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Blood brain barrier-adapted precision medicine therapy for pediatric brain tumors

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Abstract

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Targeted chemotherapeutics provide a promising new treatment option in neuro-oncology. The ability of these compounds to penetrate the blood brain barrier is crucial for their successful incorporation into patient care. “CNS Targeted Agent Prediction” (CNS-TAP) is a multi-institutional and multi-disciplinary translational program established at the University of Michigan for evaluating the CNS activity of targeted therapies in neuro-oncology. In this report, we present the methodology of CNS-TAP in a series of pediatric and adolescent patients with high-risk brain tumors, for which molecular profiling (academic and commercial) was sought and targeted agents were incorporated. Four of five of the patients had potential clinical benefit (partial response or stable disease greater than 6 months on therapy). We further describe the specific drug properties of each agent chosen and discuss characteristics relevant in their evaluation for therapeutic suitability. Finally, we summarize both tumor and drug characteristics that impact the ability to successfully incorporate targeted therapies into CNS malignancy management.

INTRODUCTION

During the last 20 years, the development of targeted therapies has been among the more promising advances in cancer therapy. Furthermore, our ability to characterize tumors on a molecular and genomic level enables the identification of specific cellular pathway aberrations that may be targeted with one or more of these therapies. Tumors in the pediatric population are more likely to have fewer mutation events, and may therefore be more amenable to these new targeted therapies.(1) However, one of the most challenging situations in neuro-oncology and drug delivery is the inability of medications to enter and remain in the central nervous system (CNS).(2–4) Less than 5% of chemical compounds that are screened during the drug development process have any CNS activity.(5) There is a variable amount of evidence regarding the blood-brain barrier (BBB) penetration and activity of most of these small molecule inhibitors in the CNS. Given this challenge, we have undertaken a thorough review of drug-specific properties and available data regarding BBB penetration and CNS activity in selecting specific therapies for pediatric CNS tumor patients who have undergone clinically integrated genomic sequencing.

Using these data, we have established the University of Michigan (UM) CNS Targeted Agent Prediction (CNS-TAP) program, a multi-institutional translational program for evaluating the CNS activity of targeted therapies in pediatric neuro-oncology. In this report, we describe the CNS-TAP program, using a series of representative pediatric brain tumor patients with unique molecular pathways driving tumor formation. These cases allow us to review the important pharmacologic and treatment considerations in selecting targeted therapies for children with high-risk or refractory brain tumors.

METHODS

Patient Selection

We performed a dual site (University of Michigan C.S. Mott Children’s Hospital and Cincinnati Children’s Hospital) prospective observational case-series of patients younger than 21 years old with a diagnosis of a “high-risk” brain tumor (those identified to have at least a 25% chance of treatment failure by their treating oncologist) for which tumor sample

was suitable for molecular profiling (DNA and/or RNA sequencing) either through an academic (e.g., Pediatric MI-ONCOSEQ for cases 1, 2, 4 and 5), or private (Foundation One (Foundation Medicine, Inc., Cambridge, MA), case 3), platform.

Pediatric MI-ONCOSEQ Study

PEDS-MIONCOSEQ is a clinically-integrated sequencing study which includes paired tumor/normal exome and tumor transcriptome sequencing, and it was approved by the Michigan Medicine institutional review board (HUM00056496) (7, 9, 10). All patients were seen by a physician investigator and a genetic counselor. This study was initiated in May 2012 and continues as of March 2017. All patients or their parents or legal guardians provided informed consent (written assent if >10 years) and received mandatory pre-enrollment genetic counseling regarding the potential risks of incidental genetic findings. A “flexible default” consent model was used to mandate disclosure of findings that directly influenced the current cancer management strategy, but patients or parents could choose whether to receive incidental results associated with high risk of hereditary cancer syndromes in patients and other family members. However, incidental genetic findings did not include disclosure for conditions other than cancer.(6–8) Once enrolled, a patient’s clinical course was captured quarterly in order to document clinical status and treatment decisions made by the primary team since the last follow-up.

Study methodology has been described previously.(7, 9, 10) Briefly, a neuro-pathologist evaluated histologic sections for estimation of tumor content before submitting tissue for sequencing. Nucleic acid preparation and high-throughput sequencing were performed using standard protocols in our sequencing laboratory, which adheres to the Clinical Laboratory Improvement Amendments (CLIA).(7, 9, 10) Paired-end whole-exome libraries from tumor samples that were matched with normal DNA and with transcriptome libraries either from polyadenylated tumor RNA (PolyA + transcriptome) or from total RNA captured by human all-exon probes (capture transcriptome) were prepared and sequenced using the Illumina HiSeq 2000 and 2500 (Illumina Inc). Aligned exome and transcriptome sequences were analyzed to detect putative somatic mutations, insertions and deletions (indels), copy-number alterations, gene fusions, and gene expression as described previously.(7, 9, 10)

Pathogenicity of germline variants was determined through a review of the published literature, public databases including but not limited to ClinVar, the Human Genome Mutation Database, the Leiden Open Variation Databases, and variant specific databases (eg, International Agency for Research on Cancer TP53 Database, International Society for Gastrointestinal Hereditary Tumors mutation databases). Only variants that had been previously described as pathogenic were considered for disclosure. Variants with conflicting pathogenicity reports and variants not previously reported were considered to be of uncertain significance and were not considered for disclosure. Following disclosure, familial testing was recommended. Clinical relevance of somatic variants was investigated using an integrated approach incorporating technical considerations (e.g., recurrence, variant allele fraction, expression levels, and predictive algorithms for pathogenicity), variant specific information (e.g., ClinVar, published literature, and curated gene specific resources), as well as published correlations of drug and variant sensitivity profiles. Considerations of tumor

heterogeneity, including clonal vs sub-clonal mutation were addressed by comparing variant allele fractions and copy-number estimates for each of the mutations to post-sequencing estimates of tumor content derived from single-nucleotide variation and copy-number analyses. Each of the aberrations for which clinical action was based in this study were judged to be clonal.

