Clinical targeted exome-based sequencing in combination with genome-wide copy number profiling: precision medicine analysis of 203 pediatric brain tumors


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Abstract

Background. Clinical genomics platforms are needed to identify targetable alterations, but implementation of these technologies and best practices in routine clinical pediatric oncology practice are not yet well established.

Methods. Profile is an institution-wide prospective clinical research initiative that uses targeted sequencing to identify targetable alterations in tumors. OncoPanel, a multiplexed targeted exome-sequencing platform that includes 300 cancer-causing genes, was used to assess single nucleotide variants and rearrangements/indels. Alterations were annotated (Tiers 1–4) based on clinical significance, with Tier 1 alterations having well-established clinical utility. OncoCopy, a clinical genome-wide array comparative genomic hybridization (aCGH) assay, was also performed to evaluate copy number alterations and better define rearrangement breakpoints.

Results. Cancer genomes of 203 pediatric brain tumors were profiled across histological subtypes, including 117 samples analyzed by OncoPanel, 146 by OncoCopy, and 60 tumors subjected to both methodologies. OncoPanel revealed clinically relevant alterations in 56% of patients (44 cancer mutations and 20 rearrangements), including \( \text{BRAF} \) alterations that directed the use of targeted inhibitors. Rearrangements in \( \text{MYB-QKI}, \text{MYBL1}, \text{BRAF} \), and \( \text{FGFR1} \) were also detected. Furthermore, while copy number profiles differed across histologies, the combined use of OncoPanel and OncoCopy identified subgroup-specific alterations in 89% (17/19) of medulloblastomas.

Conclusion. The combination of OncoPanel and OncoCopy multiplex genomic assays can identify critical diagnostic, prognostic, and treatment-relevant alterations and represents an effective precision medicine approach for clinical evaluation of pediatric brain tumors.
Importance of the study

Clinically validated genomics platforms are required for the implementation of precision medicine, including in pediatric neuro-oncology. In this study, we demonstrate the utility of a targeted exome-sequencing platform in combination with genome-wide copy number profiling using aCGH in a clinical setting. These platforms detected clinically relevant alterations in 55% of all patients, including identification of driver genomic mutations. In addition, we identified alterations that confer diagnostic and prognostic significance, such as those that allow subtyping of medulloblastoma. Our results indicate that genomic profiling of pediatric brain tumors is feasible and clinically useful.

Methods

Ethics Statement

Ethics approval was granted by the human institutional review board committees of the Dana-Farber/Harvard Cancer Center. Informed consent was obtained from all patients and/or their guardians. Targeted exome sequencing was performed on tumors from patients enrolled on the Profile clinical study.

Clinical Data and Review of Histology

Clinical data were extracted from clinical charts and de-identified. A histological diagnosis for each patient was rendered for the study by at least one pediatric neuropathologist for all cases (S.H.R.) and most cases were reviewed by a second neuropathologist.

Clinical Array Comparative Genomic Hybridization

High resolution array comparative genomic hybridization (aCGH) (Agilent Sure Print G3 Human 1 × 1M feature array) was performed on clinical biopsy samples from formalin-fixed paraffin embedded material in a CLIA-certified laboratory as part of clinical care using a methodology previously described. Two micrograms of patient and reference DNA (Promega) were fragmented and hybridized to each microarray. The average resolution of the aCGH platform across Reference Sequence genes is 1.8 kb, and across the genome 2.1 kb.

Analysis was performed as previously described and clinical reports were generated by teams of board-certified clinical cytogeneticists, neuropathologists, and molecular pathologists.

Targeted Next-Generation Sequencing—OncoPanel

For patients enrolled on the Profile clinical trial, detection of mutations and gene rearrangements in tumors was achieved using targeted next-generation sequencing (OncoPanel). This assay (Agilent SureSelect for target capture and Illumina HiSeq sequencing) surveys exonic DNA sequences of 300 cancer genes, including 91 introns across 30 genes (Supplementary Table 1) for mutation and rearrangement detection in tumor-derived DNA (minimum 50 ng, formalin-fixed paraffin embedded–derived tissue). Neuropathologists review each tumor specimen and estimate the number of neoplastic cells in the submitted sample (tumor purity). OncoPanel results were interpreted and reported by molecular pathologists, and alterations were...
classified into 4 tiers (Tier 1 to Tier 4) to provide therapeutic and prognostic significance (Supplementary Table 1), with Tier 1 alterations having well-established published evidence confirming clinical utility. BreaKmer, an algorithm that predicts insertions, deletions, tandem duplications, and gene rearrangements, was implemented in April 2014 and detected aberrations in 70/120 (58%) patients.

