Original Research

The "SEED" Study: The Feasibility of Selecting Patient-Specific Biologically Targeted Therapy with Sorafenib, Everolimus, Erlotinib or Dasatinib for Pediatric and Young Adult Patients with Recurrent or Refractory Brain Tumors

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Abstract

Background: Pediatric brain tumors are the leading cause of cancer death in children and represent a variety of diseases and molecular subtypes. This study sought to evaluate a rapid immunohistochemistry testing panel to aid in therapy selection at the time of malignant tumor recurrence. **Methods**: With IRB approval and appropriate informed consent, we conducted a single-institution prospective clinical trial of selected kinase inhibitor therapy. A laboratory-developed immunohistochemical testing panel was performed on tumor tissue, and therapy with one of four small molecule inhibitors was recommended in combination with oral chemotherapy consisting of temozolomide and etoposide. **Results**: All 20 enrolled subjects were assigned to Everolimus (n = 4), Erlotinib (n = 6) or Dasatinib (n = 10); 90% (18/20) within the pre-specified 14-day feasibility time period. Only two subjects elected treatment on study, 8 received targeted treatment based on testing results either alone (n = 5) or in combination with chemotherapy (n = 3). Other subjects received chemotherapy alone (n = 7), surgery alone (n = 2) or no further therapy (n = 3). Immunohistochemical targets were associated with correlative genetic changes in 28% (5/18) of those evaluated. **Conclusions**: It was feasible to rapidly select targeted therapy in recurrent pediatric brain tumors, but not feasible to treat with a uniform combination treatment regimen.

Keywords: targeted; chemotherapy; pediatric; brain tumor; clinical trial

1. Introduction

Pediatric brain tumors are the leading cause of cancer death in children, with no standard effective therapy for patients who relapse. That being said, pediatric brain tumors are not a single uniform group, but are instead a heterogeneous group of rare neoplasms with many molecular drivers and subtypes, which have been defined in recent years [1]. The sheer number of new molecular diagnoses in pediatric brain tumors can make it challenging to design clinical trials, since many molecular subtypes are uncommon, and it may be challenging to enroll sufficient numbers of patients to evaluate efficacy within a specific histologic tumor type. At the same time, histology agnostic therapies have emerged such as PD-1 inhibitors and NTRK-inhibitors which have proven effective for a variety of tumor types [2,3]. Many other tyrosine kinase inhibitors have also been developed and basket trials such as the Pediatric MATCH are underway to evaluate the tumor response of these drugs in wide range of tumors (ClinicalTrials.gov Identifier: NCT03155620). The median progression free survival for pediatric patients with recurrent CNS tumors enrolled on phase II studies of single-agent therapy is less than two months [4–9].

Temozolomide and etoposide are an attractive combination as backbone chemotherapy because of their oral route of administration, and different mechanisms of action with preclinical data suggesting synergy between alkylating agents and topoisomerase inhibitors [10–12]. A phase I study established safe dosing of this combination even in heavily pre-treated patients, with hematologic dose-limiting toxicity [13]. In a prospective trial of this combination in 11 pediatric and young adult patients with high-grade brain tumors, five patients responded with tumor shrinkage, and an additional five patients achieved prolonged stable disease. Responding patients included diffuse midline glioma, CNS embryonal tumor, NOS, glioblastoma, and anaplastic astrocytoma [14].

In a review of pediatric patients with recurrent gliomas treated with oral etoposide and temozolomide using various dosing schedules, Korones *et al*. [15] reported responses in 7 of 11 patients, including a partial response in a patient with brainstem glioma and a complete response in a patient with glioblastoma. The rate of response in these preliminary combination studies appears to be much better than

single-agent studies of either drug [8,16–18].

The primary objective of this study was to determine the feasibility of utilizing data from rapid immunohistochemical studies performed on patient tumor tissue to inform treatment decisions in the setting of relapsed or refractory pediatric brain tumors. Secondary objectives were to estimate the objective response rate in patients who received biologically directed therapy in combination with chemotherapy, estimate event free and overall survival for pediatric patients who receive biologically directed therapy, and to further describe toxicity and tolerability of the combination regimens used in this study. Given prior experience with targeted next-generation sequencing [19,20], correlative testing was performed to compare clinical sequencing to clinical immunohistochemical testing.

