PRECISION MEDICINE

original reports abstract

Comprehensive Genomic Profiling of High-Risk Pediatric Cancer Patients Has a Measurable Impact on Clinical Care

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PURPOSE Profiling of pediatric cancers through deep sequencing of large gene panels and whole exomes is rapidly being adopted in many clinical settings. However, the most impactful approach to genomic profiling of pediatric cancers remains to be defined.

METHODS We conducted a prospective precision medicine trial, using whole-exome sequencing of tumor and germline tissue and whole-transcriptome sequencing (RNA Seq) of tumor tissue to characterize the mutational landscape of 127 tumors from 126 unique patients across the spectrum of pediatric brain tumors, hematologic malignancies, and extracranial solid tumors.

RESULTS We identified somatic tumor alterations in 121/127 (95.3%) tumor samples and identified cancer predisposition syndromes on the basis of known pathogenic or likely pathogenic germline mutations in cancer predisposition genes in 9/126 patients (7.1%). Additionally, we developed a novel scoring system for measuring the impact of tumor and germline sequencing, encompassing therapeutically relevant genomic alterations, cancer-related germline findings, recommendations for treatment, and refinement of risk stratification or prognosis. At least one impactful finding from the genomic results was identified in 108/127 (85%) samples sequenced. A recommendation to consider a targeted agent was provided for 82/126 (65.1%) patients. Twenty patients ultimately received therapy with a molecularly targeted agent, representing 24% of those who received a targeted agent recommendation and 16% of the total cohort.

CONCLUSION Paired tumor/normal whole-exome sequencing and tumor RNA Seq of de novo or relapsed/refractory tumors was feasible and clinically impactful in high-risk pediatric cancer patients.

JCO Precis Oncol 6:e2100451. © 2022 by American Society of Clinical Oncology

INTRODUCTION

The discovery that actionable genomic alterations are more prevalent in pediatric tumors following chemotherapy and disease recurrence was an initial driver for precision oncology toward novel targeted therapy in pediatric cancer.[1-4] Genomic sequencing has led to targeted agents with a demonstrable clinical benefit, including alterations activating *ABL1*,[5] *ALK*,[6] *BRAF*,[7] and *NTRK*.[8] Additionally, molecular profiling has identified therapeutic targets in de novo high-risk and chemotherapy-resistant tumors, including diffuse intrinsic pontine glioma (DIPG),[9] CNS rhabdoid tumors,[10] high-risk pediatric sarcomas,[11,12] and Philadelphia chromosome–like acute lymphoblastic leukemia.[13] The utility of tumor sequencing to refine risk stratification and to provide a molecularly targeted therapeutic approach has prompted several centers to develop sequencing pipelines for pediatric oncology patients.[14-24]

Early and current iterations of next-generation sequencing (NGS) used targeted cancer gene panels to identify tumor somatic alterations using a finite panel of potentially actionable (druggable) genes.[16,19] Whole-exome sequencing (WES) of tumor and germline DNA yields a broader genomic landscape, leading to the detection of more alterations than those captured by targeted gene panels.[14,20] Tumor RNA sequencing (RNA Seq) complements WES by detecting oncogenic gene fusions and alternative transcript variants.[25] Combining WES and RNA Seq offers an opportunity to capture the breadth of somatic and germline alterations to inform clinical decisions, including risk classification and choice of therapy.

Herein, we describe the feasibility, implementation, and impact of a comprehensive precision medicine program at a large pediatric cancer center, incorporating WES (tumor and germline) and transcriptome sequencing in combination with treatment recommendations provided by a molecular tumor board.

ASSOCIATED CONTENT

Data Supplement

Protocol

Author affiliations and support information (if applicable) appear at the end of this article.

Accepted on March 23, 2022 and published at ascopubs.org/journal/po on May 11, 2022: DOI https://doi.org/10.1200/PO.21.00451

CONTEXT

Key Objective

Genomic profiling of pediatric cancers is increasingly prevalent, but the clinical impact of such sequencing remains unclear. Our prospective observational trial used a novel impact scoring system to quantitatively assess the impact of genomic profiling across a wide range of pediatric cancers.

Knowledge Generated

Using whole-exome and whole-transcriptome sequencing of tumor tissue paired with whole-exome sequencing of germline tissue, we identified impactful findings from sequencing in 85% of samples. Sixteen percent of patients in the cohort received therapy with a molecularly targeted agent.

Relevance

The high rate of impactful findings supports the continued use of genomic sequencing for select pediatric cancer patients. Our novel scoring system may be used to prospectively validate the impact of alternative sequencing platforms such as targeted gene panels or whole-genome sequencing.