Foundation One

For the patient's tumor in this study profiled by Foundation One (case #3), sample collection and methodology was conducted per their established platform of commercial tumor profiling. Briefly, DNA was extracted from formalin-fixed paraffin-embedded tumor sections. Next generation sequencing was performed for exons of at least 315 cancer-related genes and select introns of 28 rearrangements using Illumina HiSeq 2000 (Illumina, Inc., San Diego, California). Testing was performed in a CLIA-certified, College of American Pathologists-accredited reference laboratory. The sample was evaluated for genomic alterations, including base pair substitutions, indels, copy number alterations, and rearrangements.

Brain Tumor Precision Medicine Conference

Sequencing results were reviewed in a monthly brain tumor precision medicine conference. Clinical case histories, sequencing results, and potential treatment options were discussed with a multi-disciplinary team including clinicians from pediatric and adult neuro-oncology, pediatric neurosurgery, pediatric oncology, neuropathology, pathology/cytogenetics, clinical pharmacy, and research team members including bioinformaticians, genetic counselors, and study coordinators. This conference was held at the University of Michigan and teleconferenced with clinicians/researchers from six children's hospitals in order to improve consensus treatment opinion and to generate discussion of clinical trial availability. Protected health information from cases was removed to respect the privacy of each patient. Selected findings underwent additional independent CLIA-validated testing, and summarized results were disclosed to treating oncologists and families by the clinical sequencing team, board-certified clinical geneticists, and counselors, as appropriate.

Evaluation of Targeted Agents

Mutations identified by sequencing were first categorized using a grading schema reported by Parsons *et al.* regarding clinical utility of observed somatic mutations in tumor and germline whole-exome sequencing (category I corresponding to mutations with established clinical utility, category II corresponding to mutations with potential clinical utility, category III corresponding to mutations in other consensus cancer genes, and category IV corresponding to mutations in other genes).(11) Next, the likelihood of CNS penetration was evaluated. For most targeted therapies, the amount of pre-clinical and clinical evidence regarding BBB penetration and CNS activity of these small molecular inhibitors is limited. In addition, a large number of small molecule inhibitors are substrates for efflux transporters such as the ATP-binding cassette (ABC) family, also known as multi-drug resistant (MDR) transporters, limiting the retention of these compounds in the CNS. Given these known limitations, a systematic way of evaluating targeted therapies was developed based on an extensive literature review of each potential compound's predicted ability to cross the blood

brain barrier and the level of pre-clinical and clinical evidence to support its use in any specific case. These criteria are summarized in Table 1.

To assess the likelihood of diffusion across the BBB, a compound's molecular weight, lipophilicity, and polarity were assessed using Lipinski's rule of five, a rule of thumb commonly used by medicinal chemists to predict the likelihood of a molecule being membrane permeable.(12) To satisfy these parameters, agents must be a) sufficiently small (molecular weight <500–600 g/mol); b) lipophilic (logP [partition coefficient] >1); and c) relatively non-polar (based on the number of hydrogen bond donors and acceptors).(13) Two additional characteristics included in our evaluation of each compound were the percentage of protein binding—as only unbound drug is able to cross the BBB—as well as the affinity of the compound for efflux pumps, most commonly P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP). As mentioned previously, compounds that are high-affinity substrates for these efflux pumps in general experience severely limited CNS distribution. To ensure uniform evaluation of targeted therapies the UM CNS-TAP system was developed with seven criteria to score utility of the agent, including (1) pathway targeted/relevance to sequencing results, (2) pre-clinical data, (3) pediatric phase I data, (4) clinical data in CNS tumors, (5) active clinical trials for which the patient may be eligible, (6) CNS/BBB penetration, and (7) relevant pediatric formulation (pill vs suspension). In Supplemental Figure 1, we illustrate an example checkbox developed for the purpose of evaluation of everolimus for a patient with a tumor containing a mutation in *PIK3CA*.

