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EDITED BY
Luca Giacomelli,
Polistudium srl, Italy

REVIEWED BY
Gisela Barbany,
Karolinska University Laboratory, Sweden
Xin Zhou,
St. Jude Children's Research Hospital, United States

*CORRESPONDENCE
Samara L. Potter
Samara.Potter@nationwidechildrens.org

†These authors have contributed equally to this work

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Comprehensive genomic characterization of hematologic malignancies at a pediatric tertiary care center

Ann M. Kebede[1], Elizabeth A. R. Garfinkle[2], Mariam T. Mathew[2,3,4], Elizabeth Varga[2], Susan I. Colace[1,4], Gregory Wheeler[2], Benjamin J. Kelly[2], Kathleen M. Schieffer[2,3,4], Katherine E. Miller[2,4], Elaine R. Mardis[2,4], Catherine E. Cottrell[2,3,4†] and Samara L. Potter[1,2,4*†]

[1]Division of Pediatric Heme/Onc/BMT, Nationwide Children's Hospital, Columbus, OH, United States, [2]The Steve and Cindy Rasmussen Institute for Genomic Medicine, Nationwide Children's Hospital, Columbus, OH, United States, [3]Department of Pathology and Laboratory Medicine, The Ohio State University, Columbus, OH, United States, [4]Department of Pediatrics, The Ohio State University, Columbus, OH, United States

Despite the increasing availability of comprehensive next generation sequencing (NGS), its role in characterizing pediatric hematologic malignancies remains undefined. We describe findings from comprehensive genomic profiling of hematologic malignancies at a pediatric tertiary care center. Patients enrolled on a translational research protocol to aid in cancer diagnosis, prognostication, treatment, and detection of cancer predisposition. Disease-involved samples underwent exome and RNA sequencing and analysis for single nucleotide variation, insertion/deletions, copy number alteration, structural variation, fusions, and gene expression. Twenty-eight patients with hematologic malignancies were nominated between 2018-2021. Eighteen individuals received both germline and somatic sequencing; two received germline sequencing only. Germline testing identified patients with cancer predisposition syndromes and non-cancer carrier states. Fifteen patients (15/18, 83%) had cancer-relevant somatic findings. Potential therapeutic targets were identified in seven patients (7/18, 38.9%); three (3/7, 42.9%) received targeted therapies and remain in remission an average of 47 months later.

KEYWORDS

genomics, precision medicine, next generation sequencing, therapeutic targets, hematologic malignancies, pediatric oncology

Introduction

Hematologic malignancies, particularly acute leukemias, remain the most commonly diagnosed pediatric malignancies (1–3). Significant improvements have been made in the treatment of pediatric hematologic malignancies since the 1960s, especially in acute lymphoblastic leukemia where survival rates now exceed 80-90% (4, 5). Improved outcomes for pediatric patients with leukemias and lymphomas can be attributed to several factors, including current risk stratification, conventional chemotherapeutic approaches, and improved supportive care, leading to what is considered standard of care today (3–6).

Additionally, research has established that recurrent genomic variants play a role in cancer development, including hematologic malignancies (3, 7–12). In some cases, these alterations have defined disease subtypes, thereby impacting clinical risk stratification or prognosis for pediatric leukemia and lymphoma patients (1, 3, 10, 13). The advent of next generation sequencing (NGS) combined with large-scale consortium-based clinical trials has accelerated the identification of these alterations, such that patients with pediatric leukemia and lymphoma now receive more personalized care, with improved risk stratification and increasing use of targeted agents (14–16). However, most clinical sequencing in this patient population is achieved through panel testing, due in large part to cost and reimbursement constraints. Unfortunately, panel testing, which sequences only a subset of genes and hotspots, may not identify all potential clinically applicable genomic variants including prognostic copy number alterations.

While strides have been made in the survival of pediatric hematologic malignancies, some patient populations remain difficult to treat (6, 12, 17–19). These high-risk patients may ultimately require therapy that is not considered standard of care due to disease progression, therapeutic toxicity, or genetic comorbidities. Comprehensive genomic characterization of both germline and somatic disease tissues can be especially impactful in such high-risk patients. Increasingly expansive NGS technologies can discover genomic alterations that might otherwise go undetected through standard testing approaches, namely cytogenetic studies and panel-based testing. Such findings can have important diagnostic and prognostic implications for patients. Furthermore, broad genomic profiling can suggest potential therapeutic targets for those particularly difficult to treat malignancies, which may improve outcomes (7, 8, 20–23). This knowledge could strengthen our understanding of genetic drivers of high-risk disease as well as prompt further study in clinical trials and disease interventions.

Here we report the results of 18 pediatric and adolescent/young adult (AYA) individuals diagnosed with hematologic malignancies who underwent comprehensive genomic profiling as part of this translational research protocol at a single tertiary care pediatric institution. To evaluate the clinical utility of genomic profiling for this clinician-nominated pediatric/AYA population, paired exome sequencing analysis for the detection of germline and somatic variation, as well as RNA sequencing of disease-involved samples was performed. The goal of this study was to identify genomic alterations of diagnostic, prognostic, and therapeutic impact. Notably, this cohort was largely comprised of patients with relapsed or high-risk disease. We also detail outcomes of those patients who received targeted therapy based upon sequencing results.

