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Tumor Genomic Profiling and *Ex Vivo* Drug Sensitivity Testing for Pediatric Leukemia and Lymphoma Patients

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OBJECTIVE To describe the frequency of use of tumor genomic profiling and functional *ex vivo* drug sensitivity testing in pediatric patients with hematologic malignancies at our institution, and to determine how the results affected treatment selection.

METHODS A retrospective chart review was conducted to analyze the frequency of tumor genomic profiling and functional drug sensitivity screening in our institution in pediatric patients with hematologic malignancies and to ask if the results were used to direct treatment. A case series of patients for whom these testing recommendations resulted in therapeutic interventions is reported.

RESULTS Thirty-three patients underwent tumor genomic profiling assays, functional *ex vivo* testing, or both. Nineteen patients (58%) had genomic profiling assays performed alone, 3 (9%) had functional *ex vivo* testing performed alone, and 11 (33%) had both tests performed. Twenty-one (64%) patients had potentially actionable mutations detected by the genomic profiling assay. Seven (21%) patients received at least 1 chemotherapeutic agent in accordance with the tumor genomic profiling or functional *ex vivo* drug sensitivity testing results. Three (43%) of the 7 patients who were treated with testing directed therapy had a favorable treatment response (PR or CR) to treatments selected based upon results of genomic or functional *ex vivo* testing.

CONCLUSIONS This retrospective case series demonstrates that precision medicine techniques such as genomic profiling and drug sensitivity testing can positively inform treatment selection in pediatric patients with relapsed or refractory leukemia and lymphoma.

ABBREVIATIONS ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; COG, Children's Oncology Group; DMSO, dimethyl sulfoxide; FDA, US Food and Drug Administration; HSCT, hematopoietic stem cell transplant; IV, intravenous; MPAL, mixed-phenotype acute leukemia; MRD, minimal residual disease; SJCRH, St. Jude's Children's Research Hospital; WBC, white blood cell.

KEYWORDS acute lymphoblastic leukemia; acute myeloid leukemia; drug screening assays, antitumor; genomic profiling; pediatric oncology; precision medicine

Introduction

Survival rates for pediatric patients with leukemia and lymphoma who have failed standard therapies remain low.[1–3] Conventional salvage chemotherapy regimens and hematopoietic stem cell transplant (HSCT) are often unable to achieve durable remissions, leading to serial relapses and ultimately refractory disease. Due to complex cytogenetic abnormalities, multiple genomic mutations, and multidrug resistance in relapsed and refractory disease, clinicians may turn to investigational or last-line treatment agents for patients who have exhausted all standard therapies.[1–3]

Therapeutic targeting of the BCR-ABL tyrosine kinase with imatinib in Philadelphia chromosome positive acute lymphoblastic leukemia (ALL) and of FLT3-ITD with mi-

dostaurin in FLT3-ITD positive acute myeloid leukemia (AML) as part of initial therapy has improved survival in both conditions.[4,5] Precision medicine diagnostic tools such as genomic profiling and functional *ex vivo* drug sensitivity testing may identify such novel therapeutic options for patients who have progressed despite receiving standard therapies. There are multiple possible re-induction regimens for relapsed and refractory leukemia and lymphoma, and the combination of genomic profiling and functional screening could potentially narrow treatment options to those most likely to be beneficial. Tumor genomic profiling is available as a commercial clinical test that can identify mutation-targeted treatments based on the published clinical data about the treatment options. The functional *ex vivo* drug screening assay assessed in this retrospective study is a research-

use only test. Previous studies have demonstrated the utility and feasibility of obtaining genomic profiling and functional drug screening.[6–9] However, results of these tests may or may not be implemented in the care of patients.[10–12] The primary objective of this case series is to describe the application and utility of tumor genomic profiling and functional *ex vivo* drug sensitivity screening in pediatric patients with leukemia and lymphoma, with a focus on the population with relapsed and refractory malignancies.