Whole-Genome Sequencing

Tumor and normal DNA were sequenced at the Broad Institute of MIT and Harvard. DNA was randomly fragmented, and libraries were prepared for paired-end sequencing on an Illumina HiSeq 2000. Read pairs were aligned to reference genome hg19 (Build 37) using the Burrows-Wheeler Aligner with options --q 5 -1 32 -k 2 -o 1. Reads were sorted by coordinates, normalized, and cleaned and duplicates were marked using SAMtools and Picard. Base quality score assignments were recalibrated to control for biases due to flow cell, lane, dinucleotide context, and machine cycle using the Genome Analysis Toolkit. Rearrangements were detected using Snowman, an assembly-based method (Wala et al, manuscript in preparation).

Exploratory Analysis of Copy Number Profiles

Copy number profiles can be inferred from OncoPanel profiling; however, the resolution of genome-wide aCGH is higher. Copy number profiles presented here were generated from aCGH analysis. A circular binary segmentation algorithm was used to segment copy number data for research purposes using parameters (α = 0.01, undo. splits = none, minimum width = 5). Focal versus broad somatic copy number alteration events were defined with a cutoff of 0.5x chromosome arm length and gene confidence level of 0.99. Segmented data were visually presented using Integrative Genomics Viewer 2.36 in heatmap format.

Unsupervised hierarchical clustering of arm-level copy number profiles was performed in GenePattern, using Euclidean distance as the column distance measurement and pairwise complete-linkage as the clustering method. Nonnegative matrix factorization (NMF) clustering was performed in GenePattern, with 20 clusterings per K and pairwise complete-linkage as the clustering method. Euclidean distance as the column distance measurement.

Support vector machine (SVM) multiclass analysis was performed within MatLab, using the fitcecoc module, and a one-versus-one coding design. Tumors were assigned one of the following labels: glial, embryonal, meningiomas, or not otherwise specified (NOS). The model was trained with 136 of the 146 samples; the remaining 10 samples were then used to assess the model’s ability to predict classes. This method was repeated 15 times, leaving out a different set of 10 samples each time so that the accuracy of the model was tested using every sample within the aCGH cohort.

Medulloblastoma Subtyping

DNA-based molecular subtyping was performed for medulloblastoma samples through analysis of the molecular aberrations (including both single nucleotide variants and copy number aberrations) identified through microarray and sequencing studies. Previously reported findings in large cohort series were used to guide subtyping according to criteria in Supplementary Table 4. For example, the Wingless (WNT) subgroup tumors were identified by monosomy 6 and/or CTNNB1 mutations. Sonic hedgehog (SHH)–activated tumors were categorized by the presence of at least one of the following: (i) any direct alteration in genes involved in SHH signaling, including PTCH1, SMO, SUFU, GLI1, GLI2, or (ii) more than one of the following: 9q deletion, which includes PTCH1 single copy loss, the co-occurrence of chromothripsis, and TP53 mutations. Group 3 tumors were classified by the presence of MYC amplification in the absence of isodicentric chromosome 17. Group 4 tumors were identified by the presence of isodicentric chromosome 17p11.2. Group 3/4 was assigned if tumors lacked diagnostic Group 3, 4, or WNT/SHH features or contained Groups 3 and 4 features in the same tumor.

Statistical Analysis

Unpaired 2-sided t-tests were used to determine differences in percentage of genome affected by genomic disruption across subtypes. P-values of <.05 were deemed to be significant.

Results

Assay Overview

Our institutions implemented a precision medicine program for pediatric patients with brain tumors that included next-generation sequencing and copy number profiling. The goals of this testing included determining diagnoses, clarifying prognoses, and identifying possible actionable molecular changes that could be used for assigning patients to clinical trials. For the purposes of this study we analyzed the OncoPanel data for single nucleotide variations (SNVs), insertions/deletions (indels), and rearrangements only and analyzed the OncoCopy (aCGH) data for copy number changes.