Methods

Patient recruitment and overview of translational study protocol

Nationwide Children's Hospital Institutional Research Ethics Board approved this study [IRB17-00206]. Clinical providers nominated patients for enrollment on to the Institute for Genomic Medicine Comprehensive Profiling for Cancer, Blood, and Somatic Disorders protocol. Once patients were approved for the study and informed consent was obtained, testing on available disease-involved and germline samples was initiated. Results were communicated to clinical providers and discussed within multi-disciplinary tumor boards. Upon provider request, results were also clinically confirmed using orthogonal testing. Genetic counseling and cascade testing were offered to enrolled patients as a part of this study.

Germline and somatic exome sequencing

Libraries were prepared using 100-500ng of input DNA beginning with enzymatic fragmentation, followed by end repair, 5' phosphorylation, A-tailing, and platform-specific adapter ligation using NEB Ultra II FS reagents (New England Biolabs, Ipswich, MA). Target enrichment by hybrid capture was performed using the IDT xGen Exome Research Panel v1.0 enhanced with the xGenCNV Backbone and Cancer-Enriched Panels-Tech Access (Integrated DNA Technologies, Coralville, IA). Paired-end 151-bp reads were generated on the Illumina HiSeq 4000 or NovaSeq 6000 (Illumina, San Diego, CA). Secondary analysis was performed using Churchill, a comprehensive workflow for analysis of raw reads from genome alignment through to germline and somatic variant identification (112). Reads were aligned to the human genome reference sequence (build GRCh38) using BWA (v0.7.15) (RRID : SCR_010910) and refined according to community-accepted guidelines for best practices (https://gatk.broadinstitute.org/hc/en-us). Duplicate sequence reads were removed using samblaster-v.0.1.22, and base quality score recalibration was performed on the aligned sequence data using the Genome Analysis Toolkit (v4.1.9) (RRID : SCR_001876) (113). Germline variants were called using GATK's HaplotypeCaller (RRID : SCR_001876) (114), somatic single nucleotide variation (SNV) and insertion-deletion (indel) detection was performed using MuTect2 (RRID : SCR_000559) (115) and copy number variation (CNV) was assessed using a combination of GATK (v4.2.4.1) (RRID : SCR_001876) and VarScan2 (v2.4.4) (RRID : SCR_006849) (116). Germline and somatic variation in cancer-associated genes was assessed using a gene-set curated from the published literature and genomic databases including those described by Zhang et al., as well

as genes with strong or emerging evidence of germline or somatic cancer association as documented in the Cancer Gene Census (117, 118).

RNA-sequencing

Extracted RNA from tumor tissue was subjected to DNase treatment and ribodepletion. For library preparation, the NEBNext Ultra II Directional RNA library prep kit for Illumina was employed. Paired-end 151-bp reads were then generated from the tumor RNA libraries using the Illumina HiSeq 4000 and aligned to the human genome reference sequence (GRCh38) using STAR-Fusion. For fusion analysis, RNA sequence data were processed using EnFusion (119), an ensemble approach that merges and normalizes the results from seven fusion callers: STARfusion (v.1.6.0) (119, 120), MapSplice (v.2.2.1) (121), FusionCatcher (v.0.99.7c) (122), FusionMap (v.mono-2.10.9) (123), JAFFA (v.1.09) (124), CICERO (v0.3.0) (125), and Arriba (v1.2.0) (126). Rare fusions (<5% frequency in our internal cohort) identified by at least three fusion callers were subject to further review for biological relevance. For gene expression studies, normalized read counts (TPM) were calculated for all samples from an internal NCH cohort of CNS cancers (n=508) using Salmon (v.1.9.0). An external cohort of CNS cancers (n=791) from the Treehouse Childhood Cancer Initiative at the UC Santa Cruz Genomics Institute (v.9 and v.11, University of California, Santa Cruz) was combined with the internal cohort to extend the dataset. Expression counts of protein-coding genes were log2(x+1) transformed and quantile normalization was performed. The 5,000 genes with the highest variances were used to perform a principal components analysis.

Graphical representation of data

Sankey plot was generated using the ggsankey package in R version 4.1.1. Bar graph and pie charts were generated using ggplot2 (RRID : SCR_014601) in R version 4.1.1. OncoPrint was generated using ComplexHeatmap (RRID : SCR_017270) in R version 4.1.1. Swimmer plot was generated using the swimplot package in R version 4.1.1. Code is available upon request.

