Original Investigation

Integrative Clinical Sequencing in the Management of Refractory or Relapsed Cancer in Youth

Rajen J. Mody, MBBS, MS; Yi-Mi Wu, PhD; Robert J. Lonigro, MS; Xuhong Cao, MS; Sameek Roychowdhury, MD, PhD; Pankaj Vats, MS; Kevin M. Frank, MS; John R. Prensner, MD, PhD; Irfan Asangani, PhD; Nallasivam Palanisamy, PhD; Jonathan R. Dillman, MD; Raja M. Rabah, MD; Laxmi Priya Kunju, MD; Jessica Everett, MS; Victoria M. Raymond, MS; Yu Ning, MS; Fengyun Su, PhD; Rui Wang, MS; Elena M. Stoffel, MD; Jeffrey W. Innis, MD, PhD; J. Scott Roberts, PhD; Patricia L. Robertson, MD; Gregory Yanik, MD; Aghiad Chamdin, MD; James A. Connelly, MD; Sung Choi, MD; Andrew C. Harris, MD; Carrie Kitko, MD; Rama Jasty Rao, MD; John E. Levine, MD; Valerie P. Castle, MD; Raymond J. Hutchinson, MD; Moshe Talpaz, MD; Dan R. Robinson, PhD; Arul M. Chinnaiyan, MD, PhD

IMPORTANCE Cancer is caused by a diverse array of somatic and germline genomic aberrations. Advances in genomic sequencing technologies have improved the ability to detect these molecular aberrations with greater sensitivity. However, integrating them into clinical management in an individualized manner has proven challenging.

OBJECTIVE To evaluate the use of integrative clinical sequencing and genetic counseling in the assessment and treatment of children and young adults with cancer.

DESIGN, SETTING, AND PARTICIPANTS Single-site, observational, consecutive case series (May 2012-October 2014) involving 102 children and young adults (mean age, 10.6 years; median age, 11.5 years, range, 0-22 years) with relapsed, refractory, or rare cancer.

EXPOSURES Participants underwent integrative clinical exome (tumor and germline DNA) and transcriptome (tumor RNA) sequencing and genetic counseling. Results were discussed by a precision medicine tumor board, which made recommendations to families and their physicians.

MAIN OUTCOMES AND MEASURES Proportion of patients with potentially actionable findings, results of clinical actions based on integrative clinical sequencing, and estimated proportion of patients or their families at risk of future cancer.

RESULTS Of the 104 screened patients, 102 enrolled with 91 (89%) having adequate tumor tissue to complete sequencing. Only the 91 patients were included in all calculations, including 28 (31%) with hematological malignancies and 63 (69%) with solid tumors. Forty-two patients (46%) had actionable findings that changed their cancer management: 15 of 28 (54%) with hematological malignancies and 27 of 63 (43%) with solid tumors. Individualized actions were taken in 23 of the 91 (25%) based on actionable integrative clinical sequencing findings, including change in treatment for 14 patients (15%) and genetic counseling for future risk for 9 patients (10%). Nine of 91 (10%) of the personalized clinical interventions resulted in ongoing partial clinical remission of 8 to 16 months or helped sustain complete clinical remission of 6 to 21 months. All 9 patients and families with actionable incidental genetic findings agreed to genetic counseling and screening.

CONCLUSIONS AND RELEVANCE In this single-center case series involving young patients with relapsed or refractory cancer, incorporation of integrative clinical sequencing data into clinical management was feasible, revealed potentially actionable findings in 46% of patients, and was associated with change in treatment and family genetic counseling for a small proportion of patients. The lack of a control group limited assessing whether better clinical outcomes resulted from this approach than outcomes that would have occurred with standard care.

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Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: Arul M. Chinnaiyan, MD, PhD, Michigan Center for Translational Pathology, Investigator Howard Hughes Medical Institute, Cancer Center, Room 5316, 1400 E Medical Center Dr, SPC 5940, University of Michigan, Ann Arbor, MI 48109-5940 (arul@umich.edu).

Outcomes of children and young adults with cancer have improved, primarily due to enhanced understanding of tumor biology and due to the clinical application of biological discoveries through multi-institutional clinical trials.[1-4] However, survival for many pediatric oncology patients, including those with recurrent disease or metastatic disease, remains poor.[5,6] To this end, integrative sequencing modalities offer a potentially useful platform to investigate the individual cancer genome in order to identify actionable genomic alterations that can be matched to targeted therapies.[7-9] As such, the concept of precision medicine, ie, taking individual variability into account while designing therapy, is not new; however, post–genome-sequencing-era discoveries provide renewed opportunities for personalizing care of individuals with cancer.[10] Precision medicine has been singled out as a priority initiative in the United States, with the goal of improving outcomes of hard-to-cure diseases, such as some pediatric cancers.[11]

Large-scale research projects, such as Therapeutically Applicable Research to Generate Effective Treatments (TARGET) and the Pediatric Cancer Genome Project (PCGP), are creating a catalog of genomic alterations in common pediatric cancers.[9,12-14] However, there are no prospective, pediatric studies demonstrating feasibility and utility of incorporating multiple comprehensive sequencing technologies (ie, whole-exome and transcriptome analysis) in the clinical management of children and young adults with cancer.

Following the establishment of a program called MiOncoSeq in 2011 to explore the feasibility of integrative clinical sequencing in adult patients with advanced cancer,[15] we established Peds-MiOncoSeq in 2012, which is a prospective, observational clinical case series of patients with relapsed, refractory, or rare pediatric cancer, with an aim to study the feasibility and utility of integrative clinical sequencing in the management of their cancer. Our study also sought to identify limitations of this approach and barriers in translating sequencing findings into viable therapeutic options.

Methods

Patients

This was a single-center case series with prospective data collection. Patients 25 years or younger with a suspected diagnosis of a neoplastic disorder were eligible for the study, which was approved by the University of Michigan C. S. Mott Children's Hospital institutional review board (see eAppendix Section I clinical protocol in the Supplement). All patients were seen by a physician investigator and a genetic counselor. This study was initiated in May 2012 and continues as of August 2015. All patients or their parents or legal guardians provided informed consent (written assent if >10 years) and received mandatory preenrollment genetic counseling regarding the potential risks of incidental genetic findings (eAppendix Section II consent documents in the Supplement). A "flexible default" consent model was used to mandate disclosure of findings that directly influenced the current cancer management strategy, but patients or parents could choose whether to receive incidental results associated with high risk of hereditary cancer syndromes in patients and other family members.[15,16] However, incidental genetic findings did not include disclosure for conditions other than cancer.[15,16] Once enrolled, a patient's clinical course was captured quarterly in order to document clinical status and treatment decisions made by the primary team since the last follow-up (eAppendix Section I in the Supplement).