Cohort Demographics and Sample Characteristics

Between January 2013 and June 2015, OncoPanel testing was requested on 142 pediatric brain tumor specimens. All histological subtypes of pediatric brain tumors were represented (Supplementary Table 2). The median age of children enrolled was 8 years and the range 0.15 to 25.5 years. At the time of our data analysis, clinical reports were generated for 120 patients (82% of requested); 3 specimens could not be evaluated because of technical limitations of the material submitted (2.5%). In addition, aCGH copy number testing was requested and reporting completed on 146 pediatric brain tumor specimens. Data from both assays were available for 60 patients during this time frame.
The mean reported tumor purity of samples profiled by OncoPanel was 70% (range 20%–95%), the mean coverage was 181× (range 75–634), and the mean percentage of exons with greater than 30 reads across the cohort was 96% (range 91%–99%).

**Copy Number Profiles Are Predictive of Histology in Pediatric Brain Tumors**

Embryonal tumors (medulloblastomas, primitive neuroectodermal tumors [PNETs], and atypical teratoid rhabdoid tumors [AT/RTs]) are known to exhibit distinct copy number profiles compared with glial tumors (gliomas, ependymomas, astrocytomas, and glioblastomas) and to carry specific diagnostic or prognostic implications. To determine the degree to which the profiles from the clinical aCGH assay differed across histological subtypes, we performed unsupervised hierarchical clustering of genome-wide copy number profiles of 146 pediatric brain tumors (Fig. 1A). Unsupervised hierarchical clustering revealed the tumors to separate into 2 dominant clusters of tumors, each with at least 2 further subclusters within each branch (Fig. 1A). Embryonal tumors were the most common tumor in cluster 1 (18 of 39 tumors, 46%) with high-grade gliomas (HGGs) being the next most frequent (11 of 39, 28%). Cluster 2 was enriched with glial tumors (76 of 107, 71%). Pediatric low-grade gliomas (PLGGs) clustered separately to HGGs, with PLGGs tending to cluster in Group 2A (20 of 28 gliomas, 71%) and HGGs clustering with embryonal tumors in Group 1 and Group 2B. WNT-positive medulloblastomas segregated into cluster 2B while meningiomas clustered with glial tumors in cluster 2. The 2 choroid plexus carcinomas clustered together with the embryonal tumors in cluster 2B. An independent analysis with NMF clustering suggested at least 8 copy number clusters.

To a large degree, these results were due to differing burdens of genomic alterations. In embryonal tumors, 26% of the genome was affected by arm-level copy number alterations, a significant increase compared with the frequency observed in glial tumors (10%). HGGs exhibited greater genomic disruption than LGGs (Fig. 1B). Indeed, 37 tumors had no discernible copy number alterations. Comparative marker selection revealed 13 arm-level events that were significantly different between glial and embryonal tumors (q < 0.25) (Supplementary Table 3); of these 10p, 17q, 7q, 19p, 19q, and 5p were the most differentially altered (q < 0.05).

We sought to harness a machine-learning algorithm (ie, SVMs) to quantify further how well copy number profiles could differentiate between the 5 histological subgroups.

![Figure 1](image-url)  
*Fig. 1* Pediatric brain tumors can be distinguished by copy number profiles. (A) Unsupervised hierarchical clustering of 146 pediatric copy number profiles (arm-level events). (B) Percent of genome disruption in glial tumors (low-grade and high-grade) and embryonal tumors. Values represent mean level of disruption (± SEM). (C) Support vector machine classification of pediatric brain tumors.
(glioma, embryonal tumor, menigioma, choroid plexus carcinoma, and NOS). We anticipated that there would be significant error due to the limited number of tumors in some subgroups and significant levels of heterogeneity within these broadly defined subgroups. However, there was interest in using this large cross-cancer dataset to explore the extent to which lineage identification by copy number might be possible. We trained an SVM classifier on 136 tumors spanning these subgroups. The in-sample classification error was 15%; glioblastomas were predicted as glial in 88% of instances and embryonal tumors as embryonal in 50% of instances. The remaining 50% of embryonal tumors were classified as glial (Fig. 1C). Cross-validation of the classification using 10-fold validation revealed an out-of-sample classification error of 33%. By contrast, random histological assignment revealed an out-of-sample classification error of 86%. SVM classification was unable to accurately distinguish HGGs from LGGs, classifying 58% of HGGs as LGGs and 26% as embryonal tumors.