Results

Cohort

Twenty-eight patients with hematologic malignancies were nominated by clinicians; 23 of these were enrolled (Figure 1A). Eighteen patients underwent paired somatic disease-germline comparator exome sequencing and RNA sequencing of a disease-involved sample. Germline only exome sequencing was performed for two additional patients, both of whom were enrolled following remission of their hematologic malignancies, and for whom other, non-hematologic tumor samples were sequenced as a part of this protocol. Five patients were excluded after nomination due to inadequate specimen, decline of consent onto the study, death prior to enrollment, transfer of clinical care, or sequencing not completed on their hematologic malignancy. Sequencing was not completed on the hematologic malignancy of one patient due to lack of a disease-involved sample, and two patients with prior history of hematologic malignancy had only non-hematologic malignancies sequenced (retinoblastoma and osteosarcoma in one individual and malignant peripheral nerve sheath tumor in the other individual). As summarized in Figure 1B, diagnoses included acute lymphoblastic leukemia, acute myeloid leukemia, Hodgkin lymphoma, and non-Hodgkin lymphoma. Most patients were male (72.2% vs 27.8%) and ranged in age from 0 to 26 years, with the majority being younger than 15 years old (Supplementary Table 1).

Rationale for patient nomination is detailed in Supplementary Table 2. The presence of relapsed or refractory disease was the most common reason for clinician nomination (9/18, 50%). Two individuals (2/18, 11.1%) were nominated due to having multiple malignancies; one with concurrent Burkitt Lymphoma and Neuroblastoma at initial diagnosis, the other with a history of Hodgkin Lymphoma at initial diagnosis later followed by Ewing Sarcoma/PNET. Clinicians sought potential therapeutic options for individuals with poor prognoses in two others; one with T-ALL and CD4/CD8 double negative immunophenotypeand one with infantile B-ALL (2/18, 11.1%). Clinicians nominated another two patients due to uncertainty of diagnosis. One patient was negative for c-myc on fluorescence *in situ* hybridization (FISH) analysis, and another had insufficient sample to confirm diagnosis by routine methods (2/18, 11.1%). Individuals were also nominated due to unusual neurologic symptoms (1/18, 5.6%), unusual clinical presentation (1/18, 5.6%), and significant therapeutic toxicity (1/18, 5.6%).

Clinical utility of testing was evaluated based on diagnostic, prognostic, and therapeutic impact. Diagnostic variants were categorized by effect on prior histologic diagnosis, wherein variants were found to confirm, change, or have no effect on histologic diagnosis. Amid genomic characterization that led to a changed histologic diagnosis, these variants were sub-categorized into those that challenged a prior histologic diagnosis, and those that refined a pathologic diagnosis (Supplementary Table 3). Genomic characterization led to confirmation of diagnosis for the majority of the cohort (14/18, 77.8%) (7, 24–85). One patient (1/18, 5.5%) had a fusion that refined diagnosis from anaplastic large cell lymphoma (ALCL) to ALK-fusion positive ALCL (IGMCH0009, Figure 1C, Supplementary Table 4) (58–60). No relevant diagnostic findings were found in three patients (3/18, 16.7%) (Figure 1C). For one patient (IGMCH0423), a translocation involving chromosomes 8 and 22 (t(8;22)(q24.2;q11.2)) involving the *MYC* and *IGL* locus, recurrently observed in Burkitt lymphoma, was identified on standard of care karyotyping (Supplementary Table 4). The expected resultant rearrangement, however, was not detected by RNA-sequencing, likely due to the nature of the genomic breakpoints. Single nucleotide variants (SNVs) were the most identified diagnostic alterations, followed by fusions, and then copy number variants (CNVs). Less common were insertions/deletions (indels) and internal tandem duplication (ITD).

