Multi-Dimensional Omics for Precision Therapy of Children and Adolescent Young Adults with Relapsed and Refractory Cancer: Report from Pediatric Oncology Branch, NCI

Wendy Changabc, wendy.chang@nih.gov
Andrew S. Brohld, andrew.brohl@moffitt.org
Rajesh Patidarh, rajesh.patidar@nih.gov
Sivasish Sindiria, sivasish.sindiri@nih.gov
Jack F. Shernab, john.shern@nih.gov
Jun S. Weiia, weij@mail.nih.gov
Young K. Songa, songyo@mail.nih.gov
Marielle E. Yoheabc, marielle.yohe@nih.gov
Berkley Grydera, berkley.gryder@nih.gov
Shile Zhanag, shilez@gmail.com
Kathleen A. Calzonei, calzonek@mail.nih.gov
Nityashree Shivaprashad, shivaprasadn@mail.nih.gov
Xinyu Wena, wenxi@mail.nih.gov
Thomas C. Badgett, tom.badgett@uky.edu
Markku Miettineng, markku.miettinen@nih.gov
Kip R. Hartmanhi, hartmankr2@gmail.com
James C. League-Pascualh, james.league-pascual@nih.gov
Toby N. Trahairj, toby.trahair@sesias.health.nsw.gov.au
Brigitte C. Widemannb, widemanb@mail.nih.gov
Melinda S. Merchantb, melinda.merchant@astrazeneca.com
Rosandra N. Kaplank, rosie.kaplan@nih.gov
Jimmy C. Linh, jimmy.lin2@nih.gov
Javed Khan#, khanjav@mail.nih.gov

a Oncogenomics Section, Genetics Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health. Bethesda, MD 20892, USA
b Pediatric Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health. Bethesda, MD 20892, USA
c Department of Pediatrics, Molecular Genetics, Columbia University Medical Center. New York, NY 10032, USA
d Sarcoma Department, Moffitt Cancer Center. Tampa, FL 33612, USA
e Genetics Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health. Bethesda, MD 20892, USA
f Pediatric Hematology-Oncology, Kentucky Children’s Hospital. Lexington, KY 40536, USA
g Laboratory of Pathology, Center for Cancer Research, National Cancer Institute. Bethesda, MD 20892, USA
h Walter Reed National Military Medical Center. Bethesda, MD 20889, USA
i Uniformed Services University of the Health Sciences. Bethesda, MD 20814, USA
j Centre for Children’s Cancer and Blood Disorders, Sydney Children’s Hospital. Randwick, New South Wales, Australia.

#corresponding author: Oncogenomics Section, Genetics Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, 37 Convent Drive, Room 2016B, Bethesda, MD 20892, USA. khanjav@mail.nih.gov

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Running Title: Multi-Dimensional ClinOmites for Precision Therapy
Statement of Translational Relevance

This study demonstrates the utility of a multi-dimensional genomics platform including whole exome sequencing (WES), whole transcriptome sequencing (WTS), and high-density single nucleotide polymorphism array in the management of children and adolescent young adults with high risk, refractory or relapsed cancers. We show that 51% of the patients have clinically actionable mutations and 12% have significant reportable germline mutations. Our study provides a roadmap for integrative genomics analysis as well as a robust bioinformatics and reporting pipeline for future precision therapy protocols for adults and children with cancers.
Abstract

Purpose

We undertook a multi-dimensional clinical genomics study of children and adolescent young adults with relapsed and refractory cancers to determine the feasibility of genome guided precision therapy.

Experimental Design

Patients with non-central nervous system solid tumors underwent a combination of whole exome sequencing (WES), whole transcriptome sequencing (WTS), and high-density single nucleotide polymorphism array analysis of the tumor, with WES of matched germline DNA. Clinically actionable alterations were identified as a reportable germline mutation, a diagnosis change, or a somatic event (including a single nucleotide variant, an indel, an amplification, a deletion, or a fusion gene), which could be targeted with drugs in existing clinical trials or with Food and Drug Administration approved drugs.