CLINICAL CASES

Case #1 (FGFR)

A 13-year-old previously healthy boy presented with a 2-week history of slurred speech, gait unsteadiness, right-sided facial droop, and increasing frequency of headaches. After head CT and brain MRI revealed a brainstem mass, he underwent stereotactic biopsy and subtotal resection, with pathology consistent with a diffuse intrinsic pontine glioma (DIPG), World Health Organization (WHO) grade IV. He then received 59.4 Gy of focal radiotherapy (RT) without concurrent chemotherapy. Sequencing of his tumor revealed an activating mutation in fibroblast growth factor receptor (*FGFR3*, category II/III), a K27M mutation in *H3F3A* (Histone H3.3, category III, and the defining mutation of pediatric midline glioma), and loss of *BCOR* (category III). (14) Four studies compiled by the cBioPortal cancer genomics database have identified 10 CNS cases with observed mutations in *FGFR3* in glial tumors, and *FGFR1* mutations, internal duplications, and fusions are seen in over a third of pediatric diffuse gliomas. (15–17)

Application of our CNS-TAP platform and discussion in our multi-institutional CNS Precision Medicine Tumor Board led to selection of the tyrosine kinase inhibitor, ponatinib, as adjuvant oral therapy after RT. No relevant clinical trials were available, thus the agent was prescribed off-trial. The patient received daily ponatinib for 28-day cycles, starting at 15 mg and increasing to 45 mg with toleration (the patient was adult size). (18) He tolerated the drug relatively well, but ultimately decreased to 30 mg daily due to gastrointestinal symptoms including diarrhea and abdominal pain.(19) He maintained stable disease for six

months on this therapy (10 months from diagnosis), but ultimately developed progressive disease and underwent re-irradiation.

Ponatinib is a multi-tyrosine kinase inhibitor (TKI) approved for patients with chronic myeloid leukemia and Philadelphia chromosome-positive acute lymphoblastic leukemia resistant or intolerant to other BCR/ABL TKIs.(20) In addition to potent activity against BCR/ABL, ponatinib has potent activity against a multitude of other tyrosine kinases, including inhibition of FGFR1-4 with IC₅₀'s of < 40 nM.(21) In preclinical models of a wide variety of FGFR-amplified tumors, ponatinib displayed potent growth inhibitory activity, considerably greater than other FGFR inhibitors (e.g., dovitinib, cedirinib).(21) Although no preclinical data exists for ponatinib in DIPG, ponatinib has displayed significant activity *in vitro* against glioblastoma cells and caused significant tumor reduction and induced considerable tumor apoptosis in a murine xenograft glioblastoma model.(22)

There is minimal published experience on the use of ponatinib in pediatric patients. One case report describes the safe use of ponatinib in a 12 year old, >70 kg patient at 45 mg once daily, the FDA approved dose for adults.(19) Data regarding use in CNS malignancies is also lacking; however, ponatinib displayed excellent CNS penetration in a murine model, with a brain:plasma concentration ratio of 0.88.(23) Ponatinib is a relatively small (molecular weight = 569 g/mol), lipophilic (logP = 4.3) molecule, both ideal characteristics for CNS penetration (Figure 1).(20, 24) Although it is highly protein bound (>99%) and a substrate for P-gp and BCRP, it also *inhibits* both transporters, likely explaining the superior CNS penetration compared to other small molecules that are substrates for such efflux pumps. Because of the preclinical data demonstrating excellent CNS penetration and the *in vitro* efficacy against FGFR-driven cell lines, ponatinib was chosen as the FGFR inhibitor in this case.

Case #2 (NF1)

A 17-year-old boy presented with vomiting, weight loss, headaches, blurry vision, confusion, and abnormal orofacial movements. MRI revealed a 3 cm × 2 cm × 2 cm enhancing posterior fossa mass, areas of prominent leptomeningeal enhancement in the thoracic and lumbar spine, and subtle enhancement along the cauda equina concerning for metastatic disease. He underwent suboccipital craniotomy with partial resection and debulking of the tumor, and pathology was consistent with a glioblastoma, WHO grade IV. Lumbar spinal CSF was negative, although ventricular CSF at the time of surgery showed atypical cells suspicious for tumor involvement. He received 36 Gy of craniospinal irradiation given leptomeningeal and metastatic disease, with a boost of 23.4 Gy to areas with gross disease in the fourth ventricle and spine. RT was delivered with concurrent vorinostat, a histone deacetylase (HDAC) inhibitor and radiosensitizer, with dosing in accordance with Arm A of the previous ACNS0822 Children's Oncology Group (COG) protocol.(25–27) Sequencing of his tumor revealed the following: homozygous somatic *NF1* loss (category III), copy loss of *BRCA1* and *CDK12* (both category III), copy-neutral loss of heterozygosity in *TP53* (category III), a missense mutation (H179R) in *TP53* (category III), a K27M hotspot mutation in *H3F3A* (category III), and a frameshift mutation (D1313fs) in *ATRX* (category III).

Application of the CNS-TAP platform and discussion in our multi-institutional CNS Precision Medicine Tumor Board led to selection of combination therapy with the mitogen-activated protein/extracellular signal-regulated kinase (MEK) inhibitor trametinib (which acts downstream of NF1) and the HDAC inhibitor vorinostat based on pre-clinical data supporting its use for DIPG with H3.3 mutations, as well as the patient's previous tolerance of vorinostat during RT.(28) No relevant clinical trials were open for enrollment. Trametinib was given 1.5 mg daily (note: patient was adult size, >70 kg) and vorinostat was given at 200 mg twice daily for 7 days, every other week. The patient tolerated combination therapy quite well, and had a partial response for 5 months (8 months from diagnosis, Figure 2), followed by progressive disease.