METHODS

Study Population and Eligibility

Since May 2018, patients age < 30 years with any relapsed, refractory, or newly diagnosed high-risk leukemia, lymphoma, solid tumor (ST), or brain tumor (BT) as defined in the Data Supplement (online only) were eligible for tumor molecular profiling through the Aflac Precision Medicine Program (APMP) observational institutional Protocol (online only, AflacPM1702; Data Supplement). Sufficient tumor sample from either archived tissue or blood/bone marrow or from a new procedure performed for standard clinical care was required. For patients with relapsed disease, archived samples from diagnosis were not eligible for sequencing except for patients with BT with no ability to safely perform a new biopsy. Caregivers/patients could consent to receive research-based germline analysis results. The study was approved by the Emory University Institutional Review Board.

Tumor Genomic Sequencing

Genomic profiling with germline subtraction was performed via the GEM ExTra assay in a College of American Pathologists-accredited, Clinical Laboratory Improvement Amendments (CLIA)–certified laboratory through Ashion Analytics and included DNA (paired tumor-germline WES) and RNA (tumor whole-transcriptome sequencing) sequencing (Data Supplement).[26] Peripheral blood (STs and BTs) or saliva (leukemias) samples were obtained for germline DNA WES. GEM ExTra reports somatic variants, copy-number alterations, and structural rearrangements including gene fusions. Therapeutically targetable alterations (TTAs) were defined as somatic alterations with documented response to on- or off-label US Food and Drug Administration–approved drugs, investigational agents available via active pediatric and adult clinical trials, or for pediatric compassionate use. Additional significant alterations (ASAs) were defined as somatic changes with published evidence for confirming or changing the pathologic diagnosis, affecting prognosis or pointing to a targeted agent under early-phase clinical trial investigation limited to adults. TTAs, ASAs, chromosome arm gains/losses, and chromosome loss of heterozygosity were considered as reportable genomic alterations for purposes of our analysis. Variants of unknown significance were examined but not included in the analysis.

Germline Sequencing

Germline variant detection was based on variant call format files provided by Ashion Analytics using the FreeBayes algorithm,[27] after quality control, filtering, and alignment to the human genome (Data Supplement). Retained variants were restricted to a set of 186 cancer predisposition genes (Data Supplement).[28] Only variants previously classified as pathogenic or likely pathogenic (P/LP) were reported. A letter was provided to the primary oncologist describing the germline findings and recommendations for confirmatory testing in a CLIA-licensed laboratory if patient consented initially.

Treatment Recommendations and Impact

A multidisciplinary molecular tumor board that composed of pediatric oncologists, pathologists, molecular pathologists, cancer biologists, clinical geneticists, and bioinformaticians reviewed tumor sequencing results bimonthly. Consensus recommendations were circulated to the primary oncologist using a previously published tier system.[16] Each case was assigned an overall clinical impact score on the basis of the summation of elements weighted from 0.5 to 1.0 points (Table 1). The maximum possible score was 6. Provider perception of the impact of sequencing was independently assessed as described (Data Supplement). Data collection and management used Research Electronic Data Capture tools.[29]

Statistical Analysis

Descriptive statistics were conducted for demographics and disease characteristics. The distribution of genomic alterations and the impact of testing were categorized by disease status (de novo *v* relapsed/refractory) and by tumor

TABLE 1. Clinical Impact Scoring System

Element	Points
Targeted agent (maximum 1.5 points)	
Alteration with tier 1-2 evidence supporting a targeted agent[21]	1
Alteration with tier 3-5 evidence supporting a targeted agent[21]	0.5
Cancer predisposition (maximum 1 point)	
Pathogenic/likely pathogenic variant in known cancer predisposition gene identified in the germline AND/OR	1
Clinical or family history supports cancer predisposition evaluation	
Therapy availability (maximum 2 points)	
Consideration for enrollment in a pediatric or adolescent clinical trial	1
Consideration for compassionate use application for targeted agent with an FDA indication in adults	1
Refinement of risk stratification or prognosis (maximum 1.5 points)	
Alteration accepted as a modifier of risk stratification that would result in a change in treatment AND was not previously identified by alternative detection methods AND/OR	1
Alteration confirms a diagnosis that was unclear before sequencing or results in a change in diagnosis	
Alteration with literature supporting its role in prognosis even if not used to alter therapy AND/OR	
Alteration otherwise meeting criteria for 1 point but was already identified by prior testing	0.5

Abbreviation: FDA, US Food and Drug Administration.

group (BT, leukemia/lymphoma [L/L], or ST). Statistical analyses were conducted using SAS Enterprise Guide version 7.15 (SAS Institute, Cary, NC).