Results

Fifty-nine patients in 20 diagnostic categories were enrolled from 2010 to 2014. Ages ranged from 7-months-old to 25-years-old. Seventy-three percent of the patients had prior chemotherapy, and the tumors from these patients with relapsed or refractory cancers had a higher mutational burden than that reported in the literature. Thirty patients (51% of total) had clinically actionable mutations, of which 24 (41%) had a
mutation that was currently targetable in a clinical trial setting, 4 patients (7%) had a change in diagnosis, and 7 patients (12%) had a reportable germline mutation.

Conclusions

We found a remarkably high number of clinically actionable mutations in 51% of the patients, and 12% with significant germline mutations. We demonstrated the clinical feasibility of next generation sequencing in a diverse population of relapsed and refractory pediatric solid tumors.
Introduction

While clinical genomic analysis of tumors has been increasingly used to guide cancer care in adults, its efficacy in the pediatric setting is still under investigation. The genomic landscape of pediatric cancers at diagnosis has been noted as having a lower mutational burden than adult cancers (1-5). Current standard of care chemotherapy is particularly inadequate for the 30-40% of pediatric solid tumor patients who have metastatic, refractory or relapsed disease. Given the need for improved therapeutic strategies for these patients, we undertook a study to determine the utility and feasibility of performing comprehensive genomic analyses to identify clinically actionable mutations in pediatric and young adult patients with refractory or relapsed solid tumors.

Identification of somatic genomic events beyond single nucleotide variations (SNVs) increasingly plays a role in the diagnosis and prognosis of pediatric tumors. For example, the presence of the PAX3-FOXO1 fusion in rhabdomyosarcoma not only contributes the diagnosis of fusion-positive rhabdomyosarcoma, but also imparts a poorer prognosis (6, 7). In neuroblastoma, MYCN amplification status places patients in the high-risk stage, which leads to a significant change in clinical management (8). The paucity of actionable mutations is a reflection of the low somatic mutational burden in pediatric cancers, which is in direct contrast to adult oncology where multiple reports have described a high percentage of tumors with actionable mutations (9).

In this pilot study, we performed a multi-dimensional comprehensive genomics analysis of patients referred to the Pediatric Oncology Branch (POB) of the National Cancer Institute’s (NCI) Center for Cancer Research (CCR). This included whole exome research.
sequencing (WES), whole transcriptome sequencing (WTS) of the tumor, WES of matched germline DNA, and high-density single nucleotide polymorphism (SNP) array analysis of tumor. Our goal was to identify actionable genomic alterations defined as a reportable germline mutation, a change of diagnosis, or a somatic event, which can be targeted with drugs in existing clinical trials or with drugs approved by the Food and Drug Administration (FDA). In particular, we determined the clinical utility of a multi-genomics platform to alter the management of children and young adults with relapsed and refractory cancers.

Patients and Methods

Patients

Patients with pediatric non-central nervous system (CNS) solid cancers that were undergoing biopsies for clinical indications on treatment protocols at the POB, of the CCR, or at our collaborative institutes, were enrolled in our open-ended tumor profiling/specimen repository protocol (NCT01109394). Written consent was obtained from the patients or from their legal guardian if patients were minors. Tumor tissue was only obtained if there was sufficient tissue available after clinical management needs of the patient were met. All patients had matched whole blood collected. The protocol was approved by the Institutional Review Board at the NCI, at the National Institutes of Health (NIH). Samples were de-identified after clinical information and histologic diagnoses were compiled. Quality control genotyping for the samples was performed to ensure the match of tumor/normal pairs.
Genome sequencing and analysis

Are detailed in Supplementary Methods.

Actionable Mutations

We defined actionable mutations as: 1) a reportable germline mutation including nonsense or frameshift indels of a cancer consensus gene or pathogenic or likely pathogenic mutation of an American College of Medical Genetics (ACMG) gene (11, 12), 2) genomics alterations that changed the patient’s diagnosis, or 3) a somatic event (including single nucleotide variant, indel, amplification, deletion, or a fusion gene), which may be targeted and patient treated with FDA approved drugs or in the context of existing clinical trials according to the NCI-adult MATCH- Criteria (Supplementary Table 1). All actionable SNVs and indels reported in this manuscript were confirmed firstly by visualization on an IGV viewer, then by Sanger sequencing in a research setting. All actionable mutations for which clinical decisions were made the mutations were validated with the same tumor from the pathology laboratory and the sequencing was performed in a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory.