2. Materials and Methods

2.1 Retrospective Biomarker Assessment

Potential protein biomarkers were initially screened using immunohistochemical stains to archived formalin-fixed paraffin-embedded tissue microarrays. Tissue microarrays included a variety of high grade primary pediatric brain tumors including initial diagnosis of medulloblastoma (n = 28), ependymoma (n = 17), high-grade glioma (n = 10), and CNS embryonal tumors, NOS (n = 6). In addition, 13 paired tumors obtained at the time of relapse included 2 medulloblastoma, 8 ependymoma, 1 high-grade glioma and 2 CNS embryonal tumors. Laboratory-developed immunohistochemical testing was performed for the following proteins: EGFR, HER2, CKIT, PDGFRA, pS6, pERK and scored using the methods detailed below. All patients included in these initial screening studies had appropriate consent and IRB approval was obtained for this analysis. Survival analysis was performed using data extracted from the electronic medical record system. The log-rank test was used to evaluate the significance of associations between survival, tumor type, age at diagnosis, extent of resection, and immunohistochemical staining pattern. Stepwise Cox regression was used to conduct multivariable analysis. Hazard of death associated with each biomarker was adjusted for tumor histology, age, and extent of resection.

2.2 Prospective Clinical Trial Ethics Approval

This study was conducted as a prospective phase 1 clinical trial. FDA risk determination was requested regarding complex laboratory-developed testing, and the immunohistochemical panel was determined to pose a nonsignificant risk in the context of this study. The study was subsequently approved by the Seattle Children's Institutional Review Board (IRB). Informed consent, and assent where applicable, was obtained from study participants or their legal guardians according to institutional standards, including IRB-approved process for phone consent for patients not residing in the region.

2.3 Eligibility Criteria

Eligibility for tumor testing included age less than 30 years, Karnofsky or Lansky performance score ≥50%, and documented progression or recurrence of brain tumor by MRI or CSF since completion of last tumor-directed medical therapy, for which there was no known curative therapy. Histologic confirmation of brain tumor was required from either initial diagnosis or relapse. Most recent surgical tissue was preferred and utilized for study treatment selection if discrepant results were found between initial diagnostic and relapse tissue. Additional eligibility criteria to begin study therapy included adequate organ function and recovery from prior therapy. Adequate organ function included bone marrow function defined as absolute neutrophil count >750/uL and platelet count >75/uL; renal function defined as normal creatinine for age; liver function defined as bilirubin and alanine aminotransferase ≤1.5 times upper limit of normal; and neurologic function defined as well-controlled seizures on non-enzyme inducing antiepileptics, and no increase in steroid dose within past seven days. Adequate recovery from prior therapy was considered three weeks from surgery or myelosuppressive chemotherapy; seven days from hematopoietic growth factors or biologic therapy; 12 weeks from craniospinal radiation and 2 weeks from focal radiation therapy. Exclusion criteria consisted of pregnancy, breastfeeding, uncontrolled infection, within one year of allogeneic transplant, bleeding disorder or more than punctate intratumoral hemorrhage, other anti-neoplastic agents, immunosuppressive agents, or strong CYP3A4 inducers or inhibitors.

2.4 Treatment Plan

Treatment was recommended but not required for patients consented to study. Treatment consisted of 28-day cycles of oral temozolomide 150 mg/m² daily on days 1–5, oral etoposide 50 mg/m² daily on days 1–12 and continuous kinase inhibitor based on arm assignment to either Sorafenib (Arm A) 150 mg/m²/dose twice a day, Everolimus (Arm B) 3 mg/m²/dose daily, Erlotinib (Arm C) 85 mg/m²/dose daily, or Dasatinib (Arm D) 60 mg/m²/dose twice a day. Eligible subjects were permitted to begin study treatment with temozolomide and etoposide alone prior to laboratory testing results if treatment was deemed to be urgent based on disease status.