Integrative Clinical Sequencing

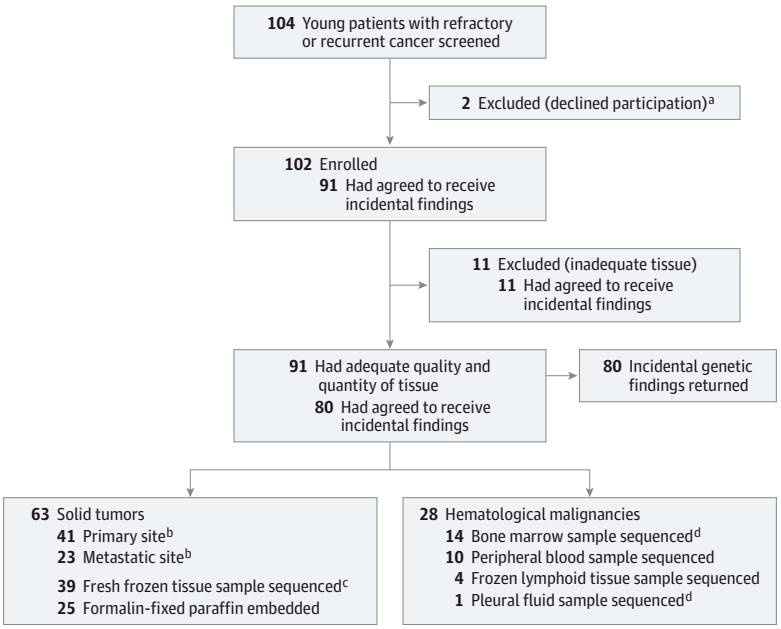
Board-certified pathologists (R.R. and L.P.K.) evaluated histologic sections for estimation of tumor content before submitting tissue for sequencing. Nucleic acid preparation and high-throughput sequencing were performed using standard protocols in our sequencing laboratory, which adheres to the Clinical Laboratory Improvement Amendments (CLIA).[17,18] Paired-end whole-exome libraries from tumor samples that were matched with normal DNA and with transcriptome libraries either from polyadenylated tumor RNA (PolyA + transcriptome) or from total RNA captured by human all-exon probes (capture transcriptome) were prepared and sequenced using the Illumina HiSeq 2000 and 2500 (Illumina Inc). Aligned exome and transcriptome sequences were analyzed to detect putative somatic mutations, insertions and deletions (indels), copy-number alterations, gene fusions, and gene expression as described previously and detailed in eMethods in the Supplement.[17,18] Summaries of sequencing depth and quality control parameters are presented in eTable 2 in the Supplement.

Pathogenicity of germline variants were determined through a review of the published literature, public databases including but not limited to ClinVar, the Human Genome Mutation Database, the Leiden Open Variation Databases, and variant specific databases (eg, International Agency for Research on Cancer *TP53* Database, International Society for Gastrointestinal Hereditary Tumors mutation databases). Only variants that had been previously described as pathogenic were considered for disclosure. Variants with conflicting pathogenicity reports and variants not previously reported were considered to be of uncertain significance and were not considered for disclosure. Following disclosure, familial testing was recommended. Clinical relevance of somatic variants was investigated using an integrated approach incorporating technical considerations, (eg, recurrence, variant allele fraction, expression levels, and predictive algorithms for pathogenicity), variant specific information (ie, ClinVar, published literature, and curated gene specific resources), as well as published correlations of drug and variant sensitivity profiles. Considerations of tumor heterogeneity, including clonal vs subclonal mutation were addressed by comparing variant allele fractions and copy-number estimates for each of the mutations to postsequencing estimates of tumor content derived from single-nucleotide variation and copy-number analyses. Variant allele fractions and tumor content estimates are shown in eTable 1 in the Supplement. Each of the aberrations for which clinical action was based in this study were judged to be clonal.

Precision Medicine Tumor Board Activity

A weekly, multidisciplinary precision medicine tumor board interpreted and deliberated on sequencing results for each patient. The tumor board included pediatric and adult oncolo-

Figure 1. Overview of the Peds-MiOncoSeq Clinical Study



[a] One patient declined participation because the patient did not have available tissue, so the family declined research biopsy. The second patient's cancer was in remission at the time of screening, so the family chose not to pursue the study using archived tissue.

[b] One patient had both his primary and metastatic tumor site samples sequenced.

[c] One patient's sample was sequenced twice using frozen tissue.

[d] One patient with leukemia had both bone marrow and malignant pleural fluid sequenced.

gists, geneticists, pathologists, biologists, bioinformaticians, bioethicists, genetic counselors, study coordinators, and ad hoc expertise (eFigure 1 in the Supplement). Selected findings underwent additional independent CLIA-validated testing, and summarized results were disclosed to treating oncologists and families by the clinical sequencing team, board-certified clinical geneticists, and counselors, as appropriate. A representative tumor board presentation is included in the eAppendix Section V in the Supplement.

For the purposes of this study, *potentially actionable findings* were defined as any genomic finding discovered during sequencing analysis that could lead to a change in patient management by providing a targetable molecular aberration, a change in diagnosis or risk stratification, or a change in patient or family counseling by identifying cancer-related germline findings that inform patients and families about the potential risk of various cancers.

Results

Feasibility of Integrative Clinical Sequencing

We screened 104 patients and enrolled 102 patients (mean age, 10.6 years; median age, 11.5 years; range, 1-22 years) between May 2012 to October 2014 (**Figure 1**). Two patients declined participation. Because no tissue was available for the first patient, the family declined research biopsy. The second patient's cancer was in remission at the time of screening, so the family chose not to pursue the study using archived tissue. The patient population included common pediatric diagnoses including hematological malignancies, solid tumors, and brain tumors (**Table 1**). Eighty-one patients had refractory or relapsed disease at the time of enrollment, had exhausted all proven therapeutic options, and were seen in clinic for experimental therapeutic options. The rest of the 21 patients were enrolled at the time of their original diagnoses when they presented with either a very rare cancer or an atypical presentation for a pediatric age group (eTable 1 in the Supplement).

Tumor tissue used for sequencing was obtained by pediatric radiologist (J.R.D.), using image-guided percutaneous core needle research biopsy from 7 patients. The procedure was well tolerated and yielded adequate tissue. For 95 patients, tissue was obtained by standard of care diagnostic or therapeutic surgical procedures done either at the time of enrollment or from earlier procedures (eTable 1 in the Supplement). Overall, from 91 patients (89%), we were able to obtain adequate quality and quantity of tumor to perform full sequencing analysis including 28 hematological malignancies (31%) and 63 solid tumor cases (69%). These 91 patients were used as a denominator for all subsequent analyses (Figure 1). Details on the type of tissue used (ie, frozen vs formalin-fixed paraffin embedded) as well as site of tissue collection (ie, primary vs metastasis), are summarized in Figure 1 and eTable 1 in the Supplement.

Genetic counseling at study enrollment was well received by patients and families, with 91 of the 102 (89%) enrolled in the study choosing to receive optional incidental genetic findings. Overall, 80 (78%) actually received results because these patients had both agreed to receive incidental genetic findings and had an adequate tumor sample to complete sequencing.

The median turnaround time from study enrollment to case presentation at the precision medicine tumor board for the overall cohort was 53 days, with a mean of 54 days (range, 15-114 days), which was longer than our anticipated 3- to 4-week timeline.[15] The primary reasons for delay included waiting for bioinformatics analysis as well as wait times for the next board,

Table 1. Patient Demographics (102 patients)

Diagnosis	No. of Patients			Age, y			No. of Patients Sequenced	
	Total	Male	Female	Range	Mean	Median	At Relapse or Progression	At Diagnosis
All patients	102	52	50	0-22	10.6	11.5	81	21
All hematological malignancies	32	17	15	1-22	10.6	9	26	6
Leukemia	25	12	13	1-22	11.2	12	22	3
Lymphoma	4	3	1	2-20	11	11	3	1
Other	3	2	1	4-7	5	4	1	2
All solid tumors	70	35	35	0-22	10.6	12	55	15
Brain tumors	8	4	4	1-13	7.1	7.5	6	2
Neuroblastoma	9	4	5	5-15	7.2	6	9	0
Sarcoma	29	14	15	0-22	11.1	12	21	8
Renal tumors	3	2	1	18-22	20	20	1	2
Liver tumors	7	4	3	0-17	7.3	5	5	2
Ovarian tumors	3	0	3	9-13	11.7	13	2	1
Other solid tumors	11	7	4	0-18	13.7	15	10	1

which often delayed return of results by 2 weeks. However, over the course of the study, the turnaround times improved from a mean of 60 days for the first 51 patients to a mean of 48 days for the last 51 patients. We also assessed the actual costs of integrative clinical sequencing from the time a sample was obtained and estimated the costs at approximately $6000 per patient including supplies, labor, and bioinformatics analysis (eTable 3 in the Supplement). However, patients or their families were not charged for the sequencing and analysis.