Recurrent Activating Mutations and Genomic Rearrangements Are Detected by Targeted Exome Sequencing

OncoPanel data obtained from 117 brain tumors were analyzed to assess for both gene mutations and rearrangements. As part of the clinical reporting workflow, variants were included in reports based on the presence of both >5 variant reads and an allelic fraction greater than 1%. OncoPanel does not include matched normal DNA controls. Thus, private germline alterations could not be excluded and were often reported as Tier 4 variants, representing SNVs not previously reported to be pathogenic in cancer.

Excluding 2 glioblastoma (GBM) patients with a hypermutator phenotype, a mean of 4 SNVs (range 1–13) were detected in each patient. OncoPanel revealed clinically relevant alterations in 56% of patients (64/115). Across the 115 OncoPanel patients (excluding the 2 GBM patients with a hypermutator phenotype), 44 (38%) were found to have Tier 1–3 mutations (42 of these patients had Tier 1 or Tier 2 mutations) and an additional 20 (17%) were found to have clinically actionable rearrangements. The remaining patients were observed to harbor Tier 4 alterations (Fig. 2A); this was the most frequent tier of alteration detected across the entire cohort (Fig. 2B).

Mutations were observed in a number of genes of direct relevance to pediatric brain tumor diagnosis and treatment, including Braf, Fgfr1, Ntrk1, Atrx, Tp53, and Idh1 in glial tumors, and Cttnb1 and Pch1 mutations in medulloblastoma (Fig. 2D). We also observed driver mutations previously reported: KIT in germinoma, Vhl mutations in hemangioblastomas; and single case of primary CNS melanoma in association with neurocutaneous melanocytosis was driven by a mutation of Nras (p.Q61K) as previously described.

The identification of alterations for which small molecule inhibitors exist influenced clinical management of the patients. Across the entire cohort of patients profiled with OncoPanel, 37 had tumors with an alteration for which a small molecule inhibitor is in early phase clinical investigation (Supplementary Table 2). Of these 37 patients, 8 children (22%) were treated with a targeted small molecule inhibitor based on the mutation identified by OncoPanel, and more patients are likely to be treated in the future upon tumor progression.

OncoPanel Detects Clinically Relevant Rearrangements

OncoPanel detected rearrangements in 25 of 115 pediatric brain tumors analyzed and reported for rearrangements. These included rearrangements involving Braf, Fgfr1, Tacc1, and Myb family members previously reported in PLGGs,5,29,30 The most frequent rearrangement/indel detected was the Braf-Kiaa1549 rearrangement found in PLGGs (Fig. 2C).5,29,31,30 We also detected EwSri1 rearrangements in 2 tumors: an integrase interactor 1–deficient AT/RT with EwSri1-Plag1 fusion and a spinal ependymoma with EwSri1-Bend2 fusion. We further validated the presence of the EwSri1-Plag1 rearrangement in the first tumor using whole-genome sequencing (Supplementary Figure 2). Rearrangements involving EwSri1 have recently been suggested as defining a subclass of central PNETs,26 and Plag1 genes have been previously implicated as a driver in gliomas.39 This alteration raises the question whether EwSri1 fusions occur in multiple tumor types or whether there may be potential overlap between central PNET and “classic” AT/RT.

Medulloblastoma Subtyping Achieved Through Integrated Mutation and Copy Number Analysis

Medulloblastoma has been categorized by the most recent World Health Organization (WHO) 2016 CNS tumor classification update4,28,35 as having 4 biological subtypes: Wnt, Shh, Group 3, and Group 4 tumors.25,36–38 These subtypes have been defined most commonly using expression profiling and most recently methylation profiling.25,36–38 While these technologies have significantly advanced the understanding of the biology of medulloblastoma, the most appropriate approach to subtyping medulloblastoma in the clinical setting remains unclear.