2.5 Treatment Assignment

All cases were reviewed by the study pathologist to confirm the diagnosis. The schema for assignment of treatment arm (see Fig. 1) was based on specificity of targeted agent with more specific agent selected if target was present. If more than one tumor specimen was available from different surgical procedures, the results of the most recent relapse specimen were prioritized. Additionally, if a sample was positive for more than one marker the drug priority was first Erlotinib, then Dasatinib, Everolimus, and

last Sorafenib. Priority was based on drug specificity from most specific to less specific.
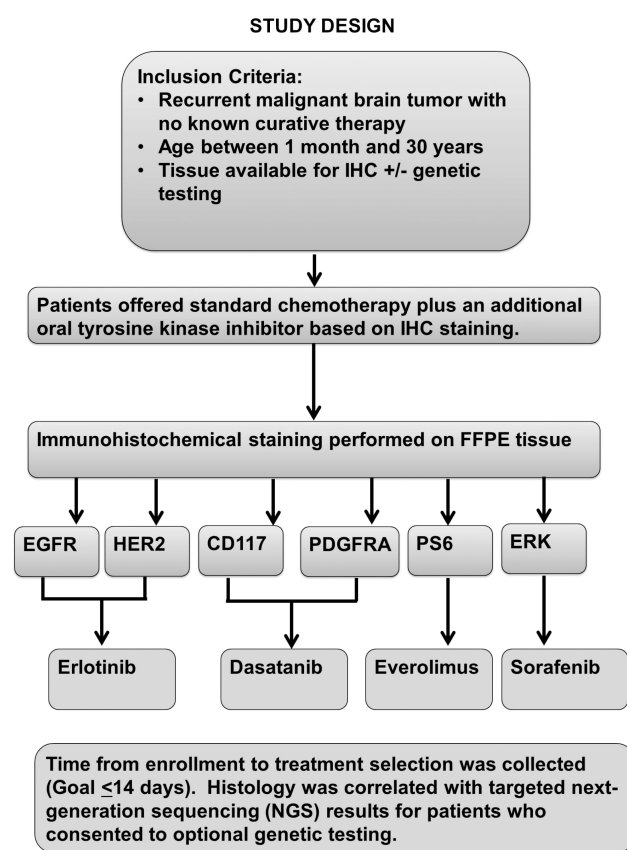
Fig. 1. CONSORT diagram with study arm assignment.

Four-μm-thick paraffin sections of each case were stained using a Ventana Benchmark Stainer (Tucson, AZ). Sections were incubated with primary antibodies in the following concentrations: EGFR, 1:100 (Invitrogen, Carslbad, CA, USA); CD117, 1:2000 (Dako, Carpinteria, CA, USA); pERK, 1:50 (Cell Signaling Technology, Danvers, MA, USA); PDGFRA, 1:100 (Santa Cruz Biotechnology Inc., Dallas, TX, USA); and pS6, 1:150 (Cell Signaling Technology, Danvers, MA, USA) diluted in phosphate-buffered solution (PBS). Slides were incubated with biotinylated secondary antibodies, followed by incubation with the streptavidin and biotinylated peroxidase complex. Sections were counterstained with hematoxylin and mounted. For HER2 testing only, unstained slides were sent to Phenopath Laboratories, Seattle, WA and stained with HER2. All testing was performed in a CAP accredited CLIA approved setting with appropriate control tissues.

2.6 Immunohistochemistry Methods

Stains were interpreted as either positive or negative according to the following algorithm. Each stain was first scored according to both staining intensity (weak, moderate, to strong) and based on the percentage of tumor cells

staining positive (0 for no staining, 1+ for <10%, 2+ for 10–50, 3+ for 50–90% and 4+ for >90%). Tumors with no staining were scored as 0. A score of 1+ or greater was considered positive for PDGFRA, EGFR, CD117, and HER2. A score of 2+ or greater was considered positive for pERK and pS6.

2.7 Next Generation Sequencing

The study was subsequently amended to offer optional correlative targeted tumor Next Generation Sequencing (NGS). Sequencing was performed using UW Oncoplex, a clinically validated method as previously reported [21]. This panel was designed to detect most classes of mutations, including single nucleotide variants, small insertions and deletions, gene amplifications, and selected gene-fusions. Sequencing libraries were prepared from DNA samples and hybridized to a custom set of complementary RNA (cRNA) biotinylated oligonucleotides targeting the exons of 262 cancer related genes and select intronic regions. NGS was performed using a HiSeq 2500 instrument system (Illumina, San Diego, CA, USA).