Materials and Methods

Study Population. A retrospective chart review was conducted to describe the frequency of tumor genomic profiling and functional drug screening and whether the results were used in pediatric leukemia and lymphoma patients at our institution. For this case series, the following patients were included in the chart review: those 0 to 25 years of age with hematologic malignancies, including ALL, AML, chronic myeloid leukemia (CML), mixed-phenotype acute leukemia (MPAL), myelodysplasia, myelofibrosis, non-Hodgkin lymphoma, and Hodgkin lymphoma; those who received treatment at this large freestanding children's hospital between November 1, 2012, and July 31, 2019; those who underwent genomic profiling, functional drug sensitivity screening, or both.

Data Collection. One of the study members (AE) reviewed the patients' electronic medical records to evaluate which patients with leukemia or lymphoma received testing and to assess if a therapy that was recommended by the tumor genomic profiling or functional drug screening was implemented. The case data were reviewed by a second member (DJK) of the study team. If the medical record could not provide clarity, then the study team approached the patient's treating physician to determine if there was an association between the testing results and the therapies implemented.

Application of either genomic profiling or functional drug sensitivity test results to clinical care of patients was the responsibility of the treating clinicians, in consultation with other members of the pediatric hematology-oncology team, the patients and their guardians. Genomic profiling was performed by Foundation Medicine (Cambridge, MA) which uses next-generation sequencing to analyze biomarkers and genomic alterations to identify potential targeted therapies. As of February 2021, the Foundation One Heme test uses DNA sequencing of 406 genes and the selected introns of 31 genes involved in rearrangements and RNA sequencing of 265 genes. This test produces a report with identified targetable mutations and recommendations for treatments and clinical trials that would potentially target these mutations. The data behind the recommendations and potential available trials are included in the report. For the purposes of this study, genomic profiling was considered to have

given a treatment recommendation if it suggested a FDA-approved therapy for the patient's tumor type with clinical benefit, a FDA-approved therapy in another tumor type with clinical benefit, or a potential clinical trial. If none of those 3 options were included in the report, then the genomic profiling was not considered to have given a treatment recommendation.

Functional drug sensitivity screening was performed by Notable Labs (Foster City, CA) using a custom robotic platform to determine the anticancer effects of FDA-approved chemotherapy and targeted agents against individual patient's tumor cells (Supplemental Figures S1–S4). This test produces bar graphs where the Y-axis represents the different screened compounds or combinations. They are ranked based on the degree of blast reduction (fractional blast reduction relative to control), which is measured on the X-axis. The compounds and combinations with a higher degree of blast reduction (higher on the Y-axis in the figures) are considered to have a higher chance of producing a therapeutic response. The platform and procedure for the Notable Labs platform, which is a research use only test, has been previously described.[13] As a research use only test, the findings themselves are not clinical recommendations, but may have been considered informative to the treating clinicians within the full context of each patient's care.

Analysis. All patients who had tumor genomic profiling, functional drug screening, or both were included in the analyses. Results of the genomic profiling assays and functional drug screening were compared with the subsequent chemotherapy treatments the patients ultimately received to determine the impact testing had on therapy and outcome. Patients who received a therapy that was in accordance with results of the testing platforms are described in detail.

Results

Patient Characteristics. Between November 1, 2012, and July 31, 2019, 377 patients were diagnosed with leukemia and 98 patients were diagnosed with lymphoma at this institution, including both newly diagnosed and relapsed patients. Thirty-three patients were identified who had genomic profiling assays, functional *ex vivo* testing, or both performed. The patients' demographic and clinical data were collected (Table 1). The results are summarized using descriptive statistics. The demographic characteristics (age, sex, race/ethnicity, and diagnoses) of the patients who had received such testing are detailed in Table 2. The average age of patients who underwent either tumor profiling or *ex vivo* drug sensitivity testing was 9.4 years. Ten patients (30%) had testing performed at the time of initial diagnosis, 20 (61%) patients had relapsed disease, and 3 (9%) had refractory disease when testing was performed. Of the 33 patients, 19 (58%) patients had genomic profiling assays performed alone, 3 (9%) had *ex vivo* drug