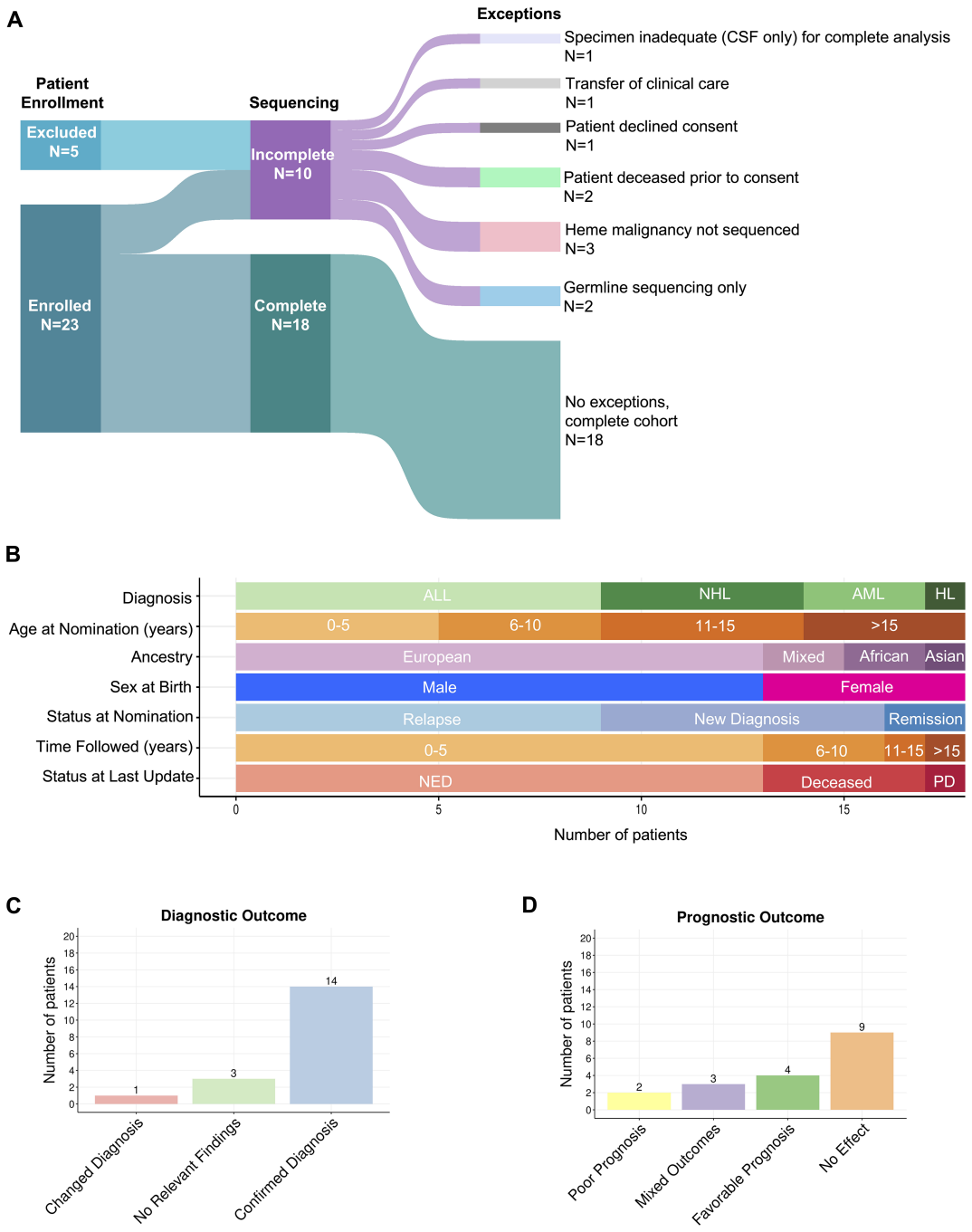

FIGURE 1
Overview of cohort. **(A)** Sankey plot displaying number of patients that were enrolled and sequenced. Lines flowing between columns represent the relationship between the blocks and their width corresponds to the number of patients. **(B)** Graphical representation of cohort demographics. **(C)** Number of patients and their diagnostic impact from protocol enrollment. **(D)** Number of patients and their prognostic impact from protocol enrollment.

Prognostic variants were categorized as conferring favorable prognosis, poor prognosis, mixed/intermediate prognosis, and no effect (Supplementary Table 5; Figure 1D). Additionally, each patient was denoted as having an overall clinical prognostic category (i.e. favorable, poor, or mixed) based on a cumulative assessment of their combined variants. Variants conveying favorable prognosis were identified in 22.2% of the cohort (4/18), including *NOTCH1 SNVs, NPM1::ALK* fusion, and copy number gains (52–54, 75, 77, 86, 87). The mixed clinical prognosis category included patients with multiple variants conferring different prognostic implications (i.e. favorable, poor, intermediate), and variants that have conflicting evidence for prognostication. Three patients (3/18, 16.7%) had variants that resulted in mixed prognosis (35, 43, 45, 46, 51–54, 64, 65, 86–92). Two patients were noted to have overall poor clinical prognosis based on their cumulative variants. (2/18, 11.1%). In one patient with B-ALL, this included an SNV in *NT5C2* and a *KMT2A* fusion. In

another patient with myeloid sarcoma and history of treatment-related AML, this included an *ASXL1* indel and *FLT3* ITD (52, 53, 82–87, 93–96). Nine patients did not have genomic findings that affected prognosis (9/18, 50%).

Sequencing identified 61 somatic variants in 15 patients. Of these patients, seven were newly diagnosed with malignancy, whereas eight patients had relapsed/refractory disease. Six of the seven (85.7%) newly diagnosed patients were advanced stage (Stage III or IV) or high-risk based on age or diagnostic features. Variations in *CDKN2A* were most frequent (5 variants) (Figure 2).

Seven patients had at least one therapeutic target identified (7/18, 38.9%), and to date, three (3/7, 42.9%) of those patients received targeted therapy based upon these results (28, 40, 41, 58–60, 85, 97) (Table 1). Variants identified as targets included fusions, SNVs, and CNVs. The three patients who received targeted therapy had no evidence of disease at last update, an average of 47 months' duration (Figure 3A).

Germline alterations included variants associated with cancer predisposition syndromes, such as *CHEK2* and Noonan syndrome (*PTPN11*). Additionally, non-cancer predisposition-associated carrier state was identified in two patients, including carrier for Cystic Fibrosis and Gaucher Disease. One variant was identified as a medically actionable finding and was associated with WHIM (Warts, Hypogammaglobulinemia, Infections, and Myelokathexis) syndrome (Supplementary Table 6).

Complete patient information, including somatic variants, germline findings, and results of testing prior to comprehensive genomic profiling, is available in Supplementary Tables 3-5 and Supplementary Tables 7, 8. Supplementary Table 8 includes clinically significant findings. Testing done on samples prior to NGS, including cytogenetics and molecular testing, is included when available, and NGS results that were known prior to comprehensive sequencing denoted in bold.