Results

Patient Demographics

We present a unique cohort of 59 pediatric patients with solid tumors outside of the CNS, who were referred to the NIH from 2010 to December 2014. A total of 64
children and adolescent young adults (AYA) with non-CNS solid tumors had sufficient tumor tissue and blood available for genomic analysis and were consented or enrolled on NCT01109394. Of these, 59 (92%) had successful complete multi-dimensional genomics performed on the tumor and germline DNA. Three patients were excluded due to partial completion of the multi-omics studies. Two patients were initially sequenced using older poor quality NGS technology, resulting in insufficient material available for the newer sequencing technologies. Patient demographics of this cohort are summarized in Supplementary Table 2. The median age at biopsy was 15 years, ranging from 7-months to 25-years-old. Gender distribution was 39% (n=23) female and 61% (n=36) male. Importantly, our cohort contained 20 different clinically aggressive solid tumor diagnoses reflecting the protocols and trials that were open at the POB during recruitment for this study. The most common diagnoses in our cohort were Ewing sarcoma (n=10, 20%), and neuroblastoma (n=10, 20%). Seventy-three percent (n=43) of the patients had received chemotherapy prior to enrolling in this study and were referred for clinical trials involving novel agents. Lastly, 15% (n=9) of the cases had multiple metastases from the same time point and 8% (n=5) of the cases included tumors from sequential time points (Supplementary Table 2).

**Multidimensional Genomics Platform: General Somatic Discoveries**

On average, WES generated 86 million reads per sample to a median depth of 68X (mean depth of 75X), and WTS had an average 227.6 million reads per sample (Supplementary Table 2). We identified a total of 703 high confidence SNVs and 67 indels in our dataset (Supplementary Table 2) with a median of 8 (range 0-387, average 22.2) somatic mutations per exome pair.
The number of somatic point mutations differed by diagnosis: metastatic melanoma had the highest number of somatic variants per sample, while atypical teratoid rhabdoid tumors and fusion gene-driven cancers had the lowest number of SNVs per sample, similar to what has been described in the literature (Fig. 1) (1, 4, 5). In keeping with previous genomics studies of relapsed pediatric cancers by our group and others, mutations increased with respect to tumor time (Fig. 1) (17-19). For neuroblastoma, we found a median somatic mutation relapse rate of 28.5 in the exome, which is about twice the median of 12-18 previously reported (2, 20). For Ewing sarcoma, we previously reported an average mutation rate of 6-7 somatic protein altering mutations per tumor, whereas in our current study with relapsed samples, we found approximately 3 times the rate with an average somatic protein altering mutation rate of 15. Of interest when a transcriptome filter with a variant allele frequency (VAF) of 10% was applied to somatic WES mutations, across the samples we found that 51% of the DNA mutated genes were expressed at the transcript level. In 44% the somatic variants were expressed, and in 7% only the reference alleles were expressed (Fig. 1). We and others have reported the same phenomenon that approximately 50% of DNA somatic mutations are expressed in the RNA (22, 23).

Overall in our index cases, we found 125 somatic alterations in known cancer consensus genes. This included 12 chimeric genes, 54 non-synonymous SNVs in 42 genes, 8 truncating or frameshift mutations including stopgains or indels in 7 genes, 6 amplifications in 2 genes, 4 homozygous gene deletions in 2 genes, and 27 cases of loss of heterozygosity in 5 genes (Fig. 2). The somatic SNVs were enriched for cancer pathways including signaling, transcription factors, splicing, DNA repair and epigenetic
modifiers. As expected from the demographics of children and AYA patients seen at the POB, who were primarily diagnosed with sarcomas, 26 (44%) of the patient’s tumors had gene fusions. All were identified with high confidence with exact breakpoint detection, placing WTS as an appropriate method for detection of these diagnostic fusion driver genes (Fig. 2 and Supplementary Table 2).