The combination of OncoPanel and aCGH led to the identification of genomic changes previously associated with transcriptome-defined subtypes (Wnt, Shh, Group 3, and Group 4) in 89% (17/19) of medulloblastomas (Fig. 3A and 3B, Supplementary Table 4). Of the 31 medulloblastoma tumors in our study cohort, 30 were analyzed by aCGH and 19 were assessed by both OncoPanel and aCGH. We focused on the integrated analysis of somatic copy number alteration and SNV data across these 19 tumors in order to identify recurrent alterations and genomic subtyping performance. To categorize these tumors, we defined a classification scheme of “genetic Wnt,” “genetic Shh,” “genetic 3,” “genetic 4,” or “genetic 3 or 4” based upon the presence of genetic features previously associated with these subtypes (see Methods, Fig. 3A, and Supplementary Table 4).

For example, we classified tumors with Ctnnb1 mutations or monosomy 8 as genetic Wnt; SHH pathway alterations (Patched 1 [Pch1] mutations, or 9q loss, or chromothriptis combined with tumor protein 53 [Tp53] loss) as genetic Shh; 2 or more of MYC amplification, Group 3 associated
polysomies, DEAD (Asp-Glu-Ala-Asp) box 31, or growth factor independent 1–family alterations as genetic 3; and structural alterations, including isochromosome 17q, as genetic 4. Criteria used to subtype each individual tumor are included in Fig. 3B.

CTNNB1 mutations and monosomy 6 co-occurred in all tumors with mutations in either gene and were present in 16% (3/19) of tumors, consistent with genetic WNT (Fig. 3A). Four tumors harbored PTCH1 mutations, defining them as genetic SHH. MYC amplification was detected in 60% (3/5) of tumors, consistent with genetic 3, and Idic(17) (p11.2) was detected in 3 tumors, classified as genetic 4. Two tumors (10%) could not be definitively assigned to a subclass. However, these tumors lacked definitive WNT or SHH features and therefore likely belonged to Group 3 or Group 4 tumors. One exhibited focal gain/subclonal amplification of OTX2, favoring a Group 3 tumor.

Clinically Actionable Alterations in PLGGs with Implications for Targeted Therapy

We performed comprehensive genomic profiling (CGP) of 18 PLGGs using both aCGH and OncoPanel and identified diagnosis-relevant alterations in 55% of PLGGs (Fig. 4A). Among pilocytic astrocytomas (PAs), 88% (7/8) harbored KIAA1549-BRAF rearrangements, including all cerebellar (6/6) and cerebral (1/1) PAs. BRAF V600E mutations were
detected in all gangliogliomas and in no other tumors (3/3). CGP identified driver events in all 3 tumors designated LGG (NOS), including 2 \( \text{FGFR1} \) events (rearrangement and mutation) and a \( \text{BRAF-KIAA1549} \) rearrangement. An optic pathway PA showed no detectable alterations, including in \( \text{BRAF} \) or \( \text{NF1} \), suggesting the need for broader analysis via whole-exome or whole-genome sequencing.

**Genomic Profiling-Aided Treatment Planning with Targeted Therapies**

A key goal of precision medicine and genomic profiling is to better guide the use of targeted therapies in patients. While no targeted therapies are currently FDA approved for pediatric brain tumors, several ongoing clinical trials are evaluating such treatments. In our cohort the identification of \( \text{BRAF} \) alterations guided the use of targeted inhibitors in the clinical setting (Fig. 4B). Of the 12 patients with \( \text{BRAF} \)-altered PLGGs, 8 were treated with surgery alone and did not require any other tumor-directed therapy. Four required further therapy, 2 of whom received treatment with a targeted inhibitor (\( \text{BRAF} \) inhibitor for one patient with a \( \text{BRAF} \) V600E mutation, and an inhibitor of mammalian target of rapamycin for a patient with a \( \text{BRAF-KIAA1549} \) rearrangement). The patient with the \( \text{BRAF} \) V600E mutation was a 7-year-old girl who presented with seizures and was subsequently diagnosed with disseminated ganglioglioma. Pathology revealed a glioneuronal neoplasm, for which immunohistochemistry was used to identify the \( \text{BRAF} \) \( \text{V600E} \) mutant protein (Fig. 4C). OncoPanel subsequently validated the presence of the \( \text{BRAF} \) V600E mutation. The patient was started on treatment with a \( \text{BRAF} \) inhibitor (dabrafenib), and a response to treatment was observed within 3 months of initiation of therapy (Fig. 4D).