Potentially Actionable Findings

One of the study aims was to estimate the prevalence of potentially actionable findings after completion of sequencing analysis. We identified 42 patients (46%) (**Table 2** and **Table 3** and eTable 1 in the Supplement), including 9 patients (10%) with significant incidental germline findings (Table 3).

Actionable Findings in Pediatric Hematological Malignancies

Potentially actionable findings were identified in 15 of 28 patients (54%) with hematological malignancies (Table 2). In patient 3, a girl with precursor-B acute lymphoblastic leukemia (Table 2 and eTable 1 in the Supplement), RNA sequencing revealed an actionable, cryptic gene fusion involving *ETV6* and *ABL1* (eFigure 2C in the Supplement) that was not detected by other standard diagnostic tests including cytogenetics and fluorescence in situ hybridization for the *BCR-ABL* fused genes. As predicted,[19,20] preclinical in vitro assays on this patient's primary leukemia cells demonstrated their sensitivity to imatinib, a tyrosine kinase inhibitor (eFigure 2E, F in the Supplement). With all standard therapeutic options failing the patient, imatinib and chemotherapy were initiated. She was unable to tolerate ongoing cytotoxic chemotherapy with imatinib and was treated with imatinib alone for most of her course. She maintained morphological, cytogenetic, and molecular remission for 21 months with excellent quality of life while receiving imatinib (Table 2 and eTable 1 in the Supplement). (All genes and their Accession numbers are reported in eTable 4 of the Supplement.)

Other patients with hematological malignancy with potentially actionable findings and clinical course are discussed in Table 2 and eTable1 in the Supplement, including a cryptic, actionable *EBF1-PDGFRB* gene fusion in a patient with refractory pre-B ALL and in 3 patients with hematologic malignancies who had actionable alterations in the FLT3 kinase detected by sequencing. Sorafenib has shown clinical activity in patients with refractory leukemia with FLT3 protein alterations.[21]

Actionable Integrative Clinical Sequencing Findings in Pediatric Solid Tumors

We identified potentially actionable findings in 43% (27 of 63) patients with pediatric solid tumors (Table 3). Patient 43 is a girl who was originally diagnosed with infantile myofibromatosis and subsequently (by sequencing) diagnosed with high-grade spindle cell sarcoma that tested negative for the *ETV6-NTRK3* gene fusion (eAppendix section IV, V in the Supplement).[22] Her transcriptome analysis identified a novel in-frame fusion of the *LMNA-NTRK1* genes, which preserves the functional tyrosine kinase domain of the NTRK1 protein (eFigure 3B in the Supplement). Although almost 90% of patients with infantile fibrosarcoma have the canonical *ETV6-NTRK3* gene fusion, the *LMNA-NTRK1* gene fusion reported in this index patient is functionally analogous. The *NTRK1* gene fusions in other cancers, including lung cancers, have shown sensitivity to crizotinib, an ALK and c-MET inhibitor.[23,24] Discovery of the *LMNA-NTRK1* gene fusion in patient 43 suggested the diagnosis of infantile fibrosarcoma that required changing treatment to oral crizotinib. Within 6 weeks of starting therapy, she achieved a partial remission and has since maintained a favorable response to crizotinib for more than 8 months without experiencing major toxic effects (Table 3 and eFigure 3C, D in the Supplement).

The second solid tumor case example features patient 57, a girl diagnosed with medulloblastoma, who enrolled in the study at the time of her relapse. Our analysis identified a cryptic

Table 2. Summary of 14 Patients With Hematological Malignancies Patients With Potentially Actionable Findings[a]

Patient No.	Diagnosis	Tissue Sequenced	Informative and Actionable Genomic Findings	Standard Therapy Without Sequencing	Potentially Actionable Findings	Potential Actions Based on Sequencing Results	Action Taken	Outcome
3	Pre-B ALL	Bone marrow[b] Pleural fluid[b]	Homozygous *CDKN2A* deletion; *ETV6-ABL1* fusion	Palliative cytotoxic therapy for relapsed ALL or phase 1 clinical trials	Homozygous *CDKN2A* deletion; *ETV6-ABL1* fusion	Imatinib targeting ABL1, CDK inhibitors (NCT01037790)	Yes	Sustained clinical remission for 21 mo on imatinib
9	MDS, AML	Bone marrow[b]	*NRAS* (p.G13R) Mutation; Loss at Chr6q and Chr20q	Palliative cytotoxic therapy or azacitidine or phase 1 clinical trials	*NRAS* p.G13R Mutation	MEK inhibitor (NCT01907815)	No	Patient died of progressive disease; no MEK inhibitor available for clinical trial at the time; no dosing information for off-label use
14	Pre-B ALL	Bone marrow[b]	*TP53* (p.R196*), *NRAS* (p.Q61H), *MED12* (p.R1467*), *MLL2* (p.S4042fs); *CDKN2A/2B* homozygous deletion, chr7p 1 copy loss; *CRLF2* overexpression	Palliative cytotoxic therapy or phase 1 clinical trials	*NRAS* (p.Q61H), *CDKN2A/2B* homozygous deletion	CDK inhibitors (NCT01037790), MEK inhibitor (NCT01907815)	No	Patient died of progressive disease; no MEK or CDK inhibitor available for clinical trial; no dosing information for off-label use
30	AML	Bone marrow[b]	*CSF3R* p.T640N and p.Q768* point mutations, *EIF4A2-MECOM* fusion; chr7q copy losses of *EPHA1, EPHB6, EZH2, MLL3, MNX1, RHEB, SHH, BRAF, CREB3L2, GRM8, PRSS1, SMO*, chr3q copy gain, chr21 copy gain	Palliative cytotoxic therapy, azacitidine, or phase 1 clinical trials	*CSF3R* p.T640N and p.Q768* point mutations	Targeting *CSF3R* p.T640N and p.Q768* point mutations with JAK2 inhibitor ruxolitinib	No	Patient could not be treated with ruxolitinib on clinical trial or off label due to rapid progression before availability of results
38	T-ALL	Peripheral blood[b]	*CDKN2A* and *CDKN2B* deletions, *PRSS1* deletion, *NTRK1* overexpression;	Palliative cytotoxic therapy or phase 1 clinical trials	*CDKN2A* and *CDKN2B* deletions	CDK inhibitors (NCT01037790)	No	Patient died of rapid progressive disease; no CDK inhibitors available for clinical trial; no dosing information for off-label use
41	ETP-ALL	Peripheral blood[c]	*FLT3* ITD mutation; Chr16p gain, Chr16q loss; *FLT3* overexpression	Allogenic BMT, no adjuvant therapy postBMT	*FLT3* ITD mutation, *FLT3* overexpression	FLT3 inhibitor	Yes	Patient in clinical remission post-BMT, receiving sorafenib post-BMT for 15 mo
49	Pre-B ALL	Peripheral blood[b]	*FLT3* nonframeshift deletion; *BLK* and *FLT3* overexpression	Allogenic BMT for relapsed ALL, no adjuvant therapy postBMT	*FLT3* nonframeshift deletion, *FLT3* overexpression	FLT3 inhibitor	Yes	Patient in clinical remission for 9 mo post-BMT, received sorafenib for 6 mo
53	LCH	Metastatic axillary LN[b]	*BRAF* p.V600D mutation	Steroids, vinblastine for 6 mo	*BRAF* p.V600D mutation	BRAF inhibitor	Not required	Patient in clinical remission after chemotherapy; eligible for BRAF inhibitors in case of posttreatment
54	AML	Bone marrow[b]	*NF1* (p.Y333*) mutation, *NF1* frame-shift deletion, *TSC2* stop-gain insertion (truncated after a.a.646); *CBFB-MYH11* fusion	Allogenic BMT for AML, no adjuvant therapy postBMT	*NF1* (p.Y333*) mutation, *NF1* frame-shift deletion	MEK inhibitors (NCT02049801)	Not required	Patient in clinical remission following BMT; eligible for MEK inhibitor in case of posttreatment