2.8 Treatment Response Evaluation

Response was evaluated by MRI imaging every two cycles. For subjects with measurable disease, protocol-defined tumor measurements included the product of the longest diameter and the next longest perpendicular measurement. Complete response was defined as the disappearance of all abnormal signal, partial response was defined as ≥50% decrease, minor response was defined as ≥25% decrease and progressive disease was defined as >25% increase in two-dimensional tumor measurement. Disease was considered stable if no more than 25% increase or decrease in tumor measurement.

2.9 Feasibility Analysis

The statistical design of the study defined two feasibility endpoints, namely (1) the feasibility of rapid testing, and (2) the feasibility of subjects receiving treatment on assigned study arm. Biologic selection by immunohistochemical testing was defined as feasible if at least 80% of study subjects received results of testing within two weeks of study enrollment. To meet this endpoint, tumor blocks were identified locally or acquired from another institution, tissue quality had to be adequate in amount and quality for staining and interpretation by study pathologist. Treatment on study was considered to be feasible if at least 50% of subjects started therapy with an assigned treatment arm. The treatment feasibility endpoint therefore depended on both the presence of at least one of the pre-specified protein targets as well as patient and/or treating physician decision-making.

3. Results

3.1 Retrospective Biomarker Results

In the archival tissue microarray studies eighty-nine percent of patients (54 of 61) evaluated had at least one positive immunohistochemical marker. The percent of positive tumors was 16% for EGFR, 25% for HER2, 31% for KIT, 73% for PDGFRA, 43% for pERK and 54% for pS6. Each marker was positive in a subset of tumors of each histologic type, with the exception of HER2, which was negative in all high-grade gliomas; and EGFR, which was negative in all ependymomas. In addition to tumor histology, survival was associated with extent of resection and expression of 3 of the 6 antigens evaluated: EGFR, PDGFRA and pS6 (log rank $p < 0.05$). Multivariable analysis was conducted to evaluate the independence of clinical and IHC variables. The magnitude of association between EGFR, PDGFRA and pS6 positivity and survival persisted when adjusted for tumor type, age, and extent of resection (**Supplementary Table 1**).

3.2 Subject Demographics

Twenty subjects were enrolled on this phase 1 clinical trial. One additional subject was screened and found to be ineligible due to no documented disease progression following most recent medical therapy. Subject demographics and prior therapy are detailed in Table 1. Median age at the time of enrollment was 8 years (range 1–28 years), and 11 subjects (55%) were male. All patients had received prior multi-modality therapy. The number of prior surgeries was one in eight subjects, two in eight subjects, and three in four subjects. All 20 subjects received prior radiation therapy, including seven (35%) with prior craniospinal radiation and six subjects (30%) who had received two prior radiation therapy courses. Fifteen subjects (75%) received at least one prior chemotherapy regimen. The subjects without prior chemotherapy included diagnoses of ependymoma (n = 4) and chordoma (n = 1) who had received treatment with surgery and radiation.

3.3 Feasibility of Rapid Testing

The rapid return of testing results was considered feasible, as results were returned within the 14-day study target goal in 90% of subjects (n = 18/20). Median time to return of results was 11.5 days (range 6–22 days). Only seven study subjects (35%) had tissue available at the primary study institution, and tissue was obtained from an outside institution for the remaining 13 subjects (65%). Testing was returned for the two subjects who did not meet the 14-day study target goal at 19 and 22 days. The delay to testing results was due to time to obtain tissue from outside institution in both cases. Targeted sequencing was performed for 18 subjects (90%), and oncogenic mutations or copy number changes were found in 15 of 18 tested (83%). Details of patient characteristics, prior treatment, and testing results are provided in Table 1. The highest levels of