In addition to \( \text{BRAF} \) alterations, OncoPanel detected previously reported driver alterations in PLGGs,\(^{29,30}\) including those affecting \( \text{FGFR}, \text{NTRK}, \text{MYB} \) family members, and \( \text{IDH1} \). While \( \text{IDH1/2} \) mutations are less common in pediatric compared with adult brain tumors, we identified \( \text{IDH1} \) (R132H) variants in diffuse astrocytoma (2/3), oligoastrocytoma (1/1), oligodendroglioma (1/1), and anaplastic astrocytoma (1/5). The co-occurrence of \( \text{IDH1/2}, \text{TP53} \), and \( \text{ATRX} \) mutations in PLGGs aligns with the signature previously reported in adult lower-grade astrocytomas.\(^{39}\) These findings highlight the necessity of broad multiplexed assays to genomically profile...
tumors, particularly in the adolescent age group, to distinguish adult versus pediatric diffuse astrocytoma types, which have dramatically different clinical outcomes and prognoses. Adult type astrocytomas are diagnosed in late adolescence, harboring genomic alterations more consistent with those seen in the adult disease (IDH, ATRX, TP53). Thus, such tumors should be regarded as young adult astrocytomas, analogous to those arising in older adults.

**Profiling of High-Grade Gliomas Reveal Alterations with Diagnostic and Therapeutic Implications**

We examined a cohort of 12 pediatric HGGs, defined as WHO grades III and IV tumors, and observed alterations in genes previously reported to be associated with pediatric HGG in 9 of 12 patients (Fig. 5). Two of the 3 tumors for which we did not identify driver alterations were infant HGGs. TP53 mutations were detected in 40% of high-grade astrocytomas, including 2/5 anaplastic astrocytomas and 4/10 GBM tumors. H3F3A mutations were detected in 2/10 cases of GBM and included p.G34R (aka p.G35R) and p.K27M variants. NF1 and ATRX alterations were detected in 30% of GBM with 2 tumors harboring both an ATRX and an NF1 mutation. The hypermutator phenotype was detected in 2 untreated GBM patients with an average of 147 variants per tumor, often with multiple alterations per gene.

Comprehensive genomic analysis revealed clinically targetable mutations (Fig. 5B). Small molecule inhibitors for 6 targets (BRAF, PDGFRA H3F3A [histone deacetylase inhibitors], cell cycle inhibitors, and phosphatidylinositol-3 kinase inhibitors) have entered early phase clinical trials in pediatrics. In addition, brain-penetrant small molecules directed specifically against IDH1 (R132H) are currently in clinical trials for adults and likely to enter the pediatric arena in the near future, while pediatric clinical trials are currently being designed for FGFR inhibitors. These data suggest that CGP provides meaningful data that can guide precision medicine for children with HGGs.

**Discussion**

We present the largest cohort of pediatric brain tumors that have undergone genomic profiling in a CLIA-certified setting. In this cohort we observe clinically relevant alterations (Tiers 1–3 mutations and rearrangements) in 56% of patients. The genomic profiles also had diagnostic relevance. Embryonal tumors were found to harbor distinct

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**Fig. 4** OncoPanel guides the use of targeted inhibitors in PLGG. (A) Landscape of genomic alterations identified in 18 PLGGs profiled with both OncoPanel and aCGH. (B) Treatment of patients with BRAF-altered PLGG. (C) Hematoxylin and eosin BRAFV600E stains of gangliogioma with BRAFV600E mutation. Scale bar represents 1.2 μm. (D) Axial postcontrast MRI of tumor at diagnosis, postsurgery, and following treatment with a BRAF inhibitor.
copy number profiles compared with glial tumors. The combination of targeted sequencing with genome-wide copy number profiling revealed subtype-relevant alterations in 89% of all medulloblastoma.