(continued)

Table 2. Summary of 14 Patients With Hematological Malignancies Patients With Potentially Actionable Findings[a] (continued)

Patient No.	Diagnosis	Tissue Sequenced	Informative and Actionable Genomic Findings	Standard Therapy Without Sequencing	Potentially Actionable Findings	Potential Actions Based on Sequencing Results	Action Taken	Outcome
55	AML	Bone marrow[b]	*CDK6* overexpression, *FLT3* (p.D835Y) mutation	No adjuvant therapy following donor leukocyte infusion	*FLT3* (p.D835Y) mutation	Next-generation FLT inhibitor (NCT02039726)	Not required	Patient in clinical remission following DLI; eligible for next generation FLT inhibitor in case of posttreatment
65	AML	Bone marrow[b] Bone marrow[c]	Chr9q loss, *WT1* (p.P376fs), *NF1* (p.K191fs and p.1596fs), *PTPN11* (p.E76Q)	Palliative cytotoxic therapy or phase 1 clinical trials	*NF1* (p.K191fs and p.I596fs)	MEK inhibitor (NCT02049801) or mTOR inhibitors	No	Patient died quickly after allogeneic BMT
66	JMML	Bone Marrow[c]	Somatic *CBL* (p.Y371H), *NRAS* (p.G12D), *NRAS* (p.G13D), *PTPN11* (p.D61Y)	Chemotherapy, 13-Cis Retinoic acid followed by allogenic BMT	*NRAS* (p.G12D), *NRAS* (p.G13D)	MEK inhibitor (NCT01907815)	Not required	Patient clinically stable and experiencing spontaneous regression without therapy; MEK inhibitors in case fails standard therapy
76	TMy Bi-Phenotypic leukemia	Peripheral blood[b]	*NRAS* (p.G60E), *PHF6* (R320*) mutations, *SPI1* frame-shift insertion (p.Q78fs); *ASXL1* frame-shift insertion, *CBLC* frame-shift insertion, *JAK3* (p.M511I) activating mutation; *JAK3* overexpression	Cytotoxic chemotherapy for relapsed leukemia, phase 1 clinical trials	*NRAS* (p.G60E), *JAK3* (p.M511I) activating mutation; *JAK3* overexpression	JAK3 inhibitor or MEK inhibitor (NCT01907815)	Yes	Patient treated with JAK3 inhibitor tofacitinib but could not tolerate full dose due to GI toxicity, died of progressive disease
92	ALL	Bone marrow[b]	Rearrangement of T-cell receptors and immunoglobulins detected; *ZCCHC7-PAX5*, *EBF1-PDGFRB* fusions	Allogenic BMT for elevated MRD	*EBF1-PDGFRB* fusion	PDGFRB inhibitors (imatinib)	None so far	Patient in clinical remission post-BMT, treating physician in process of getting approval for imatinib
98	Pre-B ALL	Peripheral blood[c]	Rearrangement of B and T cell receptors; *BCR-ABL1* fusion, *RUNX1-MSH6* (loss of *RUNX1* function), *MSH6-RUNX1* (reciprocal; functional *RUNX1*), *HBS1L-MYB* (out-of-frame) fusions; *FLT3* overexpression	Imatinib therapy postallogenic BMT	*BCR-ABL1* fusion	Imatinib, dasatinib targeting *BCR-ABL1* fusion	No	Patient already receiving imatinib post-BMT

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BMT, bone marrow transplant; DLI, donor lymphocyte infusion; ETP-ALL, early T-cell precursor acute lymphoblastic leukemia; indel, insertiondeletion; GI, gastrointestinal; JMML, juvenile myelomonocytic leukemia; LCH, Langerhan cell histiocytosis; LN, lymph node; MDS, myelodysplastic syndrome; MRD, minimal residual disease.

[a] Tissues are fresh frozen.

[b] Tissue used for sequencing was after relapse or refractory or progressive disease following treatment with surgery, chemotherapy, radiation, or biologic therapy.

[c] Tissue used for sequencing was prior to starting any treatment.

Table 3. Summary of 27 Patients with Solid Tumors With Potentially Actionable Findings[a]