Patient highlights

IGMCH009

IGMCH0009 presented to our tertiary care center in 2015 and was diagnosed with ALCL. The patient received initial therapy per ANHL12P1 with dexamethasone, cyclophosphamide, ifosfamide, methotrexate, cytarabine, etoposide, doxorubicin, and intrathecal methotrexate/hydrocortisone and had negative end of therapy scans (98). The patient relapsed six months following diagnosis and was treated with brentuximab (anti-CD30 monoclonal antibody) for 24 cycles, achieving second remission. Approximately two years later, the patient relapsed a second time, prompting nomination and enrollment on our translational research protocol (Figure 3B, Image #1). RNA-sequencing identified an *NPM1::ALK* fusion. *ALK* encodes the anaplastic lymphoma kinase, a receptor tyrosine kinase belonging to the insulin receptor family (99). The *NPM1::ALK* fusion encompasses the ALK protein kinase domain and is predicted to result in overexpression, constitutive dimerization, and increased ALK kinase activity, thereby promoting tumorigenesis (100–102). The patient received brentuximab and bridging therapy with ifosfamide, carboplatin, and etoposide (ICE) followed by a matched unrelated donor hematopoietic stem cell transplant (HSCT) in 2018, achieving third remission thirty-five months following initial presentation. Third relapse was five months after the second relapse and two months after transplant (Figure 3B, Image #2). The patient was restarted on brentuximab and eventually transitioned to an ALK-inhibitor, crizotinib, based upon the fusion identified, achieving remission (58–60). Fourth relapse was six months after the third relapse. The patient was transitioned back to brentuximab for five months, at which point crizotinib was restarted, which is the patient's current therapy. The patient remains without evidence of disease at last update, four years and four months since starting targeted therapy.

IGMCH0092

IGMCH0092 presented to our tertiary care center in 2019 at the time of second relapse of B-acute lymphoblastic leukemia (B-ALL); all prior treatment was completed at an outside hospital. Following enrollment on our translational research protocol, sequencing identified a *CD74::PDGFRB* fusion. *CD74* encodes the Cluster of Differentiation 74 protein which is involved in antigen presentation and therefore highly expressed in many hematolymphoid lineages (103). *PDGFRB* encodes a cell surface tyrosine kinase receptor for the platelet-derived growth factor family (104). This *CD74::PDGFRB* fusion is predicted to encode a chimeric protein containing the N-terminal region of CD74 fused to the transmembrane, juxtamembrane, and tyrosine kinase domains of PDGFRB, resulting in activation of downstream targets leading to leukemogenesis (105, 106). The patient received vincristine and dexamethasone bridging therapy followed by CAR-T therapy in 2019, but subsequently relapsed within one month of initiation. This third relapse was treated per AALL1131 with the addition of a tyrosine kinase inhibitor, dasatinib, based upon the fusion identified (41, 107, 108). Patient subsequently received a matched unrelated donor HSCT and remains in remission at last update, three years and 11 months since starting targeted therapy.

IGMCH0096

IGMCH0096 first presented to our tertiary care center in 2018 and was diagnosed with ALCL (Figure 3C, Image #1). The patient achieved remission with initial therapy on study per ANHL12P1, which included crizotinib (Figure 3C, Image #2) (98). First relapse was seven months after diagnosis, at which point the patient was nominated and enrolled on our translational research protocol Figure 3C, Image #3). Sequencing identified an *NPM1::ALK* fusion. Subsequent treatment included brentuximab and ICE followed by matched sibling donor HSCT two months after first relapse, achieving second remission (Figure 3C, Image #4). Second relapse was eight months after the first relapse, and six months after transplant (Figure 3C, Image #5). The patient was restarted on brentuximab and then transitioned to the ALK-inhibitor, crizotinib, which is the patient's current therapy (58–60). The patient remains without evidence of disease at last update, three years and seven months since starting targeted therapy (Figure 3C, Image #6).