Trametinib is a reversible inhibitor of MEK 1 and 2 with an IC₅₀ of 0.92 nM and 1.8 nM, respectively.(29) It is approved for use in combination with the BRAF inhibitor dabrafenib in patients with unresectable or metastatic melanoma and a BRAF V600E mutation, including those with brain metastasis.(30) While pre-clinical evidence of efficacy in glioblastoma xenograft models is lacking, *in vitro* MEK1/2 inhibition has been found to reduce proliferation of oligoastrocytomas and astrocytomas with *NF1* loss and concomitant BRAF mutations.(31) *In vivo* growth inhibition has also been demonstrated in solid tumor xenografts.(29, 32) Furthermore, there are case reports of efficacy in patients with brain metastasis in melanoma as well as in a patient with *NF1*-associated glioblastoma.(33, 34)

Trametinib is a 615 Da, moderately lipophilic, and 97% protein-bound molecule (Figure 1). (30, 35) The drug has been shown to be substrate for both P-gp and BCRP, with a brain-to-plasma area under the curve (AUC) ratio of 0.15 in murine models.(36) However, based on mean day 15 plasma C_{max} of 22.2 ng/mL in patients receiving 2 mg of trametinib, the proportion of drug crossing the BBB would be predicted to be above the IC₅₀ desired for MEK1/2 inhibition.(37) Thus, trametinib was selected as the most promising NF1-targeting therapy for this patient.(34)

Case #3 (PI3K)

A 5-year-old male initially presented with head tilt and difficulty swallowing that lasted for a week. Brain MRI revealed a mass in the fourth ventricle. He underwent a subtotal resection of the mass that was read as a rosette-forming glioneuronal tumor (RGNT), WHO grade I. Testing for BRAF V600E mutation by real-time PCR was negative. The patient was treated as per the COG trial A9952 induction, a regimen based on carboplatin and vincristine, and was noted to have locally progressive disease on week 7 of induction. He subsequently underwent a second subtotal resection, and tumor pathology was still consistent with a low-grade glioneuronal tumor. The patient received bevacizumab (10mg/kg every 2 weeks) for 36 weeks, and treatment was stopped electively following the parents' preference and in view of stable disease. During a routine follow-up 4 months later, a brain MRI showed further tumor growth with extensive ventricular dissemination (complete filling of the fourth ventricle, cerebral aqueduct, posterior third ventricle with associated brainstem invasion and involvement of the atrium of the left lateral ventricle). An MRI of the spine was done at that time and was negative for disease.

Sequencing revealed a *PIK3R1* mutation (category III).(11) *PIK3R1* encodes the p85-alpha regulatory subunit of the enzyme phosphatidylinositol 3-kinase (PI3K). The alteration found (D464_R465insREYD), while not fully characterized, has been previously reported in the context of oncogenesis.(38) Unlike other low-grade gliomas where genomic aberrations involving the MAPK pathway predominate, FGFR and PI3K mutations have been mostly reported in RGNT, albeit a very rare tumor in children.(39, 40) This case was discussed in the CNS Precision Medicine Tumor Board. Targeted therapy with everolimus or traditional cytotoxic chemotherapy (a regimen containing thiaoguanine, procarbazine, lomustine and vincristine) were offered to patient's family. They selected targeted treatment with everolimus 5mg/m² daily continuously in 28-day cycles. The patient remained on everolimus for 6 months with stable disease on MRI. The drug was very well tolerated without any toxicity but was stopped after 6 cycles because of parents' wish to adopt a purely complementary and alternative medicine approach to treatment.

Everolimus inhibits the mammalian target of rapamycin (mTOR) leading to inhibition of protein synthesis and cell proliferation. It is currently approved as an immunosuppressant in solid organ transplant recipients as well as a treatment option in a variety of metastatic solid tumors and in patients with tuberous sclerosis complex (TSC) with subependymal giant cell astrocytoma (SEGA).(41) The mTOR protein is downstream of PI3K, and thus represents a potential option for therapy in tumors with PI3K mutations. Pre-clinical models in breast cancer cell lines with *PIK3CA* mutations demonstrated sensitivity to mTOR inhibition with everolimus.(42) In a phase I trial in pediatric patients with recurrent solid tumors, mTOR inhibition was attained at doses of 3–5mg/m² (AUC 200ng/mL·h).(43) In a phase III clinical study in pediatric and adult patients with TSC-associated SEGA, everolimus use resulted in a 50% reduction in tumor size in 35% of the patients.(44)

While everolimus is a relatively large molecule (960 Da), its moderate lipophilicity and large concentration of unbound drug in the plasma allows for some drug penetration across the BBB (Figure 1).(41, 45) When tested in murine models, brain levels were found to be 1.5% of those in plasma.(46) Although everolimus is a substrate for P-gp, it is also an inhibitor of this efflux pump.(41) This property, coupled with the agent's longer half-life in the CNS, results in accumulation of the drug in the brain over time. This dose-dependent accumulation results in concentrations above the IC₅₀ in glioblastoma cell lines for 12 and 24 hours in PTEN⁺ and PTEN⁻ cells, respectively.(46) Because of this, everolimus represents an attractive therapeutic agent in CNS tumors and is currently being tested in two clinical trials in pediatric patients with recurrent or refractory brain tumors.(47, 48)

Case #4 (HIST1H3B K27M)