**Actionable Genomic Alterations**

1) **Reportable Germline Mutations**

In the germline, 12 patients (20%) had nonsense or frameshift indels of cancer genes, with one patient harboring a non-frameshift 2 base substitution, and an additional 5 patients with rare non-synonymous SNVs. We found a total of 20 alterations in 18 genes in 16 patients (Fig. 2 and Supplementary Table 2). All but one was validated by orthogonal sequencing methods. Overall 9 alterations in 8 genes (*ATM*, *BRCA1*, *PMS2*, *PTEN*, *RET*, *TP53*, *TSC1*, and *TSC2*) in 7 (12%) patients were considered reportable (Table 1). However, only five of these alterations would have been reported with strict adherence to the ACMG guidelines (Supplementary Table 1) (11, 12). In an adolescent patient with melanoma (NCI0072), we discovered a germline frameshift mutation in *ATM*, but interestingly, this patient’s tumor had only one detected somatic alteration of *BRAF* (V600E), which is a known driver mutation. A second patient with metastatic congenital melanoma (NCI0211) had germline mutations in *TSC1* and *TSC2* and no somatic mutations. Although neither of these germline mutations alone was considered reportable by ACMG guidelines because these are variants of unknown significance, the mutation found in the *TSC2* gene (T246A; see Table 1) is reported as a Human Gene Mutation Database (HGMD) disease-causing mutation (CM087814 (24,
Taken together, although the overall effect of these two mutations in combination was unknown, we considered them reportable. Our results indicate that children with melanomas who have germline mutation have a low somatic burden compared with the high mutational burden seen in adult melanomas.

Two patients (NCI0152, and NCI0226) had germline TP53 mutations, and had loss of heterozygosity (LOH) with complete loss of the wild type allele in the tumor, rendering the mutations likely pathogenic and reportable. The latter presented with a diagnosis of metastatic adrenocortical carcinoma, in which TP53 mutations are considered the driver (26). Sequencing of a sibling and both parents showed this to be a de-novo germline mutation (Table 1 and Supplementary Fig. 2). The remaining ACMG reportable mutations were found in a patient with neuroblastoma (NCI0010) that had frameshift mutations in two cancer predisposition genes: BRCA1 (ovarian and breast cancer) and PMS2 (Lynch syndrome and mismatch repair cancer syndrome) (27, 28). Another patient with a neuroendocrine tumor (NET) had a frame shift mutation in PTEN (R14fs) which is associated with hamartoma tumor syndromes. The final patient with medullary thyroid carcinoma had a well-described mutation in RET (M918T) associated with multiple endocrine neoplasia 2B (29). In summary, we report 7/59 (12%) patients with reportable germline mutations, which is considerably higher than the 2-4% in expected incidental findings, but corresponds to a similar rate described in recently published reports in germline mutations for both cancer predisposition genes in pediatric cancer and in whole exome sequencing of trios across clinical indications (30, 31).
2) Changes in Diagnosis

Cancers of specific types have diagnostic gene expression signatures, as reported by our group and others (32). By hierarchical clustering of the 2,000 most highly differentially expressed genes, we found all the cancers clustered with their own subtype, with the exception of alveolar soft part sarcomas, which clustered with renal cell carcinoma samples (Supplementary Fig. 3). Interestingly, both of these tumors shared a common oncogenic fusion gene, *ASPCR1-TFE3*, indicating that gene caused a dominant gene expression signature, which has important biological implication. A tumor from a second patient (NCI0108) who was initially referred to the NIH with a diagnosis of neuroblastoma clustered with neuroendocrine tumors. A change in diagnosis was histologically confirmed after anatomic pathology review.