RESULTS

Study Feasibility and Cohort

`From May 2018 to March 2020, 129 patients were enrolled onto the APMP Study. Three patients were not evaluable. One patient had two de novo STs sequenced (adenocarcinoma and alveolar rhabdomyosarcoma), resulting in successful sequencing in 127 samples from 126 patients (Fig 1A). The median time from sample submission to return of the results was 13 days (range, 8-27 days). WES and RNA Seq were successfully completed in 118/127 (92.9%) samples, with 9 (7.1%) samples having only WES because of inadequate RNA. Copy-number variation was not reported in 7/127 (5.5%) samples because of high derivative log ratio. Demographics of the cohort reflect the institutional population diversity, with 35.7% Black and 16.7% Hispanic patients (Table 2; Data Supplement). Median age at enrollment was 12.1 years (range, 0.2-25.7 years). Patients with relapsed/refractory disease accounted for 52.4% of enrollments. STs represented 42.5% of all tumors sequenced and half of the relapsed/refractory tumors, whereas L/L accounted for 39.3% of de novo tumors (Fig 1B). Within the BT, L/L, and ST groups, the most common diagnoses were medulloblastoma (13/28; 46.4%), T-cell acute lymphoblastic leukemia/lymphoma (T-ALL/LLy; 18/45; 40.0%), and nonrhabdomyosarcoma soft tissue sarcomas (13/54; 24.1%; Fig 1C). Specific diagnoses data are in the Data Supplement.

Somatic Mutation Landscape

Reportable somatic alterations were found in 95.3% (121/127) of samples. Of these, 61/121 (50.4%) were from de novo patients and 60/121 (49.6%) from patients with relapsed/refractory disease. Tumor mutational burden was low in 95.3% of samples (Data Supplement). In 7/121 samples (5.8%), the only somatic alterations were chromosome gains/losses and/or chromosome loss of heterozygosity (Data Supplement).

Eighty-nine of 127 tumor samples (70.1%) had reportable single-nucleotide variants or indels (Data Supplement). TTAs were found in 69/127 samples (54.3%), with *CDKN2A* and *CDKN2B* deletions being most common, with representation across all tumor groups (Fig 2A). Potentially targetable receptor tyrosine kinases (RTKs) were activated by missense mutations (*ALK, FGFR1,* and *BRAF*; Fig 2A). Common variants identified within the PI3K/MTOR pathway (Fig 2B) included activating mutations (*PIK3CA*, *PIK3R1*, and *MTOR*) or loss of function mutations (*PTEN*) across all tumor groups (Figs 2A and 2B). Along with mutations in canonical PI3K/MTOR pathway genes, activating mutations in *ALK*, *NRAS*, *KRAS*, and *BRAF* that converge on PI3K were also common, suggesting a large cohort of TTAs are potentially targetable with a PI3K pathway inhibitor, agnostic of tumor type (27/89; 30.3%; Figs 2A and 2B). Common TTAs in genes involved in epigenetic regulation included *H3F3A*, *EZH2*, *and SETD2* mutations, as well as deletions of *ATRX* (Figs 2A and 2B). *H3F3A* missense mutations made up the most common of these and were unique to BTs (Figs 2A and 2B, Data Supplement). The most common potentially targetable genomic aberrations across all tumors clustered into seven biologic pathways: cell cycle, DNA damage repair, epigenetic, metabolic, PI3K, RTK/RAS, and transcription (Fig 2B).

ASAs were identified in 55/127 samples (43.3%) (Fig 2C). *TP53* was the most common somatic ASAs across all tumor types (Fig 2C), with the majority found in ST and BT samples. The large number of T-ALL/LLy samples in our cohort led to identification of common ASAs in *NOTCH1* (10/127; 7.9%), *PHF6*, and *SUZ12*. Notably, *CDKN2A/B* alterations commonly co-occurred in T-ALL samples harboring *NOTCH1* or *PHF6* lesions (Data Supplement). ASAs in *MED12, SUZ12*, and *RUNX1* also co-occurred in *PHF6* or *NOTCH1*-mutated T-ALL samples (Data Supplement). Although *PHOX2B* (Fig 2C) and *DICER1* (Data Supplement) somatic

alterations were identified in ST samples, no pathogenic germline variants were identified. Deletions in *MTAP* found in 2 L/L patients were deemed ASAs (Fig 2C). A somatic *MTAP* deletion was deemed a TTA, as it presented in a 25-year-old man with relapsed Ewing sarcoma, who was eligible for an adult trial of an MAT2A inhibitor (ClinicalTrials.gov identifier: NCT03435250).

Fifty gene fusions were identified; six were novel (Fig 2D). *PAX3/FOXO1* fusions were identified in all alveolar rhabdomyosarcomas. Nine fusions found in 13 patients (18%) were potentially targetable therapeutically (Fig 2D), such as an *AGRN/NRG1* fusion in an adolescent female with refractory cholangiocarcinoma, who went on to receive a human epidermal growth factor receptor 2 (HER2) inhibitor (compassionate access) and then a HER3 inhibitor (clinical trial). The *PICALM/MLLT10* fusion was the most common potentially targetable translocation found in T-ALL samples, occurring in 4/18 (22%).

No significant differences were seen by race or ethnicity in the median number of single-nucleotide variants, fusions, copy-number alterations, or TTAs and ASAs (Data Supplement).