A 5-year-old female presented with diplopia, left facial weakness, difficulty walking, and tripping. Brain MRI identified a large enhancing lesion centered in the left brachium pontis with extension into the pons and crossing the midline, with a T1 hypointense cystic component, consistent with diffuse intrinsic pontine glioma (DIPG). Stereotactic biopsy was performed with pathology showing features consistent with DIPG, WHO grade IV. The patient received 54 Gy of involved field RT without concurrent chemotherapy. Sequencing of her tumor revealed a *HIST1H3B* K27M hotspot mutation (category IV), *PIK3CA* E545K

hotspot activating mutation and C378R sub-clonal mutation (category II–III), and copy gain of *H3F3A* (category III).(28)

Application of our CNS-TAP platform and discussion in our multi-institutional CNS Precision Medicine Tumor Board led to selection of combination therapy with everolimus (targeting *PIK3CA*) and the HDAC inhibitor panobinostat (targeting *HIST1H3B*). No relevant clinical trials were available at the time for this patient. Everolimus was given 5mg/m² daily continuously in 28-day cycles, and panobinostat was given 20 mg three times weekly, every other week (patient weight = 27.6 kg). The patient tolerated combination therapy well without notable side effects. She remained intermittently on this therapy. Therapy was intermittently discontinued in order to pursue several different early-phase experimental modalities and re-irradiation (due to progression following one of these therapies). She ultimately developed further progression while on panobinostat and everolimus (18 months from diagnosis).

Valproic acid, vorinostat, belinostat, romidepsin, and panobinostat are the current FDA approved, clinically available HDAC inhibitors. Vorinostat and panobinostat have demonstrated *in vitro* activity in pre-clinical models of DIPG. The COG DIPG preclinical consortium conducted a chemical screen of 83 compounds against a panel of 14 patient-derived DIPG cell lines.(28) While traditional chemotherapies such as temozolomide, carboplatin and vincristine displayed minimal activity *in vitro*, notably the multi-HDAC inhibitor panobinostat was active against 12/16 DIPG cultures. Of the other HDAC inhibitors tested, valproic acid was not active and vorinostat was active against 4/14 patient-derived cell lines, but less potent than panobinostat *in vitro*. Panobinostat successfully prolonged survival in a murine model compared with controls, and doses of 20 mg/kg intraperitoneally resulted in CNS panobinostat levels of approximately 200 nM, higher than the IC₅₀ (approximately 100 nM).

While *in vitro* and murine data of panobinostat are promising, it is unknown if adequate CNS penetration can be achieved in humans at clinically achievable serum concentrations. Panobinostat is a relatively small and moderately lipophilic molecule which is approximately 90% protein bound (Figure 1).(49, 50) Unfortunately, panobinostat is a known P-gp substrate, likely limiting its CNS penetration. To illustrate, in a phase I dose escalation study of panobinostat in pediatric patients with relapsed/refractory acute leukemia, 4 CSF specimens were evaluated for panobinostat CSF penetration.(51) In all four samples, panobinostat was not detectable below the lower limit of quantification of 0.1 ng/mL. In adult HIV patients receiving panobinostat 20 mg three times weekly, every other week, as a part of a clinical trial, 11 individuals had CSF analyses completed. Panobinostat was not detectable in the CSF of all 11 patients.(52) Despite limited clinical evidence of panobinostat CNS penetration, panobinostat in combination with bevacizumab has been studied in a phase I trial in adults with recurrent high grade glioma; 3/14 patients had partial responses and the median PFS was 4.3 months.(53) Given the impressive *in vitro* data, panobinostat is also being studied in two phase I trials in patients with progressive DIPG. (54–56)

While panobinostat is significantly more potent than vorinostat *in vitro*, it is notable that vorinostat likely achieves superior CNS penetration based on murine data.(57) Similar to panobinostat, vorinostat is a small, lipophilic molecule that is only 71% protein bound. However, unlike panobinostat, vorinostat is not a P-gp substrate. Despite the potentially greater CNS penetration of vorinostat, it is unknown whether this is sufficient to make up for the significantly lower potency of this agent in vitro DIPG models. Because of the promising pre-clinical data of panobinostat in DIPG (28) and safety data from its use in children (58), this agent was chosen to target the *HIST1H3B* K27M mutation in this case.

Case #4 (Targeting Efflux Proteins)

Given that efflux mechanisms appear to be the main limitation to CNS penetration of panobinostat, the concomitant use of a P-gp inhibiting pharmacologic agent (everolimus) may be beneficial. A common theme in the evaluation of targeted agents in neuro-oncology is the impediment of BBB penetration due to the presence of efflux pumps. These energy-dependent transporters are expressed on the apical surface of the cells that line the vasculature and actively efflux compounds that are able to cross the membrane via passive diffusion or other methods, thus limiting CNS accumulation of promising targeted therapies. (59) The most commonly found ABC efflux transporters, P-gp (also known as ABCB1 or MDR1) and BCRP (also known as ABCG2), are responsible for the efflux of a wide variety of traditional chemotherapy agents (e.g., anthracyclines, taxanes, methotrexate).(60) While small molecule inhibitors that have been developed in the last 15 years allow for targeted therapy based on molecular abnormalities, the vast majority of these molecules are also substrates for P-gp and BCRP efflux pumps. Animal models with a variety of targeted therapies have demonstrated that inhibition of the efflux pumps can result in increased concentrations of the targeted therapy into the brain.(61–66)