Inspection of the fusion genes identified two unexpected diagnostic fusions. One was a *CIC-FOXO4* in a patient (NCI0165) presenting to the NCI with a widely metastatic scalp Ewing sarcoma, whose diagnosis was changed to an Ewing-like sarcoma, as previously reported by our group (4). A second fusion was found in a patient presenting with a clear cell sarcoma of the kidney whose diagnosis was revised to an undifferentiated sarcoma following the identification of *BCOR-CCNB3*. This fusion gene has been reported in patients with Ewing-like and undifferentiated sarcomas (33). In addition to the presence of novel fusions, the absence of a diagnostic fusion gene resulted in a change in diagnosis, as seen in patient NCI0152, who was initially diagnosed with a high-grade fibrous histiocytoma, which was then changed to a synovial sarcoma by histopathology after a hemipelvectomy. Our analysis revealed the absence of a pathognomonic synovial sarcoma *SS18-SSX* fusion gene. Instead, we
identified a germline TP53 (p.R175H) mutation. This mutation has been previously described in a variety of sporadic tumors and was found as a germline mutation in 2 patients with osteosarcoma (34, 35). The patient’s diagnosis was subsequently changed to an undifferentiated sarcoma.

3) Linking Mutation to Drug

We next applied the NCI-Adult MATCH criteria (Supplementary Table 1) to match mutations to drugs for patients enrolled in our study. We found 4 (12%) matched at Level 1, thirteen (41%) at Level 2, and 15 (47%) at Level 3 (Table 2). Overall we found a remarkably high drug-match of 32 genes (14 unique) in 24 (41%) patients in our population. This included alterations in 14 genes (BRAF, ALK, RET, PIK3CA TSC1, TSC2, GNAQ, GNA11, CDKN2A, MYCN, PTEN, SMARCB1, STAG2, and IDH1) matching to 17 drugs or classes of drugs (36-42). Of note, two of these patients had germline mutations in TSC1 and TSC2 (NCI0211), and RET (NCI0228) that would make them eligible for the NCI Adult-MATCH trial; as germline DNA is not currently sequenced on their study, and it is not possible to distinguish germline from somatic mutations if only the tumor DNA is sequenced. We found that for the identification of these targetable mutations, the combination of WES and WTS was critical and confirmatory in 22/32 (69%) matches where we required the somatic driver variant to be expressed in the RNAseq experiments. SNP arrays in combination with WTS were used to match 9/32 (28%) drugs in the cases of homozygous loss or amplification to result in gene expression suppression or overexpression. Finally, in one case, WTS alone identified the actionable driver fusion gene RANBP2-ALK. For 14/32 (44%) of the matches, a pediatric trial is currently open, which would have enabled patients to be
diverted to specific molecularly targeted therapy. For 16/32 (50%) of the matches, there was an FDA approved drug available with an adult indication.

**Sequencing of Multiple Tumor Samples per Patient in Relapsed and Refractory Pediatric Tumors**

In our cohort, we had 13 patients who had multiple biopsies performed. Of these patients, 9 samples were obtained from multiple metastases from the same time point and 5 biopsies were from different sequential time points (Supplementary Table 2). We found that every tumor sample from the same patient shared a set of common mutations (average 67.5% overlap; Fig. 3 and Supplementary Table 2), demonstrating that these tumors shared a common ancestral clone in the patient. Eight of the 14 cases were fusion-positive sarcomas, and the identical fusion was present in the matched samples. There were a significant number of non-overlapping mutations in the matched samples, and the majority of the driver mutations were common. The development of new mutations during tumor progression has been previously reported by our group, where we showed that RAS pathway mutations were enriched for in the relapsed setting, and that many of these mutations were not present in diagnostic samples, indicating tumor evolution or selection during tumor progression (17). Similarly, in our study, there was one notable example (NCI0167), who presented with a refractory Ewing sarcoma lung metastasis, which was fully resected and found to have two likely driver mutations, an *EWSR1-FLI1* fusion and a *PIK3CA* (p.D1017G) somatic mutation. After 16 months of treatment on a vaccine trial, the patient relapsed in the lungs
bilaterally, but both relapsed tumors lacked the \textit{PIK3CA} mutation. However, the \textit{EWSR1-FLI1} fusion transcript in both metastases remained identical to the initial fusion.

