, 16q, 17p, 20p, Xq one copy loss; **KIAA1549-BRAF (in-frame with intact BRAF kinase domain) fusion**	None	Clinical trials for relapsed brain tumors *v* salvage chemotherapy	BRAF inhibitor or MEK inhibitor targeting MAP kinase pathway	CDKN2A = 3 CDKN2B = 4 Other genes = 4 **BRAF (not V600E) = 3**	No	Patient pursuing salvage (nontargeted) chemotherapy

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Table A1. Clinically Integrated Sequencing Results for Patients (Continued)

Patient ID	Diagnosis	Age at Enrollment (years)	Informative and Actionable Genomic Findings	Incidental Germline Findings	Standard Therapy Without Sequencing	Potential Actions Based on Sequencing Results	Actionable Finding Grade[13]	Action Taken	Clinical Actions and Outcome
TP-2160	GBM, at diagnosis	39	**EGFR high amplification,** CDKN2A/2B, MTAP homozygous deletion, Chr7 gain, Chr10 loss; **PIK3CA (p.R88Q) mutation**, **EGFR in frame deletion (exon 2-7, EGFRvIII);** many fusions associated with EGFR amplification, e.g. SPTLC2-PRKD1; **EGFR overexpression**	None	Radiation, followed by consideration of maintenance temozolomide	EGFR inhibitor or EGFR antibody; PI3K inhibitor	**EGFR = 3** CKDN2A = 3 CDKN2B = 4 MTAP = 4 {Chr7, Chr10} = 4 **PIK3CA = 2/3** SPTLC2 = 4 PRKD1 = 4	No	Patient pursuing phase II clinical trial (nontargeted)

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Table A1. Clinically Integrated Sequencing Results for Patients (Continued)

Patient ID	Diagnosis	Age at Enrollment (years)	Informative and Actionable Genomic Findings	Incidental Germline Findings	Standard Therapy Without Sequencing	Potential Actions Based on Sequencing Results	Actionable Finding Grade[13]	Action Taken	Clinical Actions and Outcome
TP-2190	Anaplastic astroblastoma, recurrent	29	MEN1 (pQ64*) stopgain LOH by copy loss (Vaf 98%); TP53 (p.F113_V122 delinsL, COSMIC recurrent) LOH by copy loss (Vaf 100%); **SMARCB1 homozygous deletion**, Chr6p, 9q, 14, 17p, 20q copy loss, chr 7p, 8, 11 copy gain	None	Radiation, followed by clinical trial for relapsed brain tumors *v* salvage chemotherapy	Agents targeting INI1-deficient tumor: EZH2 inhibitor or aurora kinase inhibitor	MEN1 = 3 TP53 = 3 **SMARCB1 = 3** Other genes = 4	Yes	Patient completed radiation; adjuvant therapy with alisertib (aurora kinase inhibitor) compassionate use for 1 week, then developed progressive disease
PO-3205	Granular cell astrocytoma, at diagnosis (diagnosis unclear from pathology)	16	No DNA lesions; FGFR2, GRM3, CDK18 outlier increased expression	None	No additional therapy	Not actionable	None	N/A	Patient in surveillance, without evidence of recurrence

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Table A1. Clinically Integrated Sequencing Results for Patients (Continued)