The concept of efflux pump inhibition to enhance CNS concentrations of the desired targeted agents has been shown to be effective pre-clinically, as well as in several patient cases.(62–67) Elacridor, a dual P-gp and BCRP inhibitor, was developed as such a pharmacokinetic modulator; however, this agent is not FDA approved or currently available clinically. There are few known, FDA-approved agents that inhibit both P-gp and BCRP, yet have minimal adverse pharmacologic effects. Everolimus, discussed previously in case #3, inhibits both P-gp and BCRP. In a preclinical study, everolimus was added to vandetanib, an oral multikinase inhibitor and P-gp and BCRP substrate with minimal CNS penetration but demonstrated *in vitro* activity in preclinical non-small cell lung cancer and brain tumor cells. (62) Concomitant administration of everolimus resulted in a 3–4-fold increase in the murine brain to plasma concentration ratio of vandetanib. Based on these findings, as well as identified targetable RET mutation, a group of investigators reported treating a patient with non-small cell lung cancer with brain metastases using concomitant everolimus and vandetanib on a study protocol (ClinicalTrials.gov #NCT01582191), ultimately achieving both a systemic and intracranial response by PET/CT and MRI, respectively.(67) Similarly, in pre-clinical models, by modulating P-gp and BCRP with the epidermal growth factor (EGFR) inhibitors erlotinib and canertinib, brain accumulation of pazopanib, a multikinase inhibitor approved for renal cell carcinoma and soft-tissue sarcoma, was increased 2–2.5-fold.(63)

While mTOR inhibitors possess the largest degree of evidence as successful inhibitors of BBB efflux pumps both clinically and pre-clinically, alternative P-gp and BCRP inhibitors without significant “off-target” pharmacologic effects may be ideal (e.g., avoidance of mTOR inhibitor mucositis, diarrhea, metabolic abnormalities, and rash). Few agents display such characteristics. Rolapitant, a neurokinin-1 antagonist approved as an antiemetic in patients receiving highly emetogenic chemotherapy, is a strong inhibitor of both P-gp and BCRP and has minimal adverse effects; thus, despite the lack of data, this agent may represent an ideal future candidate therapy for efflux pump modulation in neuro-oncology. (68) Finally, while inhibition of efflux pumps is a promising theoretical concept for optimizing BBB penetration, care should be taken to avoid other drug interactions (e.g., CYP inhibition) that may result in increased systemic exposure of the targeted therapy of interest and ultimately greater toxicity. Future studies are still needed to characterize the safety and efficacy of efflux pump modulation to optimize efficacy of targeted therapies in CNS malignancies.

Case #5 (CDK4/6)

A 5-year-old previously healthy boy presented with frontal headaches, nausea, vomiting, and transient blurry vision. Head CT identified a heavily calcified 3.8 cm × 3.5 cm × 3.6 cm right basal ganglia mass, and brain MRI revealed a large right thalamic mass with calcific and cystic components as well as edema and mass effect causing entrapment of the anterior horn of the right lateral ventricle. Total spine MRI showed no evidence of disease. He underwent a craniotomy with intraoperative MRI guidance, achieving subtotal resection. Pathology identified a glioblastoma, WHO grade IV. He subsequently underwent adjuvant chemoradiation with 59.4 Gy and daily temozolomide. Following completion of radiation, he began maintenance therapy with cyclic temozolomide and CCNU, but surveillance MRI and MRI spectroscopy was concerning for tumor progression as well as showed hydrocephalus, so his third cycle was held. Sequencing of his tumor revealed *CDK4* amplification and outlier expression (category III), *CCND2* copy gain and outlier expression (category III), a point mutation (p.R248Q) and one copy loss in *TP53* (category III), a K27M mutation and copy neutral loss of heterozygosity in *H3F3A* (category III), *PDGFRA* and *NTRK2* outlier expression.

Application of our CNS-TAP platform and discussion in our multi-institutional CNS Precision Medicine Tumor Board led to selection of combination of the CDK4/6 inhibitor palbociclib (targeting *CDK4* amplification) and panobinostat (targeting *H3F3A* mutation). Monotherapy with panobinostat was pursued at 20 mg three times weekly every other week (patient weight = 23.2 kg) which resulted in partial response after 12 months of therapy (24 months from diagnosis). He has tolerated therapy well with the exception of occasional and mild hyperbilirubinemia. For this patient, the plan is to add or transition to palbociclib with any evidence of tumor progression.