Table 1. Patient Data

Age
Sex
Race/ethnicity
Cancer diagnosis
Date of original diagnosis
First-line therapy
First-line cytogenetics
Time to relapse or refractory treatment
Disease state at time of testing
Time from relapse or refractory diagnosis to genomic profiling
Time from relapse or refractory diagnosis to functional screening
Results of genomic profiling assays
Results from functional screening
Treatment recommendations from testing
Implementation of recommended therapies
Duration of time receiving recommended therapy
Response to recommended therapy treatment
Reason for discontinuation of recommended therapy
Survival outcomes

Table 2. Demographics of Patients Who Underwent Genomic Profiling Assays or Functional *Ex Vivo* Testing

Demographic	Result
Age, median (range), yr	9.4 (1–21)
Sex, n (%)	
Male	16 (48)
Female	17 (52)
Ethnicity, n (%)	
African American	4 (12)
Asian	2 (6)
White: Hispanic	13 (39)
White: Non-Hispanic	14 (42)
Diagnosis, n (%)	
B-ALL	12 (36)
AML	10 (30)
T-ALL/T-LL	3 (9)
MPAL	1 (3)
CML	1 (3)
Follicular lymphoma	1 (3)
Burkitt-like lymphoma	1 (3)
Hepatosplenic T-cell	1 (3)
Lymphoma	1 (3)
Hodgkin lymphoma	1 (3)
Myelodysplasia	1 (3)
Myelofibrosis	1 (3)
Disease state at time of testing, n (%)	
Diagnosis	10 (30)
Refractory	3 (9)
Relapse	20 (61)

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; B-ALL, B-cell acute lymphoblastic leukemia; CML, chronic myeloid leukemia; MPAL, mixed-phenotype acute leukemia; T-ALL/T-LL, T-cell acute lymphoblastic leukemia/lymphoma

screening performed alone, and 11 (33%) had both tests performed (Table 3).

Implementation of Test Results. Clinicians assessed the actionability of the results through intradepartmental discussion and review of the medical literature. The clinicians used available studies, protocols, and medical literature to guide the dosing of the actionable chemotherapeutic agents. Then therapies under consideration were discussed in clinical informed consent conferences by the treating oncologists with each patient and their guardians to review information, safety concerns, and the potential benefit-risk assessments before implementation. Seven (21%) of the 33 patients received treatment based on the drug sensitivity or tumor profiling test results (Table 4). Four (29%) of the 14 patients who received *ex vivo* drug sensitivity screening had implementation of the test results into treatment. Among the 30 patients who had genomic profiling, 21 (70%) patients had potentially actionable mutations suggested by the genomic profiling assay (Table 4). Four of these 21 patients (19%) implemented at least 1 chemotherapeutic agent based on tumor genomic profiling.

Patients With Agent Selection Based on Test Results (Supplemental Table S1). *Patient 1.* A 1-year-old female initially diagnosed with infant pre-B-cell ALL with a MLL (also known as KMT2A) rearrangement. She underwent frontline therapy on AALL0631 (NCT00557193), a Children's Oncology Group (COG) protocol that included lestaurtinib.[14] After her first relapse, she began salvage therapy with clofarabine, etoposide, and cyclophosphamide followed by decitabine. The genomic profiling assay showed a BRAF D594E mutation that could be targeted with sorafenib. Forty days after completing the earlier planned salvage chemotherapy regimen, Patient 1 implemented treatment according to the genomic profiling with sorafenib 200 mg/m^2/day divided orally twice daily. On the day the sorafenib was started, the peripheral blood WBC count was 2800/μL with 9% blasts. The bone marrow aspiration on that day showed 98% blasts. Seven days into the sorafenib therapy, the WBC count had risen to 26,700/μL with 94% blasts. Due to progressive disease

Table 3. Distribution of Functional Drug Screening and Genomic Profiling According to Patient Diagnosis and Disease