Germline Sequencing Identifies Patients With Cancer Predisposition

All evaluable patients consented to disclosure of germline analysis. Known P/LP variants in cancer predisposition genes were detected in nine individuals (7.1% of the cohort); eight patients had single-allele P/LP variants in autosomal dominant cancer predisposition genes and one individual had biallelic mutation in *PMS2* (Data Supplement). Confirmatory clinical testing has been completed in 4/9 patients, which validated the findings in all cases. One additional subject with mixed phenotype acute leukemia had an apparent germline mutation in *WT1* that was not detected by clinical germline sequencing, attributed to contamination of saliva with DNA from leukemia cells. Twenty additional subjects were carriers of monoallelic known P/LP mutations in genes associated with autosomal recessive syndromes (Data Supplement).

Evaluating Clinical Impact of Tumor and Germline Sequencing

Overall, 108/127 (85.0%) reports indicated at least one impactful finding on the basis of the scoring system delineated in Table 1 (Fig 3). The median impact score was 1 (range, 0.5-4) in newly diagnosed patients and 1.5 (range, 0.5-3.5) in relapsed/refractory cases (Fig 3A). Among de novo patients (Fig 3B), the most common finding was a refinement of risk stratification/prognosis (0.5 point) in 62.3% of patients, followed by a tier 1-2 treatment consideration in 37.7%. Among relapsed/refractory patients (Fig 3C), refinement of risk stratification/prognosis (0.5 point) was noted in 42.4% of patients, followed by a tier 3-5 treatment consideration in 39.4%. Overall, a recommendation for potential targeted therapy was made in 82/127 (64.6%) sequenced tumors. To date, 20/82 patients (24.4%) have received 22 targeted agents (Table 3). A recommendation for referral to the Aflac Cancer Predisposition Program was made for 18 patients (Figs 3B and 3C) on the basis of germline sequencing (n = 10) or somatic

TABLE 2. Demographics of the Study Cohort

Demographics	Total	BT	L/L	ST
Enrolled and evaluable	126	28	45	53
Age				
Median age, years (range)	12.1 (0.2-25.7)	10.4 (4.8-23.3)	12.1 (0.9-20.5)	12.8 (0.2-25.7)
Sex, No. (%)				
Female	51 (40.5)	10 (35.7)	18 (40.0)	23 (43.4)
Male	75 (59.5)	18 (64.3)	27 (60.0)	30 (56.6)
Race, No. (%)				
Asian/Pacific Islander	4 (3.2)	1 (3.6)	0 (0.0)	3 (5.7)
Black	45 (35.7)	9 (32.1)	16 (35.6)	20 (37.7)
White	66 (52.4)	15 (53.6)	25 (55.6)	26 (49.1)
Unknown	11 (8.7)	3 (10.7)	4 (8.9)	4 (7.5)
Ethnicity, No. (%)				
Hispanic	21 (16.7)	7 (25.0)	5 (11.1)	9 (17.0)
Non-Hispanic	105 (83.3)	21 (75.0)	40 (88.9)	44 (83.0)
Disease status, No. (%)				
De novo	60 (47.6)	16 (57.1)	24 (53.3)	20 (37.7)
Relapsed/refractory	66 (52.4)	12 (42.9)	21 (46.7)	33 (62.3)

Abbreviations: BT, brain tumor; L/L, leukemia/lymphoma; ST, solid tumor.