protein overexpression (3–4+) were often observed to be associated with gene amplifications or pathway mutations (Fig. 2). In six cases, 33% of cases sequenced, the immunohistochemical staining results identified cases with genetic alterations predicted to respond to targeted drug therapy.
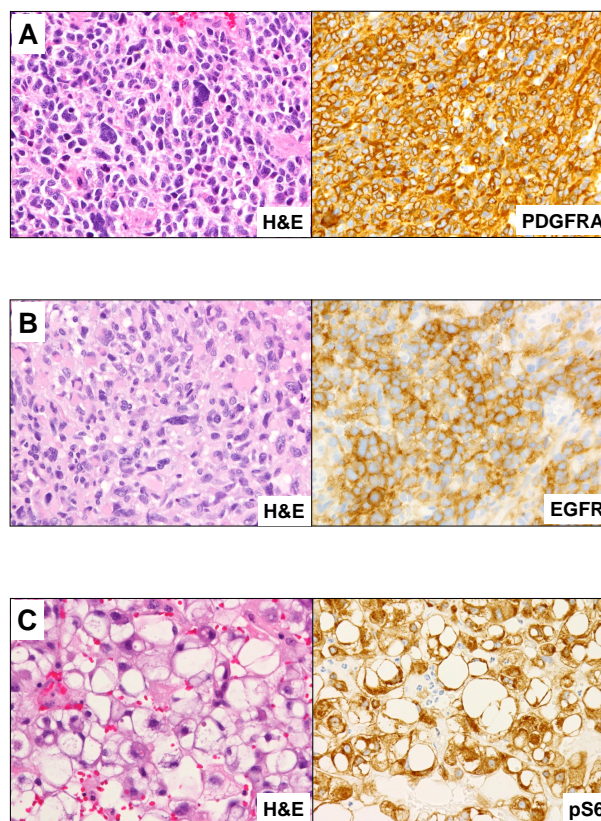
Fig. 2. Examples of high immune positivity associated with gene amplification, subject 3. (A) PDGFRA overexpression associated with gene amplification; subject 7 (B) EGFR overexpression associated with gene amplification; subject 14 (C) pS6 overexpression associated with TSC2 mutation.

3.4 Feasibility of Treatment on Study

No subjects were assigned to treatment arm A (Sorafenib). Four, six, and ten subjects were assigned to treatment arms B (Everolimus), C (Erlotinib) and D (Dasatinib), respectively. While all 20 subjects were assigned to a study treatment arm (see Fig. 1), only two subjects (10%) elected treatment with study-assigned therapy within the pre-specified four-week time-period (subjects 2 and 5). Of the two subjects who elected treatment on study, only one was able to receive kinase inhibitor; the other subject experienced a delay in start of targeted therapy due to insurance denial of targeted agent, and subsequently experienced disease progression while receiving oral chemotherapy alone. Two subjects received study-directed therapy off study, one who elected for other treatment with re-irradiation prior to

Table 1. All subjects, results, and treatment arm assignment.