Palbociclib is an inhibitor of cyclin-dependent kinases 4 and 6 (CDK4/6) and is approved by the FDA for the treatment of estrogen-receptor positive and human epidermal growth factor receptor 2-negative metastatic breast cancer. (69) Pre-clinical evidence of activity of palbociclib in CDK4/6 mutated and retinoblastoma wild-type glioblastoma and diffuse

intrinsic pontine gliomas (DIPG) tumor xenograft cell lines supported potential pharmacologic activity of this agent in this case.(70, 71) Furthermore, oral palbociclib administration at the maximum tolerated doses for mice (150 mg/kg once daily) resulted in cell-cycle arrest of a brainstem glioma cell line driven by D-type cyclin overexpression and prolonged survival in an *in vivo* brainstem glioma mouse model.(71)

Palbociclib is a relatively small and moderately lipophilic molecule which is approximately 85% protein bound, all favorable biochemical properties for BBB penetration (Figure 1).(69, 72) Unfortunately, active transport mechanisms have been found to limit the distribution of this agent into the CNS to picomolar concentrations, considerably lower than the IC₅₀ of CDK4 and CKD6 (IC₅₀, 11nM and 16nM respectively).(61, 70) An investigation of murine and human *in vitro* models revealed high affinity of palbociclib for both P-gp and BCRP efflux transporters, which resulted in a 60-fold decrease in the brain:plasma concentration ratio of drug in wild-type mice when compared to P-gp and BCRP knockout mice on *in vivo* analyses.(61) Furthermore, a separate study evaluating the brain penetration of palbociclib in an orthotropic (intracranial) xenograft model derived from a patient-derived GBM demonstrated delivery of drug to the tumor was limited by BBB efflux transport via P-gp and BCRP; at clinically achievable concentrations in the CNS at standard doses, palbociclib had minimal activity in murine models.(73) Despite these limitations, a pediatric phase I clinical trial utilizing palbociclib was initiated in children with recurrent, progressive, or refractory CNS tumors which found the drug to be safe with a maximum tolerated dose of 75mg/m².(74) Other CDK4/6 inhibitors, abemaciclib and ribociclib, are also in clinical development and may achieve greater CNS penetration than palbociclib.(75)

DISCUSSION

Precision medicine holds considerable promise in pediatric neuro-oncology; however, one of the major hurdles to successful use of targeted agents in this setting is the ability for such therapies to penetrate the BBB. As seen in the cases presented above, many of the genomic alterations in pediatric neuro-oncology have multiple available agents that modulate such pathways. For example, there are five FDA-approved HDAC inhibitors, at least seven FDA-approved agents that have activity against PDGFRA, and numerous potential inhibitors of the RAS/RAF/MEK pathway (Figure 3). Using our CNS-TAP platform to systematically evaluate all available published data and on-going clinical trials to assist in informing the potential safety, efficacy and predicted BBB penetration of relevant targeted agents, precision medicine therapies with the highest likelihood for success can be chosen in a more rational manner.

Due to the heterogeneity of our patient population, and frequent concurrence of RT or other experimental therapies, it is difficult to assess efficacy of the CNS-TAP platform. Four of five of the patients had potential clinical benefit (partial response or stable disease greater than 6 months on targeted therapy) and two (Cases #4 and #5) had longer progression-free survival than historical controls; although previous or concurrent RT makes causation difficult. Given the retrospective nature of this report, it is also challenging to accurately report on the safety and tolerability of the targeted agents selected. While no patient discontinued therapy due to intolerance, side effects were not graded using CTCAE criteria,

given the limitations with attempting to grade adverse effects retrospectively. For future patients, we are currently incorporating the CNS-TAP concepts into a computer algorithm for agent selection, which will be adapted using decisions and discussions from our precision medicine conference selected and then applied in a prospective clinical trial, using a modern design, such as an “N of 1 trial,” that allow for multiple personalized agents.(76)

Many of the patients in this case series demonstrated several promising actionable genomic alterations. To date, 40 pediatric brain tumor patients have had adequate tissue for sequencing, with sequencing revealing potentially actionable germline or tumor alterations in 25 (63%) cases. While pediatric CNS malignancies are thought to arise from relatively few mutational events compared with adult brain tumors, allowing for the promise of precision medicine in this setting, the genomic heterogeneity of CNS malignancies remains a significant consideration for therapy. Molecular testing has become an integral part of the 2016 WHO classification of tumors of the CNS. However, these results frequently do not fully reflect the intra-tumoral and inter-tumoral molecular heterogeneity of CNS tumors.(77) For example, in glioblastoma alone, the number of distinctly identified molecular subtypes continues to grow.(78, 79) Differential phenotypic expression between tumor cell sub-populations is theorized to result from different cells of origin experiencing an initial cancer-promoting (“driving”) mutation which promotes tumor growth and the development of secondary (“passenger”) mutations, which are frequently sub-clonal.(80)

The driving behavior of a mutation can be inferred from the frequency of the variant in the selected tumor reads (i.e., “tumor variant fraction”).(81–85) With multiple potential pathways affected by the landscape of molecular alterations within a tumor, this raises the important question of whether multi-agent targeted therapy—simultaneously or sequentially—is justified, both biologically and from a cost perspective. Our algorithm-based model for agent selection and future prospective trial will include variant fraction to account for heterogeneity and clonal relevance.

The timing of the biopsy is important as well, as gliomas have also been shown to develop numerous mutations in response to cytotoxic chemotherapy and irradiation.(86) Repeat biopsy or resection at time of therapy selection may yield more relevant molecular results but at the risk of additional surgical procedures. Recently, a few groups have shown the feasibility of profiling cell-free tumor DNA from the CSF of adults with CNS tumors.(87, 88) This may represent an optimal way to measure tumor evolution and treatment response in future CNS precision-medicine based trials.