We found that CGP was useful for improved clinical care of the patient. Histological diagnoses of pediatric brain tumors based on the current WHO CNS classification present diagnostic challenges, with a number of tumors that cannot be reliably distinguished. The classification of pediatric brain tumors based on genomic profiling provides both diagnostic and prognostic insight into many disease types. Thus, the ability to diagnose pediatric brain tumors based on genomic profiles is likely to be of great clinical utility. Our finding that copy number profiles broadly differ between embryonal and glial lineages holds the potential to further improve accuracy and reproducibility of diagnostics, particularly as increased numbers of tumors are profiled.

Recent large-scale efforts to profile the somatic cancer genomes of pediatric brain tumors have heralded the arrival of precision medicine in pediatric neuro-oncology. Our CGP revealed alterations in a number of genes for which inhibitors are currently in early phase pediatric clinical trials, including **BRAF**, **IDH1**, **PIK3CA**, **PDGFRα**, and **KIT**, as well as those for which inhibitors have entered, or are about to enter, early phase pediatric clinical trials including **FGFR1**, **NTRK 2/3/4**, **MYC**, or **MYCN**. Targeted therapeutics are likely to be most effective when deployed against tumors that have been characterized at a genomic level. Thus it is important that clinical practices in pediatric neuro-oncology incorporate comprehensive genomic analysis that will allow the identification of optimal patients for each targeted therapeutic agent.

Medulloblastomas have been consistently suggested to comprise at least 4 distinct biological subgroups with specific clinical and prognostic significance. These groups are robust across independent platforms, including gene-expression profiling and methylation profiling. However, these studies have been performed in a research setting, and the best methodology to translate these findings to the clinic remains unclear. In particular, RNA and methylation profiling are poorly suited to identifying therapeutic targets in specific tumors. In contrast, DNA-based genetic analysis of targeted gene panels is cost-effective, provides clinically actionable results, and tends to be more consistent across sites and experimental conditions.

Previous efforts have identified a strong concordance between genomic alterations and WHO subtype in medulloblastoma. We believe that leveraging these findings provides a more clinically useful route to tumor classification. Using our genomic approach of targeted exome sequencing combined with genome-wide copy number profiling, we identified subgroup-specific genomic alterations in almost 90% of medulloblastomas. Subgroup-specific clinical trials are now open to accrual (ClinicalTrials.gov IDs NCT01878617, NCT01601184, and NCT02212574). In addition, targeted therapeutics for specific genomic alterations in medulloblastoma are likely to have an increasing role in the treatment of children with medulloblastoma, highlighting

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**Fig. 5** Genomic alterations identified in 12 pediatric HGGs profiled with OncoPanel and aCGH. (A) Landscape of genomic alterations identified in 12 HGGs profiled with both OncoPanel and aCGH. (B) Genomic alterations for that for which either small molecule inhibitors exist or where they guide enrollment onto clinical trials.
the necessity of implementing technologies that allow the 
subtyping of medulloblastoma in the clinical setting. It will 
be useful to determine from these trials the relative value of 
genetic, transcriptomic, and methylation-based profiling 
in classifying patients. A limitation to our study is that sub-
typing of tumors in this cohort was not correlated to match 
transcriptomic subtyping from the same tumors; future 
work will be needed to complete this effort.

Three questions merit particular focus in further study. 
First, to what extent do transcriptomic and epigenetic 
profiling add to the clinical utility of genetic profiles? 
Subclassification of pediatric brain tumors based upon 
these modalities has been performed in the research setting 
and is now beginning to be implemented clinically (ClinicalTrials.gov IDs NCT02285439, NCT02212574, and NCT01878617). Second, what is the predictive and 
prognostic value of any of these assays? Formal evaluation 
of the relative impact of each assay on clinically relevant 
metrics is necessary. Third, what is the impact of tumor 
heterogeneity on the validity of clinical genomic results 
and on the treatments being implemented? Heterogeneity 
has increasingly been observed across various types of 
tumors, but the impact of such heterogeneity on the robust-
ness of clinical data has not been formally evaluated.

Supplementary Material

Supplementary material is available at *Neuro-Oncology* 
online.

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