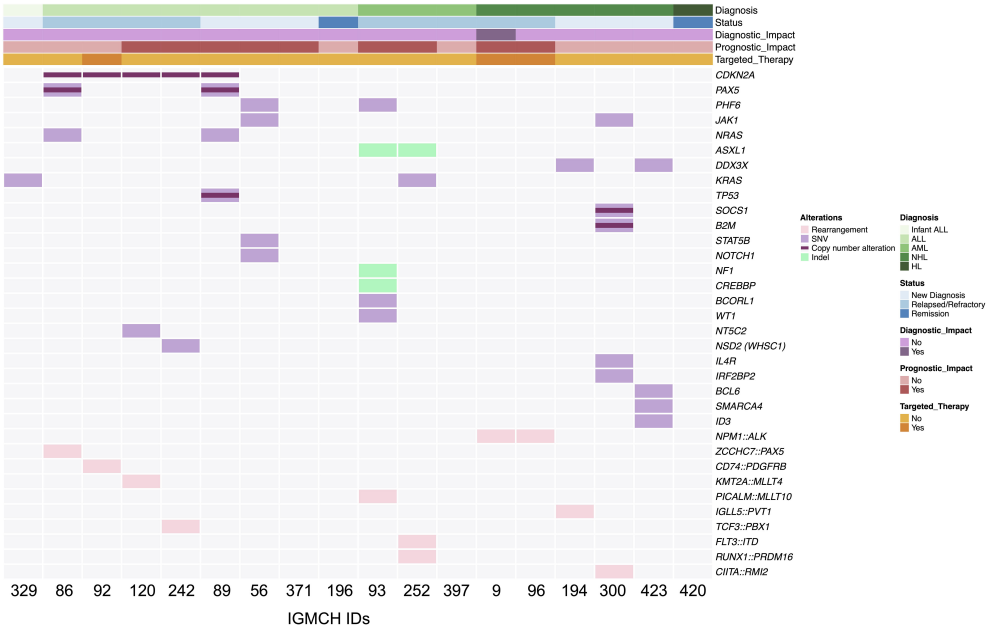

FIGURE 2
Summary of somatic findings. OncoPrint displaying an overview of somatic genetic alterations. Each column represents an individual patient (n=18) ordered by diagnosis. Status at nomination, diagnostic and prognostic impact from cohort enrollment, and use of targeted therapy are notated with annotation bars. Rearrangements, single nucleotide variants (SNV), copy number alterations, and insertions/deletions (Indel) are represented by a colored line or box. Light grey boxes represent absence of genetic alteration at that particular gene. ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; NHL, non-Hodgkin lymphoma; HL, Hodgkin lymphoma.

Discussion

Genomic characterization of pediatric hematologic malignancies in our cohort identified potential therapeutic targets in nearly 40% of patients (7/18, 38.9%), including the consideration of inhibitors targeting MEK (n=3), receptor tyrosine kinases (ALK n=2, PDGFRB n=1), FLT3 (n=1), and PD-L1 (n=1) (28, 40, 41, 58–60, 85, 97) (Table 1). FLT3 inhibitors including gilteritinib and sorafenib are often used upfront in patients with *FLT3*-mutated AML. Tyrosine kinase inhibitors including dasatinib and crizotinib were implemented in three patients (3/8, 38%), all of whom remain in remission on most recent follow up, on average approximately 4 years from initiation of targeted therapy. Of note, all of these patients also underwent HSCT. However, two of these patients (IGMCH0009, IGMCH0096) relapsed after HSCT and achieved remission after starting targeted therapy. The third patient (IGMCH0092 did not have a relapse between HSCT and starting targeted therapy; remission cannot be attributed to targeted therapy alone, as HSCT likely played a large part. These results reflect the clinical impact that comprehensive molecular profiling can have on high-risk pediatric leukemia and lymphoma patients, including those that are relapsed/refractory. Although the three patients who received targeted therapy all had fusions identified, we were also able to identify patients with other sequencing variants who could potentially benefit. Further studies, more comprehensive diagnostic testing, and access to targeted treatments are needed for pediatric patients to increase use of these therapies.

Most patients within our cohort were nominated for testing because prior/standard of care treatment failed to keep them in remission and therapeutic options were desired at relapse. Of those nominated at initial diagnosis, all but one were classified as having high risk disease. Reasons for this designation included advanced stage, older or younger age, and higher risk based upon traditional testing/standard parameters.

Somatic findings were identified in most patients in this study. While some findings were previously known from cytogenetic and molecular testing, most patients with clinically significant findings had additional results from NGS that were not previously identified (Supplementary Table 8). In terms of diagnostic utility, somatic variants were consistent with the patient's initial histologic diagnosis (14/18, 77.8%), which is expected, given standard testing capabilities. Importantly, however, one patient had refinement of diagnosis, which led to the use of a targeted therapy (see patient case #1). Overall, in this cohort, somatic variants were of limited prognostic utility—with somatic findings conferring clear prognostic utility in only six patients (6/18, 33.3%). This may be related to a paucity of information on genetic variants and their prognostic impact specific to pediatric malignancies, as well as the challenges to prognostication when genomic alterations confer a mixed prognosis. Furthermore, this was a diverse hematologic malignancy cohort which may limit yield in relation to our understanding of prognostic factors. These findings highlight the need for comprehensive genomics-based prognostication in pediatric hematologic malignancies (12, 109).

Germline variants led to cascade testing and additional genetic counseling in three patients in this cohort, underscoring the importance of paired germline testing for identification of cancer-predisposing variants (22, 23). Access to genetic counseling and

TABLE 1 Therapeutic findings.