ID	Age (years) and gender	Tumor location and histology	Prior radiation	Prior chemo-therapy	Prior surg-eries	Time to results (days)	Study Arm: Targeted therapy recommended	EGFR	HER2	CD117	PS6	PDGFRA	ERK	Mutations	Copy Changes
								Immunohistochemical testing results						Correlative targeted Sequencing	
1	5 F	PF medulloblastoma	CSI	yes	1	11	D: dasatinib	–	–	–	+	+	+	sequencing not done	
2	28 M	PF medulloblastoma	CSI	yes	3	12*	D: dasatinib	–	–	+	+	+	+	sequencing not done	
3	22 F	ST anaplastic astrocytoma	CSI	yes	2	12*	D: dasatinib	–	–	+	+	+	+	H3F3A G35R, BCOR, TP53, ATRX	PDGFRA amp, MYCN amp, CDKN2A del
4	14 M	ST anaplastic astrocytoma	focal	yes	1	9	D: dasatinib	–	–	–	+	+	+	H3F3A G35R, TP53, ATRX, PDGFRA, PTEN	CDKN2A del
5	9 F	ST anaplastic pilocytic astroctyoma	focal	yes	2	10	D: dasatinib	–	–	–	+	+	+	MSH6 biallelic, POLE, hypermutated	
6	4 F	PF ependymoma	focal, CSI	yes	3	14*	B: everolimus	–	–	–	+	–	+	RAD51C	
7	6 M	ST anaplastic astrocytoma	focal, focal	yes	2	6	C: erlotinib	+	–	–	+	–	+	NF1	EGFR amp, MYCN amp, CDKN2A del, PTEN del
8	8 M	ST glioblastoma	focal	yes	1	17	D: dasatinib	–	–	–	+	+	+	H3F3A K27M, PI3KCA, TP53	NF1 loss
9	7 M	PF ependymoma	focal, SRS	no	1	19*	B: everolimus	–	–	–	+	–	+	negative	
10	12 M	PF medulloblastoma	CSI	yes	2	14*	D: dasatinib	–	–	–	+	+	+	TP53, PBRM1	MYCN gain
11	14 M	ST glioblastoma	focal, focal	yes	2	12*	C: erlotinib	+	–	–	+	+	+	BCOR, KDM6A, MTOR, PDGFRA, PIK3CA, PIK3R1	EGFR amp, CDKN2A loss
12	8 M	PF ependymoma	focal	no	1	7*	C: erlotinib	–	+	–	+	+	+	NOTCH2	PTEN loss
13	23 F	ST anaplastic astrocytoma	focal	yes	3	6	D: dasatinib	–	–	+	+	+	+	IDH1, ATRX, TP53	
14	19 F	Clivus chordoma	focal	no	1	7*	C: erlotinib	+	–	+	+	+	+	TSC2	TSC2 loss
15	7 F	ST embryonal	CSI	yes	2	7*	B: everolimus	–	–	–	+	–	+		MYCN gain
16	13 M	PF ependymoma	focal, focal	no	3	22*	B: everolimus	–	–	–	+	–	+	negative	
17	5 M	PF medulloblastoma	CSI	yes	1	14*	D: dasatinib	–	–	+	+	+	+		FGFR3 amp, TACC3 amp, CDH1 loss
18	5 F	ST glioblastoma with embryonal features	focal	yes	2	9*	C: erlotinib	–/+	–/–	–/+	+	–/+	+	TP53 biallelic	MYCN amp, CDKN2A del
19	7 F	PF ependymoma	focal	no	2	12*	C: erlotinib	+	+	–	+	+	+	negative	
20	1 M	PF atypical teratoid/rhabdoid tumor	focal, spinal	yes	1	6	D: dasatinib	–	–	–	+	+	+	SMARCB1	

Key: M, Male; F, Female; PF, posterior fossa; ST, supratentorial; CSI, craniospinal radiation; SRS, steriotactic radiosurgery; *includes time to obtain tissue from other centers; amp: amplification; del: homozygous deletion; loss: single copy loss; gain: copy gain of <10.

initiation of study testing-directed therapy (subject 7); the other decided not to travel for study therapy but received treatment at home institution (subject 3). Both subjects remained on therapy for 9 months until disease progression. Subject 3 experienced initial response to combination study treatment which was sustained for >6 months (Fig. 3).
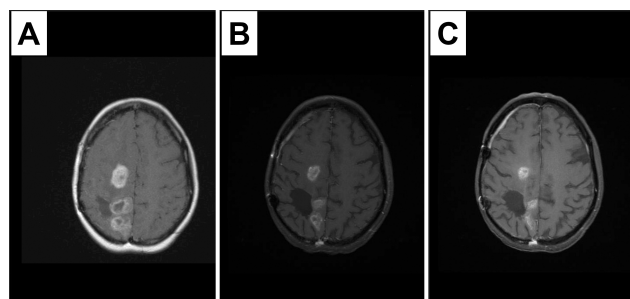
Fig. 3. Response to therapy containing dasatinib in a recurrent tumor with PDGFRA immunopositivity and amplification. MRI images at study enrollment (A), 3 months later demonstrating response (B) sustained at six months (C). Patient remained on therapy for 9 months until disease progression.

Five other subjects received targeted therapy alone based on study testing results, without recommended concurrent chemotherapy, one of whom received two sequential targeted therapies (subject 14). While a total of 8 subjects (40%) received targeted therapy based on study results, only one (5%) met the second feasibility endpoint according to study design. Details of subject therapy and follow-up are provided in Table 2.