Patient ID	Diagnosis	Age at Enrollment (years)	Informative and Actionable Genomic Findings	Incidental Germline Findings	Standard Therapy Without Sequencing	Potential Actions Based on Sequencing Results	Actionable Finding Grade[13]	Action Taken	Clinical Actions and Outcome
PO-3213	GBM, at diagnosis	17	**PDGFRA (p.V536E, p.P577R) mutation,** STAG2 p.R263W, nonrecurrent; Copy gain: chr1q, chr17; copy loss: chr1p, chr13, chr15	None	Radiation, followed by consideration of maintenance temozolomide	PDGF inhibitors	**PDGFRA = 3** STAG2 = 4 {Chr1q, Chr17 ...} = 4	Yes	Patient completed radiation; adjuvant therapy with dasatinib, partial response for 5 months, 7 months from diagnosis, then died as a result of progressive disease
PO-3214	Anaplastic oligodendroglioma, progressive (diagnosis unclear from pathology)	11	FBXW7: p.R465H; STAG2: p.R110* SETD2: splice donor, p.R2165 VAV1: p.P657A; **FGFR3: copy gain;** CDK11A/B: homozygous deletion; gain: 1q and 7q; loss: 1p, 4p15-16, 6q and 10q; **FGFR3-PHGDH** an activating in-frame fusion.	None	Resection; consideration of focal radiation	FGF inhibitors	FBXW7 = 3 STAG2 = 4 SETD2 = 3 VAV1 = 4 **FGFR3 = 2/3** CDK11A/B = 4 PHGDH = 4 {Chr1q, Chr7q...} = 4	Yes	Patient completed radiation; adjuvant therapy with ponatinib with PR for 2 months, 7 months from diagnosis of recurrence

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Table A1. Clinically Integrated Sequencing Results for Patients (Continued)

Patient ID	Diagnosis	Age at Enrollment (years)	Informative and Actionable Genomic Findings	Incidental Germline Findings	Standard Therapy Without Sequencing	Potential Actions Based on Sequencing Results	Actionable Finding Grade[13]	Action Taken	Clinical Actions and Outcome
PO-3227	DIPG, at diagnosis	4	Neuropathology determined tissue inadequate	N/A	Radiation, followed by clinical trial for DIPG *v* surveillance	N/A	N/A	N/A	Patient completed radiation; currently in follow-up
PO-3250	DIPG, at diagnosis	7	**H3F3A p.K28M**, ATRX p.Q119* stopgain; PPM1D p.G463fs deletion, subclonal; amplification-**PDGFRA**, KIT, KDR; PDGFRA, PARP1 overexpression	None	Radiation, followed by clinical trial for DIPG *v* surveillance	PDGF inhibitors; epigenetic inhibitors	**H3F3A = 3** ATRX = 3 PPM1D = 4 **PDGFRA = 3** KIT = 3 KDR = 2/3 PARP1 = 4	Yes	Patient completed radiation; adjuvant therapy with dasatinib (< 2 months)

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Table A1. Clinically Integrated Sequencing Results for Patients (Continued)

Patient ID	Diagnosis	Age at Enrollment (years)	Informative and Actionable Genomic Findings	Incidental Germline Findings	Standard Therapy Without Sequencing	Potential Actions Based on Sequencing Results	Actionable Finding Grade[13]	Action Taken	Clinical Actions and Outcome
PO-3256	DIPG, at diagnosis	8	**H3F3A p.K28M, PIK3CA p.E545K,** TCF12 p.V650D nonrecurrent, potentially deleterious, CLIP1 p.R59* stop gain, CUL4B p.G638R missense; Olig2 overexpression	None	Radiation, followed by clinical trial for DIPG *v* surveillance	PI3K inhibitors (including mTOR inhibitors), epigenetic inhibitors	**H3F3A = 3** **PIK3CA = 2/3** TCF12 = 3 CLIP1 = 4 CUL4B = 4	Yes	Patient completed radiation; adjuvant therapy with everolimus (< 2 months)
Embryonal tumors									
PO-3057	Rhabdomyosarcoma (initial diagnosis medulloblastoma)	3	**C**hr1q, 5p copy gain, Chr7p, 16q copy loss; **PAX3-NCOA2 fusion**; MYOG, MYOD1, DES (rhabdomyosarcoma markers), FGF7, FGF8, FGF9, FGFR4, ALK overexpression	None	Clinical trial for relapsed medulloblastoma *v* salvage chemotherapy	Change in diagnosis and treatment of CNS rhabdomyosarcoma	**PAX3 = 3** **NCOA2 = 3** MYOG = 4 MYOD1 = 4 DES = 4 FGF7 = 4 FGF8 = 4 FGF9 = 4 FGFR4 = 2 ALK = 2/3 {Chr7p, Chr16q...} = 4	Yes	Patient pursued therapy for CNS rhabdomyosarcoma; remained in remission 6 months, then developed progressive disease