Patient (IGMCH)	Diagnosis	Somatic Variant	Potential Targeted Agent Identified	Treatment	Outcome
0009	ALCL	*NPM1::ALK* (NM_002520, NM_004304) (58–60)	ALK-inhibitor (Crizotinib)	Chemotherapy BMT Targeted Therapy	NED
0086	B-ALL	*NRAS* c.183A>T (p.Gln61His) (NM_002524.4) (28, 97)	MEK- inhibitor (Trametinib)	Chemotherapy	NED
0089	B-ALL	*NRAS* c.35G>A (p.Gly12Asp) (NM_002524) (28, 97)	MEK- inhibitor (Selumetinib)	Chemotherapy	NED
0092	B-ALL	*CD74::PDGFRB* (41, 107)	Tyrosine Kinase- Inhibitor (Dasatinib)	Chemotherapy Radiation BMT Targeted Therapy	NED
0096	ALCL	*NPM1::ALK* (NM_002520.6, NM_004304.5) (58–60)	ALK- inhibitor (Crizotinib)	Chemotherapy Radiation Targeted Therapy	NED
0252	AML	*KRAS* c.37G>T (p.Gly13Cys) (NM_033360.4>NM_033360.3) (28, 97) *FLT3*-ITD (M_004119.3>NM_004119.2) (85)	MEK- inhibitor (Trametinib) *FLT3*-ITD (Gilteritinib)	Chemotherapy Radiation Surgery BMT	Relapse
0300	PMBCL	*JAK1* c.1872A>T (p.Leu624Phe) (NM_002227.4) (40) Chr 9 CNV (gain) (40)	PD-L1- inhibitor (Pembrolizumab)	Chemotherapy	NED

ALCL, anaplastic large cell lymphoma; B-ALL, B-cell acute lymphoblastic leukemia; AML, acute myeloid leukemia; PMBCL - primary mediastinal B cell lymphoma; CNV, copy number variant; ITD, internal tandem duplication; BMT, bone marrow transplant; NED, no evidence of disease.

cascade testing of at-risk individuals is critical when testing for pathogenic germline variants and can be a barrier for some institutions.

The present study does have limitations. First, the small overall number of patients combined with the varied diagnoses sequenced hampers our ability to draw broad conclusions for any singular diagnosis. Additionally, patients were selected for enrollment based on clinician nomination, rather than sequential diagnosis. While this resulted in a clinically interesting cohort that was largely comprised of high-risk patients, this provider variability may have skewed which patients were nominated and at which time point in their disease processes. Another difficulty we faced in this study was due to the implementation of paired tumor-normal exome sequencing. This methodology allows for analysis of genomic variation within the protein-coding regions of the genome, while also providing data surrounding the etiology of the variant (germline vs. somatic). Paired analysis of hematologic disease can be challenging, particularly in relation to the need for acquisition of a germline comparator sample absent of disease. Selection of germline sample comparator in patients with hematologic diseases needs to be balanced with potential drawbacks: for example, invasiveness of skin biopsies, known admixture with saliva containing disease-involved blood cells for buccal swabs, or time delay when choosing to obtain germline blood sample after initial treatment (when disease is expected to be absent or minimal) (127). Current testing strategies for risk stratification and prognostication of pediatric hematologic malignancies are not uniformly comprehensive (12, 109, 110). Widespread integration of comprehensive genomic testing with standard methodologies may lead to refined characterization of pediatric hematologic malignancies, increased understanding of known and emerging molecular drivers, and improved treatment (7, 8, 10, 11, 20, 21). Through further large-scale studies, we may elucidate additional clinically important prognostic variants, as well as new therapeutic targets, that have not previously been discovered. These findings are likely to be especially impactful in patients with relapsed or refractory disease for whom progress has lagged in improving overall survival, given current limits in treatment options and poor overall prognosis in this population (6, 17–19).

Efforts to create pediatric precision oncology programs have been undertaken nationally and internationally to evaluate the clinical benefit of NGS (1, 7–9, 20, 21, 23, 109, 111). Further studies are needed to expand on the timing of comprehensive genomic testing for pediatric hematologic malignancies and given current cost constraints, which patient cohorts should be prioritized. As more pediatric patients undergo molecular characterization of their hematologic malignancies at diagnosis and relapse, we will gain important insights affecting our understanding of genetic drivers and the overall genomic landscape of these malignancies. This knowledge can only help improve therapeutic approaches and survival in this patient population.

In conclusion, we present results from comprehensive genomic profiling of pediatric hematologic malignancies at a tertiary hospital. Our cohort of 20 patients was largely comprised of high-risk and/or relapsed patients, for whom clinicians were seeking additional molecular insights. Through comprehensive NGS of both