Patient No.	Diagnosis	Tissue Sequenced	Findings		Traditional Therapy Options Without Sequencing	Potentially Actionable Findings	Potential Actions Based on Sequencing Results	Action Taken	Clinical Actions and Outcome
			Informative and Actionable Genomic	Incidental Germline					
5	SS	Primary thigh, FFPE[b] metastatic lung, FFPE[b]	*NOTCH1* (p.T1997M)		Available phase 1 clinical trial for relapsed solid tumors	*NOTCH1* (p.T1997M)	NOTCH1 inhibitor (NCT01154452)	No	Patient eligible for adult NOTCH1 inhibitor trial, but in hospice with progressive disease
13	PEComa	Primary peritoneal mass[b]	*SFPQ-TFE3* fusion		Available phase 1 clinical trial for relapsed solid tumors *mTOR* or *VEGF* inhibitors	*SFPQ-TFE3* fusion	VEGF or mTOR inhibitors	Yes	Patient receiving pazopanib therapy for Posttreatment with 90% tumor reduction for >16 mo
15	WT	Metastatic paraspinal mass[b]	*AMER1* deletion, *MYC* (p.P44L), *MAX* (p.R60Q)	*HOXB13* (p.G84E) mutation	Available phase 1 clinical trial for relapsed solid tumors, no genetic counseling	*MYC* (p.P44L), Germline *HOXB13* (p.G84E) mutation	mTOR and VEGF inhibitors, family counseling for prostate cancer risk	Yes	Patient receiving VEGF2 inhibitor (XL-184) with a partial response >15 mo; family referred to genetics clinic
21	Melanoma	Metastatic lymph node, FFPE[c]	*BRAF* (p.V600E)	*BAP1* (p.D567X)	No adjuvant therapy	*BRAF* (p.V600E), germline *BAP1* (p.D567X)	BRAF inhibitor (NCT01677741), family genetic counseling for melanoma and other cancers	Yes	Family in genetics clinic for counseling. BRAF inhibitor as available option for relapse
23	NBL	Primary adrenal mass[c]	Many CNAs, *ATRX* deletion	*BARD1* fs insertion (p.E139fs)	Available phase 1 clinical trial for relapsed NBL, no genetic counseling	Germline *BARD1* fs insertion (p.E139fs)	Genetic counseling for familial cancer	Yes	Family in genetics clinic for counseling
24	Colorectal carcinoma	Primary colon mass[b]; metastatic lymph node, FFPE[b]	*TP53* (p.C135F), *GNAS* (p.R201H), *SFPQ-TFE3* fusion		Available phase 1 clinical trial for relapsed solid tumors or *VEGF* inhibitors	*SFPQ-TFE3* fusion	VEGF or mTOR inhibitors	Yes	Action taken; patient progressed while receiving pazopanib therapy
31	ACC	Primary adrenal mass, FFPE[c]	*STAG2* (p.W706G); Chr12q copy gain, Chr22 copy loss (*CHEK2*), *MDM2* amplification and overexpression		Mitotane or available phase 1 clinical trial for relapsed solid tumors	*MDM2* amplification and overexpression	MDM2 inhibitors (NCT01901172)	No	Patient eligible for MDM2 inhibitor but decided to receive other investigational therapy
33	ARMS	Metastatic hepatic lobe[b]	Chr3p, 4q, 12q, 14p, 14q, and 16q copy losses, *MYCN* copy gain; *TP53* (p.R248W), *PAX3-FOXO1* fusion; *MYCN*, *OLIG2* overexpression *FGF8* amplification and overexpression		Available phase 1 clinical trial for relapsed solid tumors or palliative cytotoxic chemotherapy	*FGF8* amplification and overexpression	FGFR4 inhibitor (NCT01976741 NCT01703481)	Yes	Action taken; patient received FGFR inhibitor ponatinib but discontinued due to skin toxicity
37	ERMS	Metastatic peritoneal fluid[b]	MDM2, *YEATS4* amplification; *MAFB*, *CSF1R*, *SPI1* overexpression; *FGFR4* (p.V550E)		Available phase 1 clinical trial for relapsed solid tumors	*FGFR4* (p.V550E)	FGFR4 inhibitor (NCT01976741 NCT01703481)	No	No FGFR inhibitor available in clinical trials or dosing information available for off-label use for pediatric patients
39	RMS	Primary oropharynx mass[b]	*FANCD2* frame-shift deletion; *ATIC-ALK* fusion		Adjuvant cytotoxic chemotherapy	*ATIC-ALK* fusion	ALK inhibitor in combination with cytotoxic therapy	None required	Patient in clinical on cytotoxic chemotherapy. ALK inhibitor is an option after treatment
43	IF	Primary forearm mass[b]	Chr3q copy loss, chr16 copy gain; *STAG2* (p.Y355F) mutation, IL-3 indel; Homozygous deletion *CDKN2A*, *CDKN2B*; *LMNA-NTRK1* fusion; *NTRK1*, *LMNA* overexpression		Available phase 1 clinical trial for relapsed solid tumors	Homozygous deletion *CDKN2A*, *CDKN2B*; *LMNA-NTRK1* fusion	Change in diagnosis, NTRK inhibitors or CDK inhibitors (NCT01037790)	Yes	50% reduction in lung masses taking crizotinib therapy for NTRK1 inhibition, on therapy for 8 mo

(continued)

Table 3. Summary of 27 Patients with Solid Tumors With Potentially Actionable Findings[a] (continued)

Patient No.	Diagnosis	Tissue Sequenced	Findings		Traditional Therapy Options Without Sequencing	Potentially Actionable Findings	Potential Actions Based on Sequencing Results	Action Taken	Clinical Actions and Outcome
			Informative and Actionable Genomic	Incidental Germline					
44	OS	Primary leg mass, FFPE[b]	Homozygous deletions of *NF1*, *PTEN*, *FAS*, *TP53*; frame-shift insertion of *ATRX*; *WNT5B*, *WNT16* overexpression		Available phase 1 clinical trial for relapsed solid tumors or palliative cytotoxic chemotherapy	Homozygous deletions of *NF1*	MEK inhibitors (NCT01725100)	Yes	Action taken; patient treated with MEK inhibitor trametinib but progressed after 2 mo.
45	Epithelioid Sarcoma	Metastatic lymph node[b]	*KRAS*, *ALAS1*, *BIRC3*, *WNT8A* overexpression		Available phase 1 clinical trial for relapsed solid tumors	*KRAS* overexpression	MEK inhibitors (NCT01725100)	Yes	Patient started receiving MEK inhibitor in combination with mTOR inhibitor but progressed in 4 weeks
51	Cholangio-carcinoma	Primary liver mass[b]	*IDH1* (p.R132C), *TP53* splice site mutation; *KRAS* amplification, *KRAS* overexpression		Available phase 1 clinical trial for relapsed solid tumors	*IDH1* (p.R132C) mutation, *KRAS* amplification, *KRAS* overexpression	IDH inhibitors or MEK inhibitors (NCT01725100)	No	Patient rapidly progressed and died; no IDH or MEK inhibitor in clinical trials and no dosing information for off-label use
57	RMS	Primary cerebellar tumor, FFPE[c]; Primary cerebellar tumor[b]	Chr1q, 5p copy gain; chr7p, 16q copy loss; *DES* overexpression; overexpression of *FGF8*, *FGF9*, *FGFR4*, *ALK*; *PAX3-NCOA2* fusion; *MYOG*, *MYOD1* overexpression		Available phase 1 clinical trial for brain tumors or cytotoxic regimen directed at medulloblastoma	Overexpression of *FGF8*, *FGF9*, *FGFR4*, *ALK*; *PAX3-NCOA2* fusion; *MYOG*, *MYOD1* overexpression (RMS markers)	Change in diagnosis, treatment plan	Yes	Change in treatment after sequencing to RMS therapy, remained in remission 6 mo following change in management before progressing
58	PPB	Primary mediastinal mass[c]	*TP53* homozygous deletion; *MLL3* (p.G3155) mutation, *CTNNB1* frame-shift deletion; moderate *FGFR1*, *FGFR4* overexpression Somatic *DICER1* (p.G1809R) point mutation- near hotspots	*DICER1* (p.E1788X)	Cytotoxic chemotherapy and genetic counseling	Germline *DICER1* (p.E1788X)	Genetic counseling for DICER1 family of tumors	Yes	Family seen in genetics clinic for counseling for DICER1 family of tumors
68	ATRT	Primary posterior fossa, FFPE[c]	*SMARCB1* frameshift deletion, (deletion of exon 2), LOH at *SMARCB1*		No additional therapy	*SMARCB1* frameshift deletion, (deletion of exon 2), LOH at *SMARCB1*	CDK4/6 inhibitor (NCT01747876)	None required	Patient in clinical remission following chemotherapy
69	MBL	Primary cerebellum, FFPE[c]	Overexpression of *PTCH1*, *PTCH2*, *GLI1*, GL12, *MYCN* (*SHH* subtype markers); overexpression of *ERBB4*, *NTRK1*, *NTRK3*; Sonic hedgehog pathway activation		Palliative cytotoxic chemotherapy or available phase 1 clinical trial for relapsed brain tumor	*SHH* pathway activation	SHH inhibitor	No	No SHH inhibitors in clinical trial and no dosing information available for off-label use in children
70	Ovarian small-cell carcinoma	Primary ovarian tumor[c]	*SMARCA4* (p.T858K); *WT1* overexpression	*SMARCA4* (p.R979X)	Cytotoxic chemotherapy and genetic counseling	*SMARCA4* (p.T858K), germline *SMARCA4* (p.R979X)	Genetic counseling for family members for ovarian tumors	Yes	Family seen in genetics clinic for counseling for ovarian tumors
81	NBL	Metastatic right kidney[b]	Chr7, Chr17q copy gains; Chr11q, Chr1p copy losses; *MYCN* single-copy gain; RHD, *GSTM1* homozygous deletion; *CCND1*, *NTRK1* overexpression; *ALK* (p.F1174L) hotspot mutation		Available phase 1 clinical trial for relapsed NBL	*ALK* (p.F1174L) hotspot mutation	ALK inhibitor (crizotinib)	Yes	Patient was treated with crizotinib but progressed after 2 mo
86	IMFM	Metastatic neck mass[b]	*NOTCH3*, *PDGFRB* overexpression; *PDGFRB* (p.N666K)	*PDGFRB* (p.R561C)	No adjuvant therapy, no genetic counseling	Germline *PDGFRB* (p.R561C)	PDGFRB inhibitors (imatinib), family counseling	Yes	Family referred to genetics clinic for counseling. No actions at present on patient, in clinical remission following chemotherapy. Eligible for PDGFRB inhibitors in case of posttreatment