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Table A1. Clinically Integrated Sequencing Results for Patients (Continued)

Patient ID	Diagnosis	Age at Enrollment (years)	Informative and Actionable Genomic Findings	Incidental Germline Findings	Standard Therapy Without Sequencing	Potential Actions Based on Sequencing Results	Actionable Finding Grade[13]	Action Taken	Clinical Actions and Outcome
PO-3063	Medulloblastoma, at diagnosis	6	ADAM3A homozygous deletion, PTEN one copy loss; group indeterminate (over expression of genes in SHH, group 3 and WNT)	None	Radiation, followed by maintenance multi-agent chemotherapy	Not actionable	ADAM3A = 4 PTEN = 2/3 PTCHD2 = 4 DISP3 = 4 BARHL1 = 4	N/A	Patient in clinical remission after therapy for high-risk medulloblastoma
PO-3067	Medulloblastoma, recurrent	9	Gain of chr4p, 7, 17q gain; loss of chr11p, 17p; CDKN1C one copy loss, TP53 one copy loss, KCTD11 one copy loss, group 4 by expression profile	None	Clinical trial for relapsed medulloblastoma *v* salvage chemotherapy	Not actionable	{4p, 7, 17q…} = 4 CDKN1C = 4 TP53 = 3 KCTD11 = 4 RSPO3 = 4 NTRK3 = 3 GRM1 = 4 GRM8 = 4 KCNA1 = 4 KCNA5 = 4	N/A	Patient pursued multiple (nontargeted) clinical trials; died after 6 months
PO-3068	AT/RT, at diagnosis	4	**SMARCB1 frameshift deletion, (deletion of exon 2) and LOH**	None	Multi-agent chemotherapy, consideration of radiation	Not actionable	**SMARCB1 = 3**	N/A	Patient in clinical remission for 5 years after high-dose chemotherapy

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Table A1. Clinically Integrated Sequencing Results for Patients (Continued)

Patient ID	Diagnosis	Age at Enrollment (years)	Informative and Actionable Genomic Findings	Incidental Germline Findings	Standard Therapy Without Sequencing	Potential Actions Based on Sequencing Results	Actionable Finding Grade[13]	Action Taken	Clinical Actions and Outcome
PO-3069	Medulloblastoma, recurrent	4	**SHH group** by expression profile; additional overexpression of ERBB4, NTRK1, NTRK3	None	Clinical trial for relapsed medulloblastoma *v* salvage chemotherapy	SHH inhibitor	{8, 17p, 12q...} = 4	No	No SHH inhibitors in clinical trial and no dosing information available for off-label use in children. Patient died as a result of relapsed disease 13 months after sequencing results returned.
PO-3073	Medulloblastoma, recurrent	9	MLH3 (p.Q1164*) mutation; SHFM1, SAMD9, CDK6, AKAP9 amplification; Chr4, 7, 17q copy gain; Chr11p, 13p, 17p copy loss; likely group 3 or 4 by i(17q) (unable to create RNA library)	None	Clinical trial for relapsed medulloblastoma *v* salvage chemotherapy	Not actionable	MLH3 = 4 SHFM1 = 4 SAMD9 = 4 CDK6 = 3 AKAP9 = 3 Other genes {Ch4, 7, 17q…} = 4 CDKN1C = 4 RB1 = 3 BRCA2 = 3 TP53 = 3 NCOR1 = 4	N/A	Patient died as a result of progressive disease after 4 months
PO-3077	PNET, at diagnosis	12	Neuropathology determined tissue inadequate	N/A	Radiation, followed by maintenance multi-agent chemotherapy	N/A	N/A	N/A	Patient in clinical remission after therapy for high-risk embryonal tumor

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Table A1. Clinically Integrated Sequencing Results for Patients (Continued)