	Functional Drug Screening Only	Genomic Profiling Only	Functional Drug Screening and Genomic Profiling
Screen or genomic testing by diagnosis			
B-ALL (n = 12)	1	7	4
AML (n = 10)	1	5	4
T-ALL/T-LL (n = 3)	0	1	2
MPAL (n = 1)	0	1	0
CML (n = 1)	1	0	0
Follicular lymphoma (n = 1)	0	1	0
Burkitt-like lymphoma (n = 1)	0	1	0
Hepatosplenic T-cell lymphoma (n = 1)	0	0	1
Hodgkin lymphoma (n = 1)	0	1	0
Myelodysplasia (n = 1)	0	1	0
Myelofibrosis (n = 1)	0	1	0
Totals (n = 33)	3	19	11
Disease state at time of testing			
Diagnosis (n = 10)	1	7	2
Refractory (n = 3)	0	2	1
Relapse (n = 20)	2	10	8
Totals (n = 33)	3	19	11

AML, acute myeloid leukemia; B-ALL, B-cell acute lymphoblastic leukemia; CML, chronic myeloid leukemia; MPAL, mixed-phenotype acute leukemia; T-ALL/T-LL, T-cell acute lymphoblastic leukemia/lymphoma

while on sorafenib, this medication was stopped and the patient was switched to treatment with mitoxantrone, pegaspargase, dexamethasone, bortezomib, vorinostat, and intrathecal methotrexate. The patient later died due to an invasive *Candida krusei* infection with minimal response to the combination treatment.

Patient 2. A 2-year-old male diagnosed with AML who received frontline treatment for high-risk AML on St. Jude's Children's Research Hospital (SJCRH) Protocol AML08 (NCT00703820).[15] He had refractory disease from the onset of treatment and never achieved a minimal residual disease (MRD) negative remission despite 2 cycles of induction and 2 cycles of consolidation therapy. His end of consolidation 2 bone marrow MRD was 2.2%. He was then started on a salvage regimen of vorinostat and decitabine; however, he continued to be refractory to treatment with bone marrow levels of AML of 5.03%. He subsequently received another cycle of clofarabine/cytarabine with MRD rise to 17% afterward. He then received chemotherapy with fludarabine, cytarabine, and gemtuzumab, which resulted in a bone marrow MRD of 0.19%. Patient 2 subsequently underwent HSCT, but had recurrent disease on day +30. The genomic profiling assay found a PIK3CA R88Q mutation that could potentially be targeted with temsirolimus. Implementing the treatment recommendation from genomic profiling, he received 4 doses of temsirolimus given approximately weekly over 28 days at which time he was found to be refractory to treatment based on his bone marrow biopsy and later died.

Patient 3. A 5-year-old male originally diagnosed with standard risk ALL at the age of 3. Despite going into remission with frontline ALL directed therapy modeled after COG protocol AALL0932 (NCT01190930), he developed AML during maintenance as a subsequent malignant neoplasm.[16] His AML clone was found to have a MLL (11q23) rearrangement due to t(9;11). He was enrolled on a high-risk frontline AML protocol (AML08 [NCT00703820]), but was found to be MRD positive at 0.5% at the end of induction and was MRD negative after induction 2.[15] He relapsed after his third cycle of frontline AML chemotherapy. Patient 3 was then enrolled on a relapsed AML protocol (SJCRH PANAML [NCT02676323]) that included treatment with panobinostat, fludarabine, and cytarabine, but was found to be refractory after 1 cycle of treatment.[17] Tumor genomic profiling discovered the following mutations MLL, CREBBP, GATA2, but there were no targetable mutations noted. Functional drug screening on a sample drawn after treatment failure confirmed that the leukemic blasts were unlikely to be sensitive to panobinostat (Supplemental Figure S1). Furthermore, the results suggested that cytarabine, clofarabine, and calcitriol could be an effective therapeutic option. This recommendation was implemented with 1 cycle of cytarabine 1000 mg/m^2/dose IV daily for 5 doses, clofarabine 52 mg/m^2/dose IV daily for 5 doses, and calcitriol 0.02 mcg/kg/day orally divided twice daily. Four weeks later, he was found to have refractory AML on bone marrow aspiration with 59% leukemia blasts. He was then enrolled in another relapsed AML protocol