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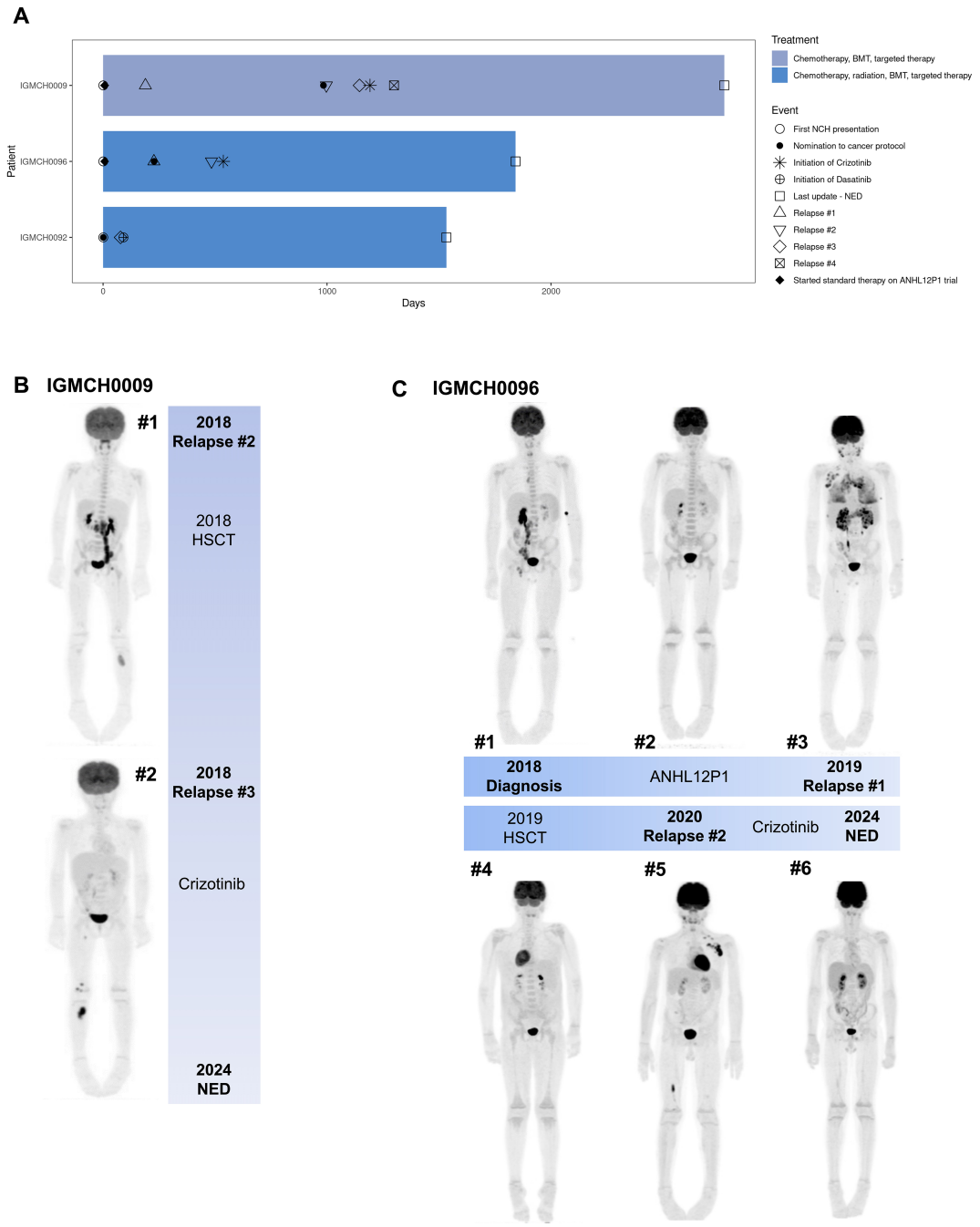

FIGURE 3
Patients treated with targeted therapy after protocol enrollment. **(A)** Swimmer plot of clinical course of treatment and outcomes for three patients who received targeted therapy after protocol enrollment. Patient IGMCH0009 and Patient IGMCH0096 were diagnosed with anaplastic large-cell lymphoma (ALCL) and received crizotinib targeted therapy. Patient IGMCH0092 was diagnosed with B-cell acute lymphoblastic leukemia (B-ALL) and received dasatinib targeted therapy. Patient IGMCH0092 was enrolled on the protocol at the time of their second relapse. Days = days from first presentation at Nationwide Children's Hospital. BMT = bone marrow transplant. NED = no evidence of disease. **(B)** PET scans for Patient IGMCH0009 at #1 second relapse and #2 third relapse. **(C)** PET scans for Patient IGMCH0096 throughout course of therapy. #1 at diagnosis, #2 after initial standard of care therapy, #3 first relapse, #4 after hematopoietic stem cell transplant (HSCT), #5 second relapse, #6 after starting crizotinib.

somatic and germline samples, we identified potentially targetable alterations in 40% of patients who underwent paired sequencing (7/18). Targeted therapies were initiated in three of these patients, all of whom remain in remission an average of 47 months (nearly 4 years) post therapy initiation. While further, larger studies are needed to evaluate the applicability of these findings more broadly, our translational research highlights the importance of genomic sequencing, especially in the relapsed setting, as testing may provide effective targeted therapeutic options and prolong survival for individual patients.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/gap/, phs001820.v3.p1.

Ethics statement

The studies involving humans were approved by Nationwide Children's Hospital Institutional Research Ethics Board. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

AK: Writing – original draft, Writing – review & editing, Data curation, Formal analysis, Investigation. EG: Visualization, Writing – review & editing, Writing – original draft. MM: Writing – review & editing, Investigation, Visualization, Writing – original draft. EV: Writing – review & editing, Data curation, Project administration. SC: Writing – review & editing. GW: Writing – review & editing, Software. BK: Writing – review & editing, Data curation, Software. KS: Writing – review & editing, Data curation, Investigation. KM: Writing – review & editing, Formal analysis. EM: Conceptualization, Supervision, Writing – review & editing, Resources. CC:Conceptualization, Supervision, Writing – review & editing, Investigation, Project administration. SP: Conceptualization, Supervision, Writing – original draft, Writing – review & editing, Data curation.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1498409/full#supplementary-material

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