Patient ID	Diagnosis	Age at Enrollment (years)	Informative and Actionable Genomic Findings	Incidental Germline Findings	Standard Therapy Without Sequencing	Potential Actions Based on Sequencing Results	Actionable Finding Grade[13]	Action Taken	Clinical Actions and Outcome
PO-3078	Medulloblastoma, recurrent	13	Chr8, chr17p loss, chr12q, 17q gain; Group 4 by expression profile		Clinical trial for relapsed medulloblastoma *v* salvage chemotherapy	Not actionable	Other genes {8, 17p, 12q...} = 4	N/A	Patient died as a result of progressive disease after 10 months
PO-3116	Pineoblastoma, recurrent	5	***DICER1* fs deletion (p.L1469fs)**	***DICER1* (p.S1585*)**	Clinical trial for relapsed embryonal tumor *v* salvage chemotherapy	Family referral to genetic counseling	*DICER1* = 3	Yes	Family referral to genetics clinic for genetic counseling on *DICER1* syndrome; patient in clinical remission after nontargeted clinical trial (chemotherapy) and surgery
PO-3132	Medulloblastoma, at diagnosis	12	Neuropathology determined tissue inadequate	N/A	In follow-up	N/A	N/A	N/A	Patient in clinical remission after therapy for high-risk medulloblastoma

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Table A1. Clinically Integrated Sequencing Results for Patients (Continued)

Patient ID	Diagnosis	Age at Enrollment (years)	Informative and Actionable Genomic Findings	Incidental Germline Findings	Standard Therapy Without Sequencing	Potential Actions Based on Sequencing Results	Actionable Finding Grade[13]	Action Taken	Clinical Actions and Outcome
PO-3137	Medulloblastoma, at diagnosis	8	Chr6 copy loss, Chr8 copy gain; CTNNB1 (p.S33F) activating mutation, KMT2D/MLL2 (p.R5454*), KDM6A/UTX (p.Q1385*) mutations; CHEK2 stopgain (p.D336_A337del,insX); WNT group by expression profile	None	In follow-up	Not actionable	{chr6, chr8} = 4	N/A	Patient in clinical remission after therapy for standard-risk medulloblastoma
							CTNNB1 p.S33F = 1		
							KMT2D = 3		
							MLL2 = 4		
							KDM6A = 3		
							UTX = 4		
							CHEK2 = 3		
							DKK2 = 4		
							WIFI1 = 4		

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Table A1. Clinically Integrated Sequencing Results for Patients (Continued)

Patient ID	Diagnosis	Age at Enrollment (years)	Informative and Actionable Genomic Findings	Incidental Germline Findings	Standard Therapy Without Sequencing	Potential Actions Based on Sequencing Results	Actionable Finding Grade[13]	Action Taken	Clinical Actions and Outcome
PO-3145	Medulloblastoma, at diagnosis (initial diagnosis unclear *v* high-grade glioma)	14	PRSS1 (p.K170E and p.S181N), BLNK (p.P246S), PATZ1 (p.531A), SOX17 (p.G201S) mutations; chr6, 7, 12, 16p, 17q, 18 copy gain, chr16q copy loss; group 4 by expression profile	None	Radiation therapy; maintenance chemotherapy for high-grade glioma	Change in therapy to high-risk medulloblastoma protocol	PRSS1 = 4 BLNK = 4 PATZ1 = 3 SOX17 = 4 {6, 7, 12, 16p…} = 4 AKT3 = 4 RSPO3 = 4	Yes	Patient in clinical remission after therapy for high-risk medulloblastoma
PO-3170	Medulloblastoma, at diagnosis	13	Chr17p copy loss, chr17q copy gain; MED12 (p.D1669H) mutation; group 4 by expression profile	None	Radiation, followed by maintenance multi-agent chemotherapy	Not actionable	{17p, 17q} = 4 MED12 = 3	N/A	Patient in clinical remission after therapy for standard-risk medulloblastoma
PO-3173	Medulloblastoma, at diagnosis	6	Chr7, 14q, 17q copy gain; chr10q, 17p copy loss; group 3 by expression profile	None	Radiation, followed by maintenance multi-agent chemotherapy	Not actionable	{7, 14q, 17q…} = 4	N/A	Patient in clinical remission after therapy for high-risk medulloblastoma