(*continued*)

Table 3. Summary of 27 Patients with Solid Tumors With Potentially Actionable Findings[a] (continued)

Patient No.	Diagnosis	Tissue Sequenced	Findings		Traditional Therapy Options Without Sequencing	Potentially Actionable Findings	Potential Actions Based on Sequencing Results	Action Taken	Clinical Actions and Outcome
			Informative and Actionable Genomic	Incidental Germline					
89	High-grade glioma	Primary brain tumor[c]	*PDGFRA, MYC, PVT1, CHIC2, RBPJ, FGF2, ING4, ZNF384* amplification; *LRP6-ETV6* fusion; *PDGFRA, MYC, PVT1, CHIC2, RBPJ, FGF2, ING4, ZNF384* overexpression		Available phase 1 clinical trial for relapsed brain tumors	*PDGFRA* amplification	Pazopanib targeting PDGFRA	No	Patient died before starting targeted therapy
91	RCC	Primary left renal mass[c]	*CDKN2A/2B* 1 copy loss; *PPM1D* frame-shift insertion (p.T506fs); *ASPSCR1-TFE3* fusion		Sunitinib, sorafennib, or pazopanib	*ASPSCR1*-TFE3 fusion	Pazopanib targeting *TFE3* fusion	Yes	Patient receiving pazopanib with stable disease for 10 mo
93	Omental mass	Primary panniculitis with infiltrate, FFPE[c]	*DICER1, FGF7* overexpression	*MITF* (p.E318K), *GJB1* (p.C179Y)	No adjuvant therapy or no genetic counseling	Germline *MITF* (p.E318K)	Genetic counseling for melanoma risk	Yes	Family genetic counseling for melanoma risk; diagnosis of X-linked CMT confirmed
94	ERMS	Primary labia mass[c]	*SMARCB1, BCR, UGT2B17* homozygous deletion; *EZH2* copy loss; *CDK8, FGF11* overexpression	TP53 (p.Y236X)	Palliative cytotoxic chemotherapy or available phase 1 clinical trial for ERMS, genetic counseling	*SMARCB1* homozygous deletion, germline *TP53* (p.Y236X)	CDK46 inhibitor (NCT01747876), family genetic counseling for Li-Fraumani family tumors	Yes	Family genetic counseling confirmed Li-Fraumani syndrome; no actions could be taken; no CDK46 inhibitor available for ERMS clinical trials in children
95	Ovarian small cell carcinoma	Primary left ovarian mass, FFPE[c]	*SMARCA4* (p.K835fs)	*SMARCA4* (p.Q415fs)	Genetic counseling for family members for ovarian tumors	Germline *SMARCA4* (p.Q415fs)	Genetic counseling for family members for ovarian tumors	Yes	Family seen in genetics clinic for counseling for ovarian tumors confirming *SMARCA4* mutation
102	NPCA	Primary nasopharyngeal mass, FFPE[c]	*KRAS* p.G12D mutation, *BRAF* p.G469E mutation		Palliative cytotoxic chemotherapy or Available phase 1clinical trial for relapsed solid tumors	*KRAS* p.G12D mutation, *BRAF* p.G469E mutation	RAF or MEK inhibitor for *BRAF* p.G469E mutation	Yes	Patient on adjuvant RAF inhibitor for 6 mo with no evaluable disease

Abbreviations: ACC, adrenocortical carcinoma; ARMS, alveolar rhabdomyosarcoma; ATRT, atypical teratoid rhabdoid tumor of brain; CNA, copy number alterations; ERMS, embryonal rhabdomyosarcoma; FFPE, formalin fixed paraffin embedded tissue; IF, infantile fibrosarcoma; IMFM, infantile myofibromatosis; indel, insertiondeletion; LN, lymph node; MBL, medulloblastoma; NBL, neuroblastoma; NPCA, nasopharyngeal carcinoma; OS, osteosarcoma; PEcoma, perivascular epithelioid cell tumor; PPB, pleuropulmonaryblastoma; PR, partial remission; RCC, renal cell carcinoma; RMS, rhabdomyosarcoma; SHH, sonic hedgehog; SS, synovial cell sarcoma; WT, Wilms tumor.

[a] Tissues are fresh frozen unless indicated as FFPE. Patient disease status for solid tumors is evaluated by response evaluation criteria in solid tumors (RECIST) 1.1.

[b] Tissue used for sequencing was after relapse or refractory or progressive disease following treatment with surgery, chemotherapy, radiation, or biologic therapy.

[c] Tissue used for sequencing was prior to starting any treatment.

fusion between the *PAX3* gene and the *NCOA2* gene (eFigure 3G in the Supplement) suggestive of a diagnosis of rhabdomyosarcoma (RMS).[25-27] Intracranial RMS is an extremely rare diagnosis (≤0.1% of all intracranial tumors) with a poor prognosis.[28] The diagnosis of RMS was confirmed by using RNA sequencing to evaluate the expression of genes associated with all 4 molecular subgroups of medulloblastoma[29] and genes associated with RMS (ie, *MYOG*, *DES*, and *FGFR4*). We detected extremely high expression of genes associated RMS and low expression for most medulloblastoma-lineage genes (eFigure 3H and eAppendix section IV in the Supplement). Furthermore, tumor stained strongly positive by immunohistochemistry for myogenin, confirming the diagnosis of RMS. The change in diagnosis for this patient resulted in a change of management as well (Table 3). Other solid tumors with potentially actionable findings are summarized in Table 3 and in eAppendix section IV in the Supplement.

Cancer-Related Incidental Germline Findings

By default, participating patients received information about cancer-related incidental genetic findings unless they opted out. Nine patients (10%) had significant incidental germline findings, potentially affecting patients and other family members (Table 3). In 4 of these families, the history was unremarkable for a familial cancer syndrome, and they would never have been otherwise referred for cancer genetics counseling. All 9 patients and families have since undergone formal counseling and genetic screening in our cancer genetics clinic. Specific mutations identified are listed in Table 2. These included mutations associated with established syndromes (*DICER1* syndrome, infantile myofibromatosis, Li-Fraumeni syndrome, and *SMARCA4*-related small-cell ovarian cancer hypercalcemic type) and in more recently described cancer risk genes (*BAP1, BARD1, HOXB13,* and *MITF*) in which cancer risk is less clearly defined.

A case example of actionable germline findings was patient 21, a girl with relapsed metastatic melanoma, in whom the canonical BRAF p.V600E protein mutation was identified, as well as a germline truncation of the BRCA1-associated protein 1 (BAP1, p.D567X) (Table 3; eAppendix Section IV in the Supplement). The BAP1 protein is a tumor suppressor gene implicated in proper BRCA1 protein function. Germline *BAP1* gene variants are implicated in cancer predisposition for malignant mesothelioma, atypical melanocytic tumors, uveal melanoma, and cutaneous melanoma.[30] This patient had a family history of cancer, including her mother who was diagnosed with ovarian cancer when she was 44 years of age. However, she was already seen in the cancer genetics clinic and was screened negative for *BRCA* gene mutations. The patient and her family agreed to be seen in our cancer genetics clinic again, this time for counseling and further testing for the *BAP1* gene in family members.