The remaining subjects who did not receive targeted treatment received either chemotherapy alone (n = 7), surgery alone (n = 2) or no further tumor-directed therapy (n = 3). One subject who did not plan initial post resection therapy was subsequently lost to follow-up after return of study results. At the time of study completion, 12 subjects had died of disease, one died of secondary malignancy (acute myelogenous leukemia), one died of infectious complications of surgery, and three were alive with disease. Only two patients were in follow-up with no evidence of disease, one of whom was later deemed to have probable pseudoprogression.

4. Discussion

We found that it was feasible to select kinase inhibitor therapy based on a limited panel of oncogenic protein targets utilizing a rapid laboratory-developed immunohistochemical panel. Although the majority of subjects on study did not have tissue available at the local study institution, it was feasible to obtain tissue suitable for rapid immunohistochemical evaluation in all patients on study, 90% within the target window of 14 days, which allowed for the incorporation of testing results when selecting ther-

apy in the setting of recurrent pediatric malignancy. Correlative targeted sequencing was also feasible to obtain in all subjects in whom it was attempted as optional testing. NGS in general takes significantly longer than immunostaining, so we were not able to use the sequencing results in assigning patients to a treatment arm, however such technologies are improving rapidly and other pediatric brain tumor trials currently in progress are using tumor sequencing to assign treatment (ClincalTrials.gov Identifiers: NCT02724579, NCT03581292).

Despite availability of testing results in a timely manner, the study did not meet the second feasibility endpoint regarding compliance with study-prescribed treatment. We found that the study "one size fits all" approach to backbone low-dose chemotherapy was not appealing to many subjects, for a variety of reasons including the desire and ability to receive care closer to home. Most subjects selected either chemotherapy alone or targeted therapy alone rather than the study combination recommended. Many local physicians were willing to prescribe off-label targeted therapies. This practice supports patients and families remaining near home rather than traveling to a tertiary treatment center. Interestingly, and perhaps because nearly all standard medical therapy in recurrent pediatric brain tumors consists of off-label medication use, in only one case was insurance denial a treatment-limiting factor. It is also of note that only one of the subjects enrolled on this trial selected treatment on a different clinical trial, likely due to lack of clinical trial options in this heavily pre-treated group.

It is a challenge to evaluate the efficacy of any precision medicine approach, and this study was not designed or powered to be able to address an efficacy question. While the study of efficacy of a single targeted therapy for rare mutations in a specific subtype is in theory feasible, it would be wholly impractical to study each rare mutation or disease separately (e.g., Everolimus in TSC-mutated chordoma). Other clinical trials are also evaluating this histology agnostic personalized approach [22,23]. This trial does demonstrate the high prevalence of potential targets for therapy in children with recurrent brain tumors [19]. Further study of the efficacy of a selected or precision medicine approach to therapy is warranted, whether through prospective novel clinical trial design or larger collaborative population studies.

This study protocol was developed during a time of controversy over the role of the FDA in regulation of laboratory-developed testing, just prior to the release of initial FDA draft guidance on laboratory developed testing (LDT). At the instruction of the local Institutional Review Board, the study team sought FDA determination regarding the risk of the LDT used in this study, and ultimately received a non-significant risk determination from the FDA allowing the trial to proceed as designed. As laboratory testing becomes more complex, the role of the FDA versus the Center for Medicaid and Medicare (CMS) Clinical

Table 2. Treatment and follow-up.