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Table A1. Clinically Integrated Sequencing Results for Patients (Continued)

Patient ID	Diagnosis	Age at Enrollment (years)	Informative and Actionable Genomic Findings	Incidental Germline Findings	Standard Therapy Without Sequencing	Potential Actions Based on Sequencing Results	Actionable Finding Grade[13]	Action Taken	Clinical Actions and Outcome
PO-3160	AT/RT, recurrent	2	Chr6q copy loss (ARID1B, FOXO3, HDAC2, etc.), Ch22 copy loss (EP300, NF2, **SMARCB1**, etc.); CDKN1C fs insertion (p.A17fs)	**SMARCB1 (deletion of exon 7)**	Radiation therapy plus consideration of clinical trials for relapsed brain tumors *v* salvage chemotherapy	Family referral to genetic counseling	ARID1B = 4 FOXO3 = 3 HDAC2 = 4 EP300 = 3 NF2 = 3 **SMARCB1 = 3** CDKN1C = 4	No	Patient previously seen in genetics clinic for genetic counseling for familial rhabdoid tumors. Patient has stable disease, currently pursuing salvage chemotherapy 13 months from diagnosis.
PO-3161	Medulloblastoma, at diagnosis (later developed secondary osteosarcoma)	14	Neuropathology determined tissue inadequate	N/A	Radiation, followed by maintenance multi-agent chemotherapy	Not actionable	N/A	N/A	Patient in clinical remission after therapy for high-risk medulloblastoma (followed by therapy for secondary malignancy)
PO-3186	Medulloblastoma, at diagnosis (later developed secondary osteosarcoma)	15	PARP4 (p.L1080F); copy gain: chr1q, 7, 12q, 17q; copy loss: chr8, 16q; LOH: chr17p; group 4 by expression profile	None	Radiation, followed by maintenance multi-agent chemotherapy	Not actionable	PARP4 = 4 {1q,12q,17q,16q…}: 4	N/A	Patient in clinical remission after therapy for high-risk medulloblastoma (followed by therapy for secondary malignancy)

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Table A1. Clinically Integrated Sequencing Results for Patients (Continued)

Patient ID	Diagnosis	Age at Enrollment (years)	Informative and Actionable Genomic Findings	Incidental Germline Findings	Standard Therapy Without Sequencing	Potential Actions Based on Sequencing Results	Actionable Finding Grade[13]	Action Taken	Clinical Actions and Outcome
PO-3241	Embryonal tumor with multilayered rosettes, at diagnosis	4	Chr 19 amplification, including CNOT3 (plus outlier expression)	None	Multi-agent chemotherapy, with or without radiation therapy	Not actionable	CNOT3 = 4 Chr19 = 4	N/A	Patient undergoing therapy with multi-agent chemotherapy
Other CNS Tumor									
PO-3002	Meningioma, at diagnosis (diagnosis unclear from pathology)	11	No DNA lesions noted (benign tumor)	None	Surveillance	Not actionable	None	No	Patient in clinical remission followed by surveillance only
PO-3124	Atypical meningioma, at diagnosis (initial diagnosis unclear *v* MPNST)	3	**NF2 one copy loss**, chr1q, 6q (partial), 22 copy loss, chr17q copy gain; **NF2 splice site mutation (after p.E270) – loss of function**; JAK2, PIK3R1 overexpression	None	Surveillance *v* consideration of focal radiation	NF2-targeted therapy (including mTOR inhibitor)	NF2 = 3 JAK2 = 3 (no specific mutation to list category 1) PIK3R1 = 3 Other genes {1q, 6q, 22, 17q} = 4	No	Patient in clinical remission followed by surveillance only; relapse at 11 months; patient in clinical remission after adjuvant proton-XRT

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Table A1. Clinically Integrated Sequencing Results for Patients (Continued)