Clinical Actions Based on Integrative Sequencing

Overall, our study revealed potentially actionable findings in tumor or germline in 42 patients (46%). Among these patients, we were able to act on results for 23 of the 91 patients and families (25%), including changing 14 patients' (15%) treatment, provide genetic counseling for future cancer risk to 9 patients (10%), and both for 1 patient (1%). Nine (10%) of these personalized clinical interventions resulted in ongoing partial clinical remission lasting between 8 and 16 months or in helping sustain complete clinical remission for between 6 and 21 months, whereas in 5 patients (5%), the treatment changes were unsuccessful. All 9 patients and families (10%) with actionable incidental genetic findings agreed to formal genetic counseling and genetic screening. The primary reasons for not being able to act on the potentially actionable findings included (1) patients in clinical remission who were receiving standard therapy and in whom the role of genomically informed adjuvant treatment to prevent relapse is not well defined, and (2) the treating physician thought that no additional therapy was necessary. Other major reasons for not being able to take clinical actions based on molecular findings included limited access to drugs, family or physician preference, or results being available too late in the clinical course to act (Table 2 and Table 3).

Overall Landscape of Molecular Alterations in the Cohort

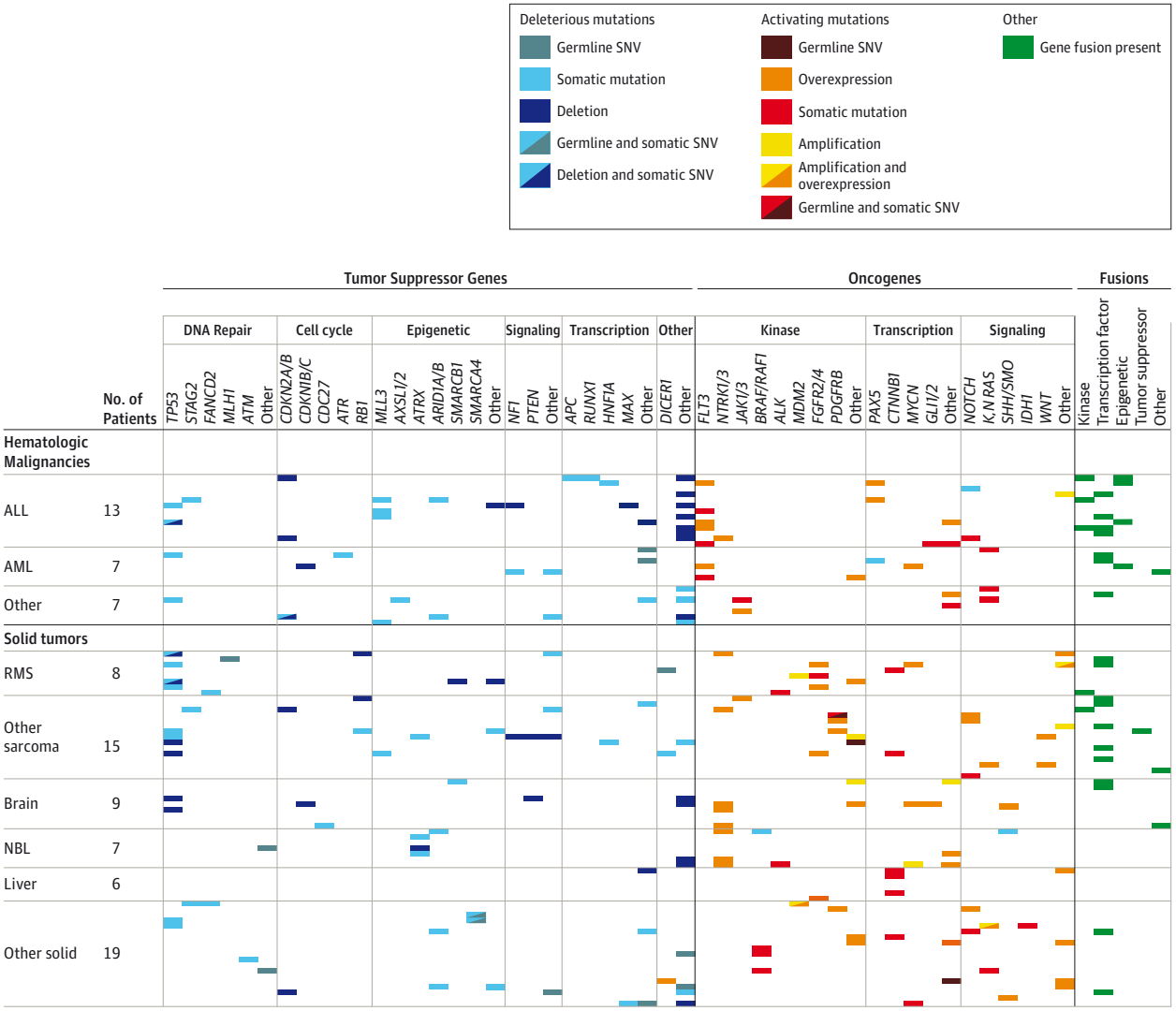
As expected, recurrent driver gene fusions were more prevalent in the hematologic malignancies (57%) vs solid tumors (27%). Among solid tumors, driving gene fusions were most prevalent in sarcomas (**Figure 2** and eFigure 7 in the Supplement). All functional fusions discovered in our study are listed in eTables 5 and 6 and eFigure 8 in the Supplement. An overview of all the classes of aberrations identified in our cohort is shown in eTable 6 in the Supplement. Thirty-six percent of patients exhibited a driving gene fusion, which indicates a potential role of including RNA sequencing (ie, transcriptome sequencing), in addition to whole-exome analysis, in the work-up of individuals with cancer. Furthermore, the presence of actionable germline findings in 10% of patients suggests a role for matched normal sequencing and mandatory genetic counseling in the management of cancer when children and young adults are diagnosed.

Discussion

To our knowledge, this is the first prospective, observational case series exploring the feasibility of integrative clinical sequencing and its potential influence in clinical decision making as well as in the management of cancer in children and young adults. Through the study, we were able to identify actionable findings in 46% of patients and we were able to take clinical actions in 25% of the patients. Overall, 10% of patients showed durable clinical responses. In another 10% of patients and their families, their care was influenced by germline results. To help other clinical sequencing efforts, the clinical protocols and consent documents are available as part of this study (eAppendix in the Supplement).