Table 4. Recommendations and Implementation of Targeted Treatment From Genomic Profiling, and Treatments Informed by Functional Drug Screening

Diagnosis	Received Genomic Profiling (n = 30)	No Treatment Recommendations From Genomic Profiling (n = 9)	Treatment Recommendations From Genomic Profiling Without Implementation (n = 17)	Treatment Recommendations From Genomic Profiling With Implementation (n = 4)	Treatment Informed by Functional Drug Screening (n = 4)
B-ALL	11	1	9	1	0
AML	9	4	2	3	4
T-ALL/T-LL	3	1	2	0	0
MPAL	1	0	1	0	0
CML	0	0	0	0	0
Follicular lymphoma	1	0	1	0	0
Burkitt-like lymphoma	1	0	1	0	0
Hepatosplenic T-cell lymphoma	1	1	0	0	0
Hodgkin lymphoma	1	1	0	0	0
Myelodysplasia	1	1	0	0	0
Myelofibrosis	1	0	1	0	0

AML, acute myeloid leukemia; B-ALL, B-cell acute lymphoblastic leukemia, CML, chronic myeloid leukemia; MPAL, mixed-phenotype acute leukemia; T-ALL/T-LL, T-cell acute lymphoblastic leukemia/lymphoma

(SJCRH VENAML [NCT03194932]) that included treatment with venetoclax and cytarabine, which was also ineffective for him.[18] In retrospect, the previous drug sensitivity assay indicated that his leukemic blasts were unlikely to be sensitive to venetoclax. The patient was later treated with a palliative chemotherapy regimen and subsequently died.

Patient 4. A 7-year-old female with a liver transplant, who developed aplastic anemia that transformed into AML with FLT3-ITD and WT1 mutations. She received frontline chemotherapy modeled after COG AAML1031 (NCT01371981) with sorafenib, but was found at the end of induction therapy to have a bone marrow MRD of 0.7%.[19] She was MRD negative after cycle 3 of chemotherapy and underwent HSCT. However, she was found to be MRD positive in her bone marrow on her 30th day after bone marrow transplant. She was then treated with azacitidine and sorafenib, and was found to have 41% AML blasts after 2 cycles. She then received 1 cycle of decitabine, after which there was still 3% blasts detected in a hypercellular marrow. Tumor molecular profiling identified the following mutations, KMT2A (MLL)-PTD, FBXO11, PTPN11, RB1, and WT1, but did not recommend any therapies targeting these mutations. The drug sensitivity assay showed likely sensitivity to bortezomib, panobinostat, and dexamethasone (Supplemental Figure S2). Of note, the assay also suggested that her prior treatment regimen of azacitidine and sorafenib

would not be highly effective. The drug sensitivity study recommended treatment was implemented with a regimen of cycles of bortezomib 1.3 mg/m^2 IV on days 2, 4, 8, 11, 22, 25, 29, and 32; panobinostat 20 mg orally on days 1, 3, 5, 8, 10, and 12; and dexamethasone 10 mg/m^2/dose orally on days 1, 2, 4, 5, 8, 9, 11, and 12. Repeat bone marrow studies showed refractory disease after cycle 1 with 16% blasts and cycle 2 with 52% blasts of this therapy, so the patient was started on CPX-351 (liposomal cytarabine with daunorubicin). After 2 cycles of CPX-351 she was in remission with no evidence of MRD by flow cytometry; however, she did not have bone marrow recovery, remained pancytopenic, and succumbed after multiple opportunistic infections with *Aspergillus fumigatus, Staphylococcus epidermidis, Enterococcus faecalis, Pseudomonas aeruginosa*, and Epstein Barr virus.