\textbf{Responses to Targeted Therapy and Development of Resistance}

We present two vignettes to demonstrate the potential utility of integrated genomic analysis of patient tumors at initial diagnosis and relapse to guide therapy decisions. Patient NCI0244 was enrolled in our study at the time of a second relapse, at which time frozen tumor tissue from the first and second relapse was also available for genomic analysis (Supplementary Fig. 4A). The patient originally presented with abdominal distention and was found by laparotomy to have mesenteric and omental tumors. The patient was diagnosed with epithelioid inflammatory myofibroblastic sarcoma (IMT), positive for ALK expression by immunohistochemistry and a \textit{RANBP2-ALK} fusion gene was confirmed by fluorescent in-situ hybridization (FISH)(43). Crizotinib therapy was initiated. Follow up imaging with positron emission tomography and computerized tomography (PET/CT) scans 8 months later documented a complete metabolic and anatomic response. Crizotinib monotherapy was continued for another 6 months, at which time the patient relapsed and was switched to ceritinib (Supplementary Fig. 4A). WTS of both recurrent tumors demonstrated the presence of the \textit{RANBP2-ALK} fusion. In addition, WTS and WES showed that both relapsed tumors acquired a secondary mutation in the \textit{ALK} coding region, c.T3512C, p.I1171T (Supplementary Fig. S4A and Supplementary Table 2). Both the fusion gene and the \textit{ALK} c.T3512C mutation was confirmed by RT-PCR and Sanger sequencing. Of note, the fusion gene was present by RT-PCR in a cell line derived from the tumor at initial
diagnosis, but the ALK I1171T mutation was absent by Sanger sequencing, indicating that mutation arose during therapy with crizotinib. This mutation has been previously described to occur in the setting of post-treatment tumor recurrence with resistance to the specific tyrosine kinase inhibitor crizotinib (44, 45). Corresponding tumor shrinkage and a reduction in radiolabeled-2-fluoro-2-deoxy-D-glucose (FDG) uptake were documented one month after treatment initiation with ceritinib. Despite the initial rapid response, local disease progression occurred after 2 months of treatment. A liver metastasis taken post-ceritinib treatment contained the same ALK mutation (c.T3512C) but no other somatic mutations were identified that could be implicated in the relapse. The patient passed away two years after initial commencement of crizotinib therapy.

In our second case, Patient NCI0155 was initially diagnosed with melanocytic neuroectodermal tumor, but the diagnosis was changed to melanoma after histological examination upon progressive disease following cytotoxic chemotherapy. The primary tumor originated from the left frontotemporal scalp and left orbit and was metastatic to regional lymph nodes, dura mater, liver, bone and lung at diagnosis. WES and WTS of the metastatic tumor revealed a GNAQ (Q209L) mutation (present in tumor DNA and RNA). The mutation, previously described as a common driver mutation of uveal melanoma and an uncommon driver of cutaneous melanoma (46), was confirmed in a CLIA-certified laboratory. The decision to treat with trametinib, a MEK inhibitor approved for use in adult uveal melanoma, was made at this time, and the patient was given one month of bridging therapy with vorinostat while awaiting the availability of trametinib. There was an initial mixed response to trametinib (Supplementary Fig. 4B). However, progressive disease of the primary tumor occurred within 5 months after initiating
therapy, and the patient was then taken off trametinib therapy to receive adoptive cell therapy on another clinical trial.

Our two clinical vignettes highlight the importance of multi-genomics analyses at initial presentation and disease progression to identify driver mutations at diagnosis and during the emergence of resistant clones.

**Discussion**

In this study, we developed a robust multi-dimensional genomics platform to comprehensively interrogate the germline and cancer genome, to determine if the management of children with high risk, metastatic refractory or relapsed cancers can be considerably altered on the basis of these assays. We used a combination of exome sequencing for both the tumor and matched normal tissue, high resolution copy number analysis using SNP arrays, and whole transcriptome sequencing for the tumor. We defined actionable mutations as a reportable germline mutation, a change in diagnosis, or a somatic or germline mutation that can be targeted with the use of existing drugs.