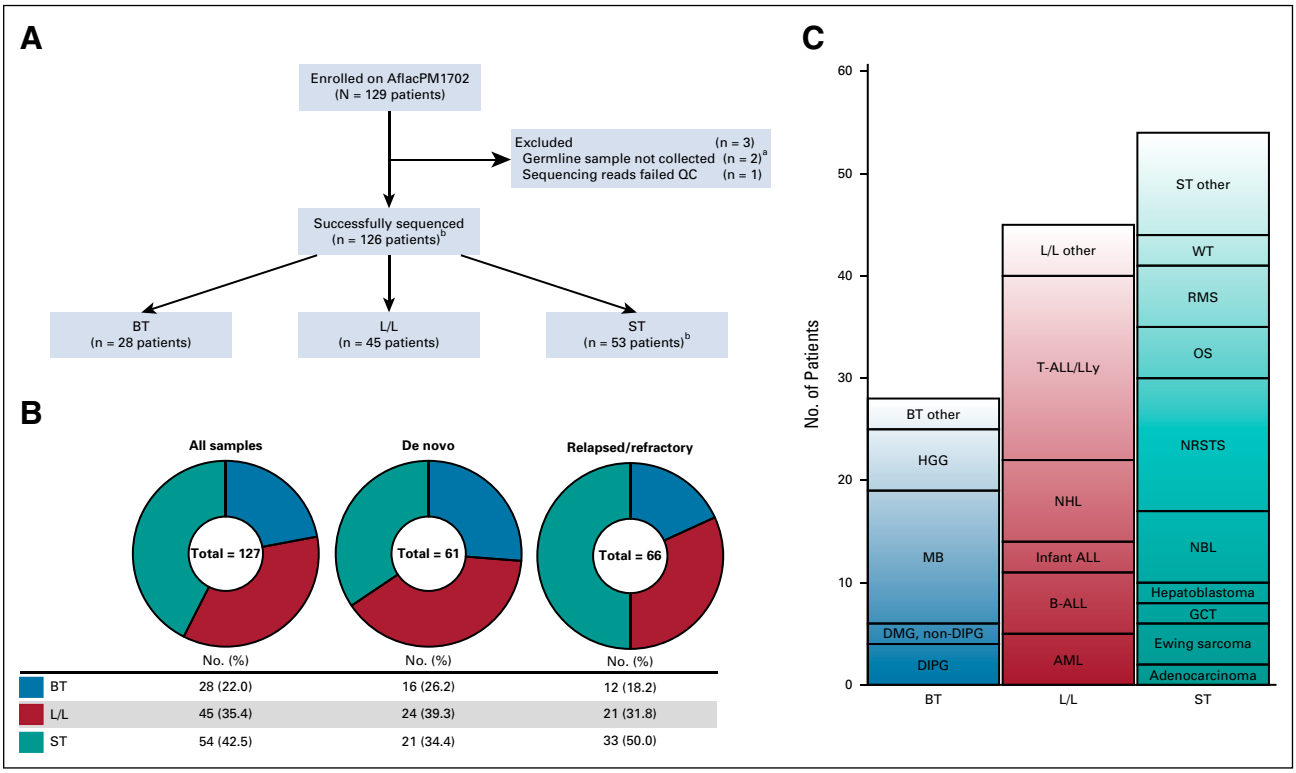

FIG 1. Aflac Precision Medicine Program enrollment and tumor demographics. Tumors were divided into three disease groups on the basis of tumor type: BT for central nervous system tumors, L/L for hematologic malignancies, and ST for extracranial STs. (A) CONSORT diagram showing enrolled and evaluable patients. (B) Distribution by tumor group for all tumors, newly diagnosed tumors, and relapsed/refractory tumors. The embedded tables present the number (%) for each tumor group. (C) Specific tumor types sequenced within each major tumor group, with the frequency of each tumor type represented. [a]One patient died and one transferred care to another institution after enrollment but before completion of sequencing. [b]One patient had sequencing performed on two separate tumor samples. ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BT, brain tumor; DMG, diffuse midline glioma; GCT, germ cell tumor; HGG, high grade glioma; L/L, leukemia/lymphoma; MB, medulloblastoma; NBL, neuroblastoma; NHL, non-Hodgkin lymphoma; NRSTS, nonrhabdomyosarcoma soft tissue sarcoma; OS, osteosarcoma; QC, quality control; RMS, rhabdomyosarcoma; ST, solid tumor; T-ALL/LLy, T-cell acute lymphoblastic leukemia/lymphoblastic lymphoma; WT, Wilms tumor.

sequencing findings, family history, and/or clinical features (n = 8); 11/18 (61.1%) patients are being evaluated to date.

Provider Perception of Sequencing Impact

Completed surveys were returned by the primary oncologist for 108/126 (85.7%) patients. Overall, providers reported a clinical decision-making impact of APMP sequencing in 33/108 (30.6%) surveys returned.

DISCUSSION

We present the implementation and findings from a cohort of 129 patients enrolled on an institutional prospective pediatric precision medicine protocol and demonstrate both high feasibility and clinical impact using WES and RNA Seq. Importantly, the incorporation of WES (tumor and germline) and RNA Seq (tumor) provides a more comprehensive approach over tumor NGS that does not require germline sample submission. This approach required at least 20% tumor content (10% variant allele fraction for heterozygous changes) to enhance sensitivity, but some alterations in known hotspots were reported with a variant allele fraction as low as 2%. The relevance of such low-frequency alterations for targeted therapy approaches remains under investigation.[30,31]

Importantly, we identified patients eligible for consortium genomic sequencing studies, including a relapsed/refractory Neuroblastoma Precision Trial (ClinicalTrials.gov identifier: NCT02868268) and a TARGET acute myeloid leukemia (AML) pilot initiative that skewed representation of relapsed neuroblastoma and AML and their commonly associated somatic alterations (ie, *MYCN* amplification, *RAS/MAPK/ALK* aberrations in neuroblastoma, and *KMT2A* or *WT1* mutations in AML).