Patient 5. A 2-year-old female diagnosed with AML. She received frontline treatment on SJCRH AML08 (NCT00703820) with vorinostat but was found to be refractory to treatment and underwent HSCT.[15] Approximately 2 years after transplant, she relapsed. She was started on treatment with vincristine, etoposide, and hydroxyurea. However, she had refractory disease in the bone marrow and furthermore developed new nasopharyngeal, parapharyngeal, extra-cranial, and intracranial chloromas. Drug sensitivity screening demonstrated that her disease might be sensitive to

bortezomib and panobinostat (Supplemental Figure S3). As panobinostat was not readily available for her age range, vorinostat, another histone deacetylase inhibitor was used. The drug sensitivity study recommendations were implemented with bortezomib 1.3 mg/m^2 IV twice a week (days 1 and 4) and vorinostat 180 mg/m^2/dose daily by mouth for 4 doses each week (days 1 to 4). With initiation of therapy, there was a significant improvement in the leukemic burden in the peripheral blood, with the WBC count dropping from 17,300/μL with 58% leukemic blasts on the day of initiation of therapy to 1.0/μL with 50% leukemic blasts 5 days later. Unfortunately, she had a respiratory decompensation after only 4 days of therapy. Bortezomib is associated with pulmonary toxicities and dyspnea, so it was held for the second week of treatment, but after pulmonary evaluation, the cause of the respiratory decompensation was attributed to a metapneumovirus infection, and bortezomib was restarted on week 3. MRI of the brain done 14 days after starting this therapy showed interval decrease in the size and resolution of the diffusion effect of the cranial chloromas consistent with treatment response. Unfortunately, 2 days later she had a further respiratory decline requiring mechanical ventilation. The vorinostat was held due to her multiple medical complications, and her WBC and leukemic blast count started to rise thereafter and she died approximately 4 weeks after initiating this therapy.

Patient 6. A 14-year-old male diagnosed with AML (FLT-ITD negative) and was given frontline AML chemotherapy modeled on COG AAML1031 (NCT01371981).[19] He relapsed 8 months after the completion of treatment and was found to be FLT3-ITD positive. He achieved a second remission with a salvage regimen of fludarabine, cytarabine, and sorafenib and subsequently underwent HSCT. He again relapsed on day 98 after HSCT. Patient 6 was then treated with sorafenib 300 mg by mouth twice daily implementing the results from FLT3 testing by conventional testing and his tumor genomic profiling assay. Tumor genomic profiling also demonstrated mutations in ETV6 and WT1, but these were not associated with therapeutic treatment recommendations. He was treated with sorafenib for 150 days. During that time he also received donor lymphocyte infusions and 3 cycles of azacitidine. He stopped therapy with sorafenib and azacitidine because of prolonged cytopenias, multiple infectious complications, and increasing MRD. The drug sensitivity assay results suggested sensitivity to bortezomib, panobinostat, and dexamethasone (Supplemental Figure S4). Of note, the same assay suggested that sorafenib would be ineffective for the patient, despite the presence of a FLT3 mutation. The treatment recommendations were implemented with cycles of bortezomib 1.3 mg/m^2 IV on days 1, 4, 8, and 11, panobinostat 20 mg by mouth on days 1, 3, 5, 8, 10, and 12, and dexamethasone 20 mg by mouth daily on days 1, 2, 4, 5, 8, 9, 11, and 12.

Cycles were repeated every 21 days.[20] After 39 days of treatment, Patient 6 achieved a complete remission and full donor chimerism. He sustained this response for 508 days until he relapsed and subsequently died due to infectious complications.