Our approach facilitated clinical decision making and enabled discovery opportunities. To balance the cost of sequencing, bioinformatics analysis, and likelihood of finding clinically actionable information, we chose to perform both exome and transcriptome sequencing but not whole-genome se-

Figure 2. Summary Results of the Peds-MiOncoSeq Study



A matrix representation of selected informative findings from the sequencing results. The presence of specific mutations, insertion/deletions, amplification/deletions, and gene fusions are indicated by colored blocks. Data are derived from all 91 patients with completed whole-exome as well as transcriptome sequencing of tumors and exome sequencing of germline DNA. Only sequencing findings with biological significance are included. ALL indicates, acute lymphoblastic leukemia; AML, acute meyloid leukemia; indel, insertion/deletion; NBL, neuroblastoma; RMS, rhabdomyosarcoma; SNV, single nucleotide variant.

quencing. We generally achieved more than 150-fold average coverage of the whole exome, which allowed us to detect subclonal populations of approximately 10%. All of the actionable findings we reported for this case series, we believe, are clonal events. Higher depths of sequencing will be required to detect minor subclones, which may affect disease progression and resistance mechanisms. Pediatric cancers have a well-known paucity of recurrent point mutations compared with adult tumors and RNA seq provided valuable insights in our patients' cancers, including structural variations leading to a new diagnosis (*PAX3-NCOA2*), novel gene fusions (*NTRK1*), and new treatment options (*ETV6-ABL1, TFE3*, and *ALK*).[31-34] These RNA sequence discoveries alone accounted for approximately 20% of the actionable findings in our study, which would have been missed otherwise.

This study also used germline sequencing, which led to about 10% of patients and families receiving formal genetic screening for familial cancer syndromes based on significant actionable incidental findings revealed by our study. Many of these families had no significant family history and would likely have not been referred to genetic counseling under routine clinical care. Eighty-nine percent of patients and families opted for disclosure of incidental genetic results, which is consistent with other studies examining parent preferences for return of research results.[35] Genetic counseling was required as an integral part, along with follow-up in our cancer genetics clinic for significant incidental genetic findings. This was well received by our participants and will be important for the future because understanding how to optimally inform families of the risks of clinical sequencing is receiving increased at-

tention from both bioethicists and empirical researchers.[36-38] Pediatric cancers, particularly leukemias after undergoing an allogenic transplant, have the added challenge of deciphering somatic alterations in the background of donor-derived cells, best exemplified by patient 3 in our study.

A key feature of this study is that we used a multidisciplinary precision medicine tumor board, which discussed, critiqued, and deliberated on genomic findings as well as assessed the feasibility of pursuing actionable findings when applicable. We believe that the unique expertise assembled on our board allowed it not only to deliberate on the scientific merit of actionable genomic findings but also to discuss possible logistical and ethical issues before sharing the existence of candidate clinical trials, potential off-label use of approved agents, and age-dependent dosing of agents with the treating team.

Furthermore, this study identified several findings that warrant further characterization and may, in some cases, suggest novel directions for research in translational science and experimental therapeutics. Among the observations of interest were an *ALK* gene fusion in rhabdomyosarcoma, a new *NTRK1* gene fusion in infantile fibrosarcoma, and a novel *YAP-MAML2* gene fusion in meningioma. This study also identified several patients with disruption of SWI/SWNF chromatin modifiers (*ARID1A/B*, *SMARCB1,* and *SMARCA4*) and tumor suppressors (CDKN2A/B and CDKN1B/C) implicating these genes in the pathogenesis of a wide variety of pediatric tumors.

This study had several limitations, many of which were inherent to the study design. A major limitation was the single institution, observational nature of the study, which did not include a control group, which limited our ability to ascertain whether the treatment changes based on the study actually improved clinical outcome compared to standard of care. In addition, several of the patients were sequenced at the time of relapse using original diagnostic material, which we realize is not ideal because it is well documented that tumors do evolve in response to treatment.

Another limitation of the study was the lack of drugs for the pediatric population, either from a dearth of clinical trials or the lack of available drugs indicated for off-label use. This was especially true in very young patients, for whom treatment formulations and dosing uncertainty created an additional barrier in using off-label agents. Although this is not surprising given the smaller number of investigational agents and clinical trials available for pediatric patients, mostly available through major consortia, it nevertheless prohibited several patients from potentially benefiting from actionable sequencing findings, for which there are drugs available for adults. Additionally, we identified aberrations in multiple pathways, which will likely require combining multiple targeted agents (with or without chemotherapy) in order to have a meaningful effect on clinical outcome.[39] Finally, longer-than-expected sequencing turnaround time also limited our ability to take clinical actions in many cases. Improvements in turnaround time can be anticipated in the future through the incorporation of rapid sequencing modes, newer streamlined library preparation and capture protocols, and the use of cloud-based computing resources for higher throughput analyses. Together, these improvements may reduce turnaround time to 2 weeks or less.

Conclusions

In this single-center case series involving young patients with relapsed or refractory cancer, incorporation of integrative clinical sequencing data into clinical management was feasible, revealed potentially actionable findings in 46% of patients, and was associated with change in treatment and family genetics counseling for a small proportion of patients. The lack of a control group limited assessing whether better clinical outcomes resulted from this approach than outcomes that would have occurred with standard care.

ARTICLE INFORMATION

Author Affiliations: Department of Pediatrics, University of Michigan, Ann Arbor (Mody, Frank, Innis, Robertson, Yanik, Connelly, Choi, Harris, Rao, Levine, Castle, Hutchinson); Michigan Center for Translational Pathology, University of Michigan, Ann Arbor (Mody, Wu, Lonigro, Cao, Vats, Prensner, Asangani, Palanisamy, Ning, Su, Wang, Robinson, Chinnaiyan); Comprehensive Cancer Center, University of Michigan, Ann Arbor (Mody, Lonigro, Robertson, Yanik, Connelly, Choi, Harris, Kitko, Rao, Levine, Castle, Hutchinson, Talpaz, Chinnaiyan); Department of Pathology, University of Michigan, Ann Arbor (Wu, Cao, Asangani, Rabah, Kunju, Robinson, Chinnaiyan); Howard Hughes Medical Institute, University of Michigan, Ann Arbor (Cao, Chinnaiyan); Department of Internal Medicine, Ohio State University, Columbus (Roychowdhury, Talpaz); Department of Pediatrics, Boston Children's Hospital, Boston, Massachusetts (Prensner); Department of Radiology, University of Michigan, Ann Arbor (Dillman); Department of Internal Medicine, University of Michigan, Ann Arbor (Everett, Raymond, Stoffel); Department of Health Behavior and Health Education, University of Michigan, Ann Arbor (Roberts); Department of Pediatrics, Michigan State University, East Lansing (Chamdin); Department of Urology, University of Michigan, Ann Arbor (Chinnaiyan).

Author Contributions: Drs Mody and Chinnaiyan had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Drs Mody and Wu are co-first authors. Drs Robinson and Chinnaiyan are co-senior authors.
Study concept and design: Mody, Wu, Roychowhury, Rabah, Raymond, Ning, Su, Roberts, Robertson, Robinson, Chinnaiyan.
Acquisition, analysis, or interpretation of data: Mody, Wu, Lonigro, Cao, Roychowhury, Vats, Frank, Prensner, Asangani, Palanisamy, Dillman, Rabah, Kunju, Everett, Raymond, Wang, Stoffel, Innis, Robertson, Yanik, Chamdin, Connelly, Choi, Harris, Kitko, Rao, Levine, Castle, Hutchinson, Talpaz, Robinson, Chinnaiyan.
Drafting of the manuscript: Mody, Wu, Lonigro, Vats, Frank, Prensner, Rabah, Raymond, Ning, Su, Robinson, Chinnaiyan.
Critical revision of the manuscript for important intellectual content: Mody, Wu, Cao, Roychowhury, Asangani, Palanisamy, Dillman, Rabah, Kunju, Everett, Raymond, Frank, Wang, Stoffel, Innis, Roberts, Robertson, Yanik, Chamdin, Connelly, Choi, Harris, Kitko, Rao, Levine, Castle, Hutchinson, Talpaz, Robinson, Chinnaiyan.
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Study supervision: Mody, Wu, Kunju, Stoffel, Innis, Robinson, Chinnaiyan.

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