Patient ID	Diagnosis	Age at Enrollment (years)	Informative and Actionable Genomic Findings	Incidental Germline Findings	Standard Therapy Without Sequencing	Potential Actions Based on Sequencing Results	Actionable Finding Grade[13]	Action Taken	Clinical Actions and Outcome
PO-3134	Anaplastic chordoma, at diagnosis	9	PTPN11 (p.G163S) mutation	**MUTYH/MYH (p.R245H) variant, no LOH (carrier)**	Focal radiation	Family referral to genetic counseling	PTPN11 = 3 **MUTYH/MYH = 3**	Yes	Family referred to genetics clinic for genetic counseling for MYH-associated polyposis. Patient in clinical remission for 8 months after radiation therapy.
PO-3150	Atypical choroid plexus papilloma, at diagnosis	8 months	ERF p.T148M. (vaf 22%) (ETS2 repressor factor); MCPH1 p.I104fs del; HTR2C-MAMLD1 fusion	None	Surveillance *v* consideration of multi-agent chemotherapy	Not actionable	ERF = 4 MCPH1 = 4 HTR2C = 4 MAMLD1 = 4	No	Patient in clinical remission followed by surveillance only

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Table A1. Clinically Integrated Sequencing Results for Patients (Continued)

Patient ID	Diagnosis	Age at Enrollment (years)	Informative and Actionable Genomic Findings	Incidental Germline Findings	Standard Therapy Without Sequencing	Potential Actions Based on Sequencing Results	Actionable Finding Grade[13]	Action Taken	Clinical Actions and Outcome
PO-3233	Choroid plexus carcinoma, recurrent	11	**NF1: Frameshift insertion, p.E2155fs, copy neutral LOH**; JAK2 (p.K207R), EPHA4 (p.A193V), TRAF3IP1 (p.A467T), MAP4 (p.Q137H), PRKD1 (p.C312Y), LRP1B (p.G4527V), EXTL1 (p.R667S), SLC47A2 (p.P412L), SLCO1B3 (p.T244P), TERT (p.T182S)	**TP53: Frameshift deletion, p.P152fs, copy neutral LOH**	Clinical trial for relapsed brain tumor *v* salvage chemotherapy	NF2-targeted therapy (including mTOR inhibitor)	**NF1 = 3** JAK2 = 3 EPHA4 = 4 TRAF3IP1 = 4 MAP4 = 4 PRKD1 = 4 LRP1B = 4 EXTL1 = 4 SLC47A2 = 4 SLCO1B3 = 4 TERT = 4	Yes	Patient underwent complete resection, followed by adjuvant therapy with trametinib for 3 months, now 6 months from diagnosis
PO-3228	Rosai Dorfman disease (isolated suprasellar), at diagnosis	17	Neuropathology determined tissue inadequate	N/A	Focal radiation	N/A	N/A	N/A	Patient in clinical remission after focal radiation

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Table A1. Clinically Integrated Sequencing Results for Patients (Continued)

Patient ID	Diagnosis	Age at Enrollment (years)	Informative and Actionable Genomic Findings	Incidental Germline Findings	Standard Therapy Without Sequencing	Potential Actions Based on Sequencing Results	Actionable Finding Grade[13]	Action Taken	Clinical Actions and Outcome
PO-3260	Clival chordoma, at diagnosis	13	Neuropathology determined tissue inadequate	None	Focal radiation	N/A	N/A	N/A	Patient in clinical remission after focal radiation

Abbreviations: AT/RT, atypical teratoid/rhabdoid tumor; DIPG, diffuse intrinsic pontine glioma; ETMR, embryonal tumor with multilayered rosettes; GBM, glioblastoma multiforme; LOH, loss of heterozygosity; MPNST, malignant peripheral nerve sheath tumor; MRI, magnetic resonence; N/A, not available; NED, no evidence of disease; PNET, peripheral neuro-ectodermal tumor; XRT, radiation therapy. Boldface denotes potantially actionable alterations.