Patient 7. An 11-year-old girl who had an orthotopic heart transplant due to dilated cardiomyopathy at 11 months of age.[21] At 6 years of age she was diagnosed with myelodysplastic syndrome. Her frontline therapy for MDS included 3 cycles of decitabine and vorinostat, after which her MDS had transformed to AML. She had tumor genomic profiling, which found an MLL-PTD (partial tandem duplication) and an RUNX1 mutation. She received AML-directed therapy with 2 cycles of cytarabine and clofarabine and achieved a morphologic remission with negative bone marrow MRD testing. She received a HSCT from a matched unrelated donor with a myeloablative conditioning regimen of busulfan and fludarabine. Unfortunately, she relapsed 6 months later with a bone marrow showing 55% blasts. As this was approximately a year after the patient's first tumor genomic profile was done, the test was repeated. On this second tumor genomic profiling test, in addition to confirming the previous findings of MLL-PTD and RUNX1 mutations, new mutations including FLT3-ITD, IDH2, and RB1 mutations were found. Midostaurin, ponatinib, sorafenib, and sunitinib were recommended to target the FLT3-ITD. Enasidenib, azacitidine, decitabine, and venetoclax were recommended to target the IDH2 mutation. To implement these recommendations, she received a novel combination treatment with azacitidine (100 mg/m^2 daily) on days 1 to 5 and sorafenib (200 mg/m^2 daily) on days 8 to 28 every 28 days. Her pancytopenia improved and bone marrow evaluation including MRD testing was negative after 8 months of treatment. She underwent a second HSCT from a new matched unrelated donor, received sorafenib for 10 months after transplant, and remains alive without evidence of relapse 35 months after transplant.

Discussion

This case series describes the use of precision medicine diagnostic tools (genomic profiling and *ex vivo* drug sensitivity testing assays) in pediatric patients with leukemia and lymphoma at a single institution. The tumor genomic profiling assessed in this study is a commercial clinical test that recommends mutation-targeting treatment based on the published clinical data about the treatment options. It also suggested therapeutic options with information about available clinical trials. The functional *ex vivo* drug screening assay assessed in this retrospective study is a research-use only test, which potentially provides insight into the potential sensitivity of the leukemic blasts to different chemotherapeutic regimens, but does not have clinical outcomes data linked to the results. In both these testing scenarios, treatment recommendations were implemented only after careful

consideration of risk, benefit, and alternative therapies.

Although in this population there was a high incidence of actionable mutations and potential treatment options identified, the majority of therapeutic recommendations were not acted upon and only 7 (21%) patients received therapy recommended suggested by the testing. In our study, the 7 patients who received the testing directed therapies all had very poor prognoses due to having failed multiple standard chemotherapeutic regimens. Furthermore, 5 of the 7 had relapsed or refractory disease after HSCT. Four of the 7 had leukemia with mutations in MLL. Three of the 6 had FLT3 ITD+ AML. Six of the 7 patients had a history of relapsed or refractory AML and only 1 had ALL. This high proportion of patients with AML is likely due to a relative lack of effective treatment options for patients with relapsed AML, whereas relapsed and refractory ALL have a number of promising therapies, specifically, FDA-approved immunotherapies including blinatumomab, inotuzumab, and CAR T-cell therapy for treatment of B-cell ALL. In this heavily pre-treated population, 3 patients had good responses to testing directed therapy. Patient 5 had a partial response demonstrated by the shrinkage of her chloromas. Patient 6 had a complete response in the bone marrow and remained in remission for 508 days. Patient 7 went into complete remission with negative MRD testing, had a second HSCT, and is currently alive in remission.

This retrospective case series was conducted to understand why these tests were ordered and how these tests were used in this practice, but this methodology has its limitations, including limited sample size and the potential for confounders. Patients with relapsed hematologic malignancies are generally considered to have a high risk of future relapses, resulting in additional testing with genomic profiling assays or functional *ex vivo* testing to look for the optimal relapse therapies. Regarding the patients tested at diagnosis, they generally had poor or unusual prognostic features at diagnosis (e.g., mixed phenotypic acute leukemia, M7-AML, T-cell ALL, or a strong family history of pediatric cancer). Possible reasons that treatments were not implemented include lack of convincing clinical data, limited clinician clinical experience with the suggested regimens, and lack of patient and parent confidence that the suggested treatments would be efficacious. Furthermore, the Notable Labs functional *ex vivo* drug screening was and is currently still an investigational research use only test, thus limiting clinician confidence in the actionability of its results. Other reasons for this lack of translation into practice included limited pediatric-specific data availability, limited dosing guidelines for pediatrics, and the restricted number of cases or publications supporting off-label uses of the drugs. Precision oncology trials such as the NCI-COG Pediatric MATCH (Molecular Analysis for Therapy Choice) Screening Protocol (NCT03155620) and the Pediatric Acute Leukemia (PedAL) Screening Trial (NCT04726241) may address these concerns in