The identification of recurrently altered targetable genes across multiple tumor types was notable. Targetable genes in cell cycle regulation, epigenetic regulation, RAS, and PI3K signaling were recurrently altered in all three tumor groups, supporting a role for tumor-agnostic molecularly targeted clinical trials in pediatric oncology, such as the NCI/COG MATCH trial (ClinicalTrials.gov identifier: NCT03155620).[32] Additionally, gene fusions identified across different tumor types confirmed diagnosis, some

FIG 2. Somatic tumor landscape of the Aflac Precision Medicine Program cohort. Pathogenic single-nucleotide variants and indels were classified as TTAs or ASAs. (A) Mutation type and frequency as well as tumor group and disease status for all TTAs found in ≥ 2 samples. The frequency plot above each column represents the number and type of genomic alterations identified in each tumor sample. The frequency plot to the right of each row indicates the number of mutations identified in each gene. (B) The most frequently altered TTAs and fusions in each of seven pathways, highlighted by tumor group. Shown are genes altered in ≥ 2 samples in our cohort. (C) Mutation type and frequency as well as tumor group and disease status for all ASAs found in ≥ 2 samples. The frequency plot above each column represents the number and type of genomic alterations identified in each tumor sample. The frequency plot to the right of each row indicates the number of mutations identified in each gene. (D) The frequency of samples with a reportable fusion, highlighted by tumor group. Novel fusions denoted with ^; therapeutically targetable fusions denoted with *. ABL, ABL proto-oncogene 1; ALK, anaplastic lymphoma kinase; ASA, additional significant alteration; BT, brain tumor; CRM1, chromosome region maintenance *1*; FGFR, fibroblast growth factor receptor; HER, human epidermal growth factor receptor; L/L, leukemia/lymphoma; NTRK, neurotrophic receptor tyrosine kinase; PD-L1, programmed death ligand 1; ST, solid tumor; TTA, therapeutically targetable alteration; UTR, untranslated region.

of which were therapeutically targetable. Novel fusions were identified that warrant further preclinical investigation to determine the functional relevance to tumor pathology and targeted drug response. Notably, the *PICALM/MLLT10* fusion was identified in 22% of T-ALL/LLy samples, in contrast to previously reported prevalence of 5%-10%.[33] Finally, we confirmed other significant genomic alterations and co-occurring lesions known to predict prognosis and therapy response, such as *PHF6* and *SUZ12* or *NOTCH1* mutations in T-ALL.[34,35]

TABLE 3. Patients Who Received Targeted Therapy on the Basis of Sequencing Results

Diagnosis	Disease Status	Gene Altered	Class of Agent	Generic Name of Agent
Brain tumor				
DIPG	Relapsed/refractory	*ARID1A*	CDK4/6 inhibitor	Abemaciclib[d]
DIPG	Relapsed/refractory	*H3F3A*	HDAC inhibitor	Panobinostat[e]
DIPG	Relapsed/refractory	*PIK3CA; H3F3A*[a]	PI3K inhibitor; DRD2 inhibitor	LY3023414[d]; ONC201[e]
DMG, non-DIPG	Relapsed/refractory	*H3F3A*	HDAC inhibitor	Panobinostat[e]
DMG, non-DIPG	Relapsed/refractory	*H3F3A*[a]	DRD2 inhibitor	ONC201[e]
Ganglioneuroblastoma	Relapsed/refractory	*KANK1/NTRK2*	NTRK inhibitor	LOXO-195[d]
HGG	Relapsed/refractory	*ATRX*	PARP inhibitor	Veliparib[d]
HGG	Relapsed/refractory	*CDK4*	CDK4/6 inhibitor	Palbociclib[d]
HGG	Relapsed/refractory	*CDKN2A*	CDK4/6 inhibitor	Abemaciclib[f]
HGG	Relapsed/refractory	High TMB[b]	PD-1 inhibitor	Pembrolizumab[f]
Medulloblastoma	Relapsed/refractory	*TERT*[c]	CK2 inhibitor	Silmitasertib[d]
Leukemia/lymphoma				
B-ALL	Relapsed/refractory	*NRAS*	MEK inhibitor	Trametinib[f]
Infant ALL	Relapsed/refractory	*KRAS*	MEK inhibitor	Trametinib[e]
T-ALL/LLy	Relapsed/refractory	*FBXW7*	mTOR inhibitor	Everolimus[d]
Solid tumor				
Adenocarcinoma	Relapsed/refractory	*MTOR, KRAS*	mTOR inhibitor	Sirolimus[f]
Cholangiocarcinoma	Newly diagnosed	*AGRN/NRG1*	HER2/EGFR inhibitor; HER3 inhibitor	Afatinib[e]; seribantumab[d]
DSRCT	Newly diagnosed	*EWSR1/WT1*	mTOR inhibitor	Sirolimus[f]
Ewing sarcoma	Relapsed/refractory	*ALK*	ALK inhibitor	Lorlatinib[f]
Mesenchymal tumor NOS	Relapsed/refractory	*TPM3/NTRK1*	NTRK inhibitor	Entrectinib[f]
NBL	Relapsed/refractory	*ALK*	ALK inhibitor	Lorlatinib[f]