Treatment and follow-up			Treatment after study arm assignment								
ID	Tumor location and histology	Study Arm: Targeted therapy recommended	Study treatment, on study	Study treatment, not on study	Targeted Only	Other chemo only	Surgery Only	No treatment	Description of Targeted Therapy	Status	Follow-up time (months)
1	PF medulloblastoma	D: dasatinib				+			none, combination chemotherapy	DOD	11
2	PF medulloblastoma	D: dasatinib				+*			none*, temozolomide/etoposide only	DOD	3
3	ST anaplastic astrocytoma	D: dasatinib		+					dasatinib with temozolomide/etoposide	DOD	9
4	ST anaplastic astrocytoma	D: dasatinib						+	none, no tumor-directed therapy	DOD	0
5	ST anaplastic pilocytic astroctyoma	D: dasatinib	+						dasatinib with temozolomide/etoposide	DOD	2
6	PF ependymoma	B: everolimus			+				everolimus	DOD	7
7	ST anaplastic astrocytoma	C: erlotinib		+					erlotinib with temozolomide/etoposide	DOD	10
8	ST glioblastoma	D: dasatinib						+	none, no tumor-directed therapy	DOD	3
9	PF ependymoma	B: everolimus					+		none, re-resection only	NED	32
10	PF medulloblastoma	D: dasatinib				+			vorinostat	SMN	13
11	ST glioblastoma	C: erlotinib				+			none, etoposide	DOD	1
12	PF ependymoma	C: erlotinib			+				everolimus	DOD	10
13	ST anaplastic astrocytoma	D: dasatinib						+	none, no tumor-directed therapy**	NED**	15
14	Clivus chordoma	C: erlotinib			+				imatinib, everolimus	DOC	6
15	ST embryonal	B: everolimus			+				everolimus	AWD	21
16	PF ependymoma	B: everolimus			+				everolimus	AWD	25
17	PF medulloblastoma	D: dasatinib				+			none, combination chemotherapy	AWD	25
18	ST glioblastoma with embryonal features	C: erlotinib				+			none, other study therapy	DOD	8
19	PF ependymoma	C: erlotinib					+		none, re-resection only	NED^	0^
20	PF atypical teratoid/rhabdoid tumor	D: dasatinib				+			none, combination chemotherapy	DOD	4

Key: PF, posterior fossa; ST, supratentorial; DOD, death from disease; DOC, death from other causes; AWD, Alive with disease; NED, no evidence of disease;*prescribed dasatinib, but insurance denied and patient never received, **progression later attributed to pseudoprogression, ^subject lost to follow-up after initial return of results.

Laboratory Improvement Amendments (CLIA) remains a controversy affecting the implementation of clinical trials of precision medicine.

While we observed a few anecdotal early signals of targeted therapy efficacy in children on this trial, the majority ultimately died from progression of recurrent therapy-resistant brain tumors, which remain the leading cause of cancer death in children. Much of brain tumor biology has been described in the past decade, and it currently remains unclear whether we need better targets for therapy and/or better therapies for known targets [24–30]. Novel mechanisms to target tumor-related proteins such as antibody therapy, vaccine therapy, and cellular immunotherapy are under evaluation against some of the same targets identified in this study, and going forward we plan to support our institution in clinical trials using CAR-T cells and other novel approaches (ClinicalTrials.gov Identifiers: NCT03638167, NCT03500991) [31]. These therapies may hold greater potential compared to the previously available small molecules without good CNS penetration. As our understanding of pediatric brain tumor biology and molecular subtypes changes, so will clinically relevant laboratory-developed testing. In addition, the decreasing cost and turnaround time of multiplexed genomic testing has gained acceptance and has been incorporated into clinical care.

5. Conclusions

In this phase 1 clinical trial we found rapid testing by immunohistochemistry was feasible for screening tumors and selecting kinase inhibitor therapy for patients. In addition, the highest levels of protein overexpression were often observed to be associated with gene amplifications or pathway mutations identified by sequencing. Despite availability of testing results in a timely manner, the study did not meet the second feasibility aim regarding compliance with study-prescribed treatment for a variety of reasons detailed in our manuscript. This work supports the further clinical development of targeted therapeutics that may inhibit growth of tumors based on these proteins, such as immunotherapy approaches.

Author Contributions

All authors contributed significantly to this project. Author SESL and KS designed the research study. Authors SESL, BLC, KS, and CML all performed parts of this research. SESL and BLC analyzed the data. SESL and BLC wrote the manuscript. All authors contributed to editorial changes, read, and approved the final manuscript.

Ethics Approval and Consent to Participate

This study was approved by the Seattle Children's Institutional Review Board (IRB). Informed consent, and assent where applicable, was obtained from study participants or their legal guardians.

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbl2707219.

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