In conclusion, we have described the establishment of the UM CNS-TAP, a multi-institutional translational program for evaluating the CNS activity of targeted therapies in pediatric neuro-oncology. The cases above highlight representative pathways and treatment courses for patients treated according to this program, as well as discussions prompted in our precision medicine conference (e.g., efflux protein inhibition). This content can be used as a platform for optimizing the use of precision medicine in pediatric neuro-oncology. We hope this will improve the outcomes for children with pediatric brain tumors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ABC	ATP-binding cassette
BBB	Blood-brain barrier
BCRP	breast cancer resistance protein
CLIA	Clinical Laboratory Improvement Amendments
CNS	Central nervous system
CNS-TAP	CNS Targeted Agent Prediction
COG	Children's Oncology Group
DIPG	Diffuse intrinsic pontine glioma
FGFR	fibroblast growth factor receptor
HDAC	histone deacetylase
MDR	multi-drug resistant transporter
mTOR	mammalian target of rapamycin
P-gp	P-glycoprotein
RGNT	rosette-forming glioneuronal tumor
RT	radiotherapy
SEGA	subependymal giant cell astrocytoma
TSC	tuberous sclerosis complex
UM	University of Michigan
WHO	World Health Organization

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AT A GLANCE COMMENTARY**Background**

Precision medicine holds significant promise in neuro-oncology; however, penetration of the blood brain barrier (BBB) limits adoption of many targeted therapies into patient care. The data regarding BBB penetration of targeted agents remains limited and challenging to interpret.

Translational Significance

The CNS Targeted Agent Prediction (CNS-TAP) algorithm was developed to evaluate the probable CNS activity of targeted therapies in neuro-oncology cases in which clinically-integrated DNA and/or RNA sequencing was conducted. A multi-institutional precision medicine conference reviewed cases and recommended targeted therapies for patients using the CNS-TAP algorithm. Four of five patients had potential clinical benefit.

Drug	Target	Properties				CNS Data				Active Clinical Trials	Pediatric Formulation Considerations
		MW (Da)	Lipophilicity (LogP)	Protein Binding (%)	Active Transport	CNS Penetration	Pre-clinical (Refs)	Pediatric Phase I (Refs)	CNS Clinical (Refs)		
Ponatinib	FGFR/PDGFR	569	4.3	>99	P-gp substrate BCRP-substrate	Brain:plasma Cmax 0.88	21, 23	None published	None published	NCT02478164	Oral tablet
Trametinib	MEK	615	3.18	97	P-gp substrate BCRP-substrate	Brain:plasma AUC 0.14	29, 31, 32	None published	33, 34	NCT02015117 NCT02124772 NCT02034110	Oral tablet
Everolimus	mTOR	960	5.01	74	P-gp substrate P-gp inhibitor	Brain to plasma Cmax 0.015 AUC 0.18	43	43	44	NCT02015728 NCT01734512 NCT01434602	Tablet; can be formulated into suspension
Panobinostat	HDAC	350	2.39	90	P-gp substrate	None detected in tissue samples of treated patients	28	58	53	NCT02717455 NCT02899715	Oral capsule
Palbociclib	CDK4/6	448	2.77	85	P-gp substrate BCRP-substrate	Minimal due to efflux	70, 71	74	None published	NCT02530320 NCT02896335	Oral capsule

Figure 1. Drug properties and CNS penetration. This figure depicts each of the five targeted agents’ abilities to cross the blood-brain barrier, based on molecular properties and clinical and pre-clinical data. Green boxes denote favorable properties or evidence that supports CNS penetration of the agent, yellow boxes indicate equivocal or mixed properties/evidence, and red boxes denote suboptimal properties or lack of data supporting CNS penetration of the agent.

Figure 2. Treatment of a patient with *NF1* loss and *H3.3* K27M hotspot mutation with combination of MEK and HDAC inhibitors. A–C: Results of MI-ONCOSEQ tumor/germline sequencing revealed homozygous somatic *NF1* loss, a missense mutation (H179R) and copy neutral loss of heterozygosity in *TP53*, an *H3.3* K27M hotspot mutation, and a frameshift mutation (D1313fs) in *ATRX*. D: The patient was treated with radiotherapy and the HDAC inhibitor, vorinostat, followed by adjuvant therapy with vorinostat and the MEK inhibitor, trametinib, resulting in a good partial response.

Figure 3. Common pathways targeted in CNS precision medicine. Here, we illustrate several cellular pathways likely involved in driving tumor growth and development in the five pediatric CNS tumor patients presented in this series. Each pathway identifies the locations of specific actionable mutations (red circles), as well as a corresponding agent which may inhibit the involved pathway (green circles), with the numbers corresponding to the cases in the order in which they have been presented.

Abbreviations: FGFR, fibroblast growth factor receptor; PDGFR, platelet-derived growth factor receptor; mTOR, mammalian target of rapamycin; HDAC, histone deacetylase; HAT, histone acetyltransferase; Ac, acetyl group; CDK, cyclin-dependent kinase; TSC, tuberous sclerosis complex

Table 1

Chemical properties associated with blood brain barrier penetration

Characteristic	Desired
Molecular weight	500 Da
Octanol-water coefficient (Log P)	5 (1–4 preferred)
Hydrogen Bonding	five H-bond donors ten H-bond acceptors
Protein binding	<90%
Affinity for P-gp or BCRP	Compound not a substrate