Abbreviations: ALK, anaplastic lymphoma kinase; CDK4/6, cyclin dependent kinase 4/6; CK2, casein kinase II; DIPG, diffuse intrinsic pontine glioma; DMG, diffuse midline glioma; DRD2, dopamine receptor D2; DSRCT, desmoplastic small round cell tumor; EGFR, epidermal growth factor receptor; HDAC, histone deacetylase; HER, human epidermal growth factor receptor; HGG, high-grade glioma; MEK, mitogen activated protein kinase kinase; mTOR, mechanistic target of rapamycin kinase; NBL, neuroblastoma; NOS, not otherwise specified; NTRK, neurotrophic receptor tyrosine kinase 1; PARP, poly(ADP-ribose) polymerase; PD-1, programmed cell death 1; PI3K, phosphatidylinositol 3-kinase; TMB, tumor mutational burden.

[a]Alteration associated with overexpression of dopamine receptor D2 *(DRD2)*, supporting the use of a DRD2 inhibitor.

[b]This patient was found to have a biallelic pathogenic germline alteration in *PMS2*.

[c]Alteration supported classification as Sonic hedgehog medulloblastoma, supporting the use of a casein kinase (CK2) inhibitor that targets the sonic hedge hog (SHH) pathway but not telomerase reverse transcriptase (TERT) directly.

[d]Obtained through clinical trial.

[e]Obtained through compassionate use.

[f]Obtained commercially.

ASAs can inform on tumor biology and influence tumor identification and risk stratification. In addition, investigational therapies targeting ASAs in early-stage adult clinical trials support the potential of ASAs to evolve into TTAs in pediatrics. For example, tumors with *TP53* deficiency may be vulnerable to WEE1 inhibition, and the WEE1 inhibitor adavosertib has demonstrated safety in pediatrics.[36] *WHSC1* encodes a histone methyltransferase and is one of the most frequently mutated genes linked to epigenetic processes in pediatric cancer,[37] and gain-of-function mutations in *WHSC1* confer sensitivity to EZH2 inhibition in preclinical studies.[38] Continued reassessment of variants classified as ASAs and advocacy for pediatric access will be important as the field of pediatric precision oncology advances.

Although disparities exist in pediatric cancer outcomes by race/ethnicity,[39,40] variation in the tumor genome is not well defined.[41,42] The racial and ethnic diversity of our cohort offers a unique opportunity. However, limitations of sample size and heterogeneity of disease types precluded subgroup analyses; stratifying by diagnostic and relapsed/refractory to account for increased somatic mutations often seen at relapse was also not possible,[4,43,44] potentially masking differences. Notably, differences in tumor microenvironment were not evaluated in this study.[45,46]