the future.[22,23]

Previous studies have demonstrated the feasibility and utility of implementing deep sequencing and functional *ex vivo* drug screening in the clinic. Pemovska et al found that molecular profiling and *ex vivo* drug sensitivity and resistance testing applied to 28 AML patient samples uncovered 5 major taxonomic drug-response subtypes, resulting in several clinical responses with therapy based upon the testing.[24] Kuusanmäki et al demonstrated that among samples from patients with lymphoproliferative disorders with overactive JAK/STAT3 signaling, drug sensitivity studies were able to define which of the many potential pathway inhibitors were most likely to be efficacious.[25] Maxson et al used deep sequencing and functional assays on primary patient cells to identify CSF3R mutations in chronic neutrophilic leukemia and atypical (BCR-ABL1–negative) CML that activate Jak signaling and are sensitive to ruxolitinib.[8] These results were later confirmed in a phase 2 study of ruxolitinib in patients with chronic neutrophilic leukemia and acute CML with CSF3R mutations.[9] Spinner et al recently published a study of 54 adult patients with myeloid neoplasms and discovered that the testing defined distinctive drug sensitivity patterns that could inform personalized therapy selection.[13] Our case series also found that genetic profiling and functional drug screening could provide potential therapeutic options in pediatric patients with leukemia when alternative standard life-prolonging therapies were not available.

Current genomic profiling tests are generally geared toward the adult population. In general, pediatric leukemia and lymphoma have fewer actionable mutations, but better outcomes with standard chemotherapy.[26] Notably, few oncologic drugs have FDA indications for use in the pediatric population and the choice of treatments is largely driven by clinician and collaborative group experiences in oncologic practice. However, pediatric clinicians and families may be willing to use novel or experimental therapies that show potential evidence of an effect in relapsed and refractory patients who have exhausted all treatment options.

Although some families may be willing to seek complementary and/or alternative therapies outside the allopathic medical world, clinicians may be cautious in their willingness to try experimental therapies. Molecular tumor boards or other forms of collective review of treatment options could be an effective means to provide proper oversight and guidance to clinicians and families regarding the use of novel therapeutic combinations; this has been shown to be effective in adults.[27-30] Consulting a pediatric molecular tumor board may be beneficial when all other treatment options have been exhausted. Given the overall lower incidence of cancer in pediatric patients than adult patients, the accumulation of direct patient experience in any particular pediatric oncology center would be slow. A virtual molecular tumor board for pediatric oncology would be an attractive model to

gather the expertise needed from multiple institutions in a robust timely manner.[28,30]

Conclusion

The results of this case series demonstrate that there are frequently actionable mutations and potential treatment options generated from precision medicine diagnostic techniques, such as genomic profiling assays and drug sensitivity testing. Testing-guided therapeutic recommendations have been implemented in a small percentage of pediatric patients with relapsed or refractory leukemia and lymphoma with limited alternative treatment therapies. Although multiple studies have demonstrated the utility of these tests, even when they are done they may not have immediate actionability, as we also observed in this cohort where most of the studies did not result in recommendations that were applied by the clinicians.[10–13,24,27] Although current levels of implementation are low, as experience with these methodologies increase, we anticipate that our institutions and collaborative groups will use the information provided by *ex vivo* drug sensitivity testing and/or genomic profiling assays upfront for patients in the future to help guide treatment.

Article Information

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