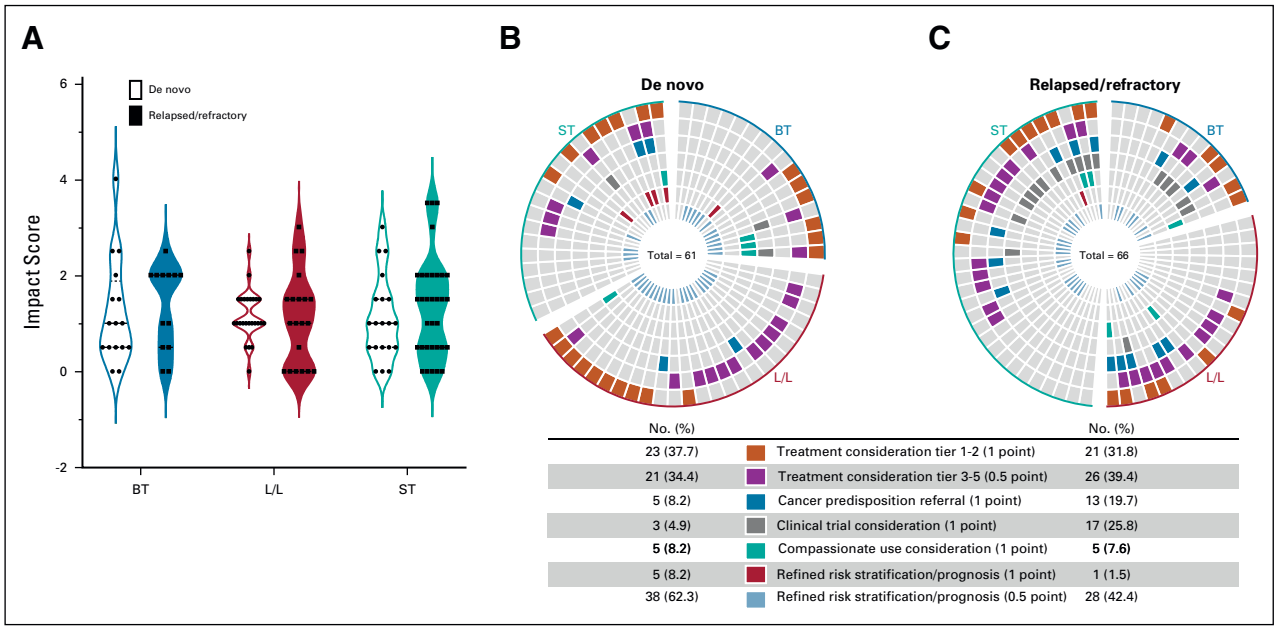

FIG 3. Impactful sequencing findings as determined by a novel impact scoring system. A total of 97 sequencing reports were reviewed by the multidisciplinary MTB, and the remaining 30 reports were scored by the study committee. (A) Violin plot showing the median and range of impact scores by disease group and disease status. Solid lines within each plot represent the median and dashed lines represent quartiles. Impact scores of individual patients with (B) de novo and (C) relapsed refractory disease. Each slice represents a single patient, and each concentric ring represents an impactful finding as defined in the Methods section. The embedded table presents the number (%) for each impactful finding. BT, brain tumor; L/L, leukemia/lymphoma; MTB, molecular tumor board; ST, solid tumor.

One challenge for precision oncology is inconsistency in defining the clinical impact of sequencing. Several pediatric studies have focused primarily on the identification of druggable alterations.[15,19,22,23] In a study of 102 children, Mody et al defined impact as any finding that was targetable, changed diagnosis or risk stratification, or identified cancer-related germline findings. Despite these broad criteria, impactful findings were noted in only 46% of patients.[14] By contrast, Marks et al[17] defined impact as any molecular test result that, when integrated with a patient's history, symptoms, and other clinical findings, could lead to a change in the assessment or management of the patient in a study of 56 pediatric patients with hematologic malignancies and reported 90% impact using WES (tumor and germline) and RNA Seq.

To standardize the measurement of impact, we developed a novel scoring tool that weights components and encompasses targetable alterations, identification of cancer-related germline findings, recommendations for clinical trials or compassionate use agents, and refinement of risk stratification or prognosis. This approach identified at least one impactful finding in 85% of our cohort. Importantly, for nearly 90% of these patients, the clinical impact included findings beyond a druggable target, highlighting the yield of a multidimensional sequencing approach. We weighted our impact score by assigning a lower point value in two groups: tier 3-5 targeted therapy recommendations and alterations with prognostic implications, which are not routinely used to alter therapy. This was done to acknowledge that such findings may inform `conversations with patients and families, yet are less likely to translate to immediate clinical care decisions. Prospective validation of this scoring system will be necessary to assess the utility of NGS approaches across platforms and pediatric oncology centers.

Identification of P/LP mutations in cancer predisposition genes in 7.1% of patients is consistent with prior pediatric literature.[14,16,20,47,48] Several patients had malignancies not typical of those reported in association with their germline genetic alteration (eg, Ewing sarcoma with a 5′ untranslated region missense mutation in *GPR161* and peripheral T-cell lymphoma with a *SMARCE1* frameshift mutation). Whether these cases represent a broader tumor spectrum associated with these gene variants remains to be determined. Expanding access to CLIA-certified germline profiling for pediatric oncology patients has the potential to impact care for many patients and family members. Partnership with genetic counseling services and a specialized cancer predisposition program is critical to implementation of germline sequencing.[49]

The primary oncologist's perception that sequencing affected clinical decision making in 31% of cases contrasts with the 85% of cases with an impact score > 0. Explanations for this discrepancy include (1) sequencing of high-risk de novo patients who received and responded to standard therapy and (2) provider survey emphasis on *therapeutic* decision making, whereas the

impact score encompasses a broader scope of clinical impact. Validated tools are needed for future uniform measurements of impact and cost effectiveness of NGS.

Study limitations include a lack of comparing our platform to a targeted gene panel to determine how many additional TTAs and ASAs were detected using a WES + RNA Seq approach. Although our current approach does not encompass methylation profiling,[22,50] it will be included in future iterations of our study for patients with CNS tumors and ST, supported by the high frequency of alterations in epigenetic regulators observed. Universal germline sequencing and pharmacogenomic profiling for all oncology patients is also germane to a broader personalized medicine approach.

In summary, we describe a feasible and impactful comprehensive tumor genomic profiling program for high-risk de novo and relapsed/refractory pediatric cancer patients. The pattern of alterations observed in relapsed and refractory patients informs targets for future tumor-agnostic molecularly targeted clinical trials. With declining costs of NGS technologies, efforts should be made to expand genomic profiling to all pediatric cancer patients.

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SUPPORT

The Aflac Precision Medicine Program is generously supported by the CURE Childhood Cancer Foundation.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/po/author-center.
Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

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No other potential conflicts of interest were reported.

ACKNOWLEDGMENT

The authors would like to acknowledge the patients and families who participated in the study as well as the research staff, Caitlin Monroe, MSc, and Taylor Lawrence, without whom this work would not have been possible.

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