We found a high percentage of reportable germline mutations in 12% of patients, which underlies the importance of performing germline WES. This high germline mutation rate is perhaps not surprising given that these cancers occur at an early age, and are often clinically aggressive. Our results are in accordance with a recent pediatric study which reported that 8.5% of children with cancer have pathogenic or likely pathogenic germline mutations (30). We were unable to confirm if the majority of the germline mutations in our patients were *de-novo* or inherited, but our findings emphasize the importance of conducting future large-scale familial cancer studies for all patients.
children presenting with cancer.

Pediatric cancers have been noted to have a generally quiet genome with a low somatic burden in comparison to their adult counterparts (4, 5, 20, 47). However, the majority of genomic studies have been performed on diagnostic pre-treatment tumor samples. In this study we found, in keeping with two recent studies in neuroblastoma, refractory or relapsed pediatric cancers not only have an increased number of mutations but also contain a higher percentage of actionable somatic mutations, coming close to the number found in adult cancers at diagnosis (17, 19, 47). Our findings also concur with another recently published study in the sequencing of relapsed cancers in young patients from a single center case series (48).

Overall, we found that the majority of patients had at least one mutation that was a previously described oncogenic driver. Furthermore, we found a total of 40 clinically actionable mutations in 30 patients (51% of total), including germline findings, changes in diagnosis, and possible application of targeted therapy. Of these, the combination of WES with WTS was important for identifying 67.5%, SNP arrays for 24.3%, and WTS alone for 8.2%. Our WTS results showed that about one-half of all SNVs are not expressed in the transcriptome, and thus can be excluded as a driver mutation. We and others have reported the same phenomenon that approximately 50% of DNA mutations are expressed in the RNA (22, 23). The cause is usually that the RNA transcript, and hence, the gene, is not expressed at the RNA level. In a small fraction of somatic mutations (7%) only the reference allele is expressed. This emphasizes the need for WTS as an important confirmatory assay for all samples, either by demonstrating the
expression of a somatic SNV, by showing loss of expression of a tumor suppressor, by displaying increased gene expression levels with amplification, or by exhibiting a change in gene expression profiles. WTS is also a sensitive and specific method to identify diagnostic, novel, and druggable fusion genes.

We show here that the use of WES as part of a comprehensive multi-omics platform allows for the detection of unexpected actionable germline and somatic tumor mutations which may be missed if panel sequencing or single-omics platforms are utilized. As the cost of sequencing drops, it will become feasible to perform WES for all patients with cancer, and many centers are rapidly moving to whole genome sequencing in combination with WTS for a more comprehensive analysis of the germline and cancer genome.

To identify targetable mutations, we used the criteria from the currently open National Cancer Institute Molecular Analysis for Therapy Choice (NCI-MATCH) tier system for adult patients. This classification has been built to be flexible as additional medications attain FDA approval, and new preclinical drugs emerge on the experimental horizon. By using the NCI Adult-MATCH criteria as a reference for matching mutation to drug, 24 patients (41%) were considered to have a mutation that was currently targetable in a clinical trial. Of these, 12% matched at level 1 in which the gene variant is approved for selection of an approved drug (e.g. BRAF V600E and vemurafenib). Forty-one percent matched at level 2a where the gene variant is an eligibility criterion for an ongoing clinical trial. The majority, 47%, matched at level 3 where preclinical data in either in vivo or in vitro models have previously provided...
biological evidence sufficient to support the use of a variant for treatment selection, but response to such therapy is currently unknown. All three levels of evidence are currently being utilized in current clinical trials in adults with cancer; however, this is proposed to be tested using similar levels of evidence in a pediatric MATCH, soon to open with the Children’s Oncology Group.

The Adult-MATCH trial does not perform germline evaluation and thus, the patient with a \textit{RET} mutation (NCI0228; medullary thyroid carcinoma) would have been eligible for the trial, since it would have been unknown if these mutations arose as somatic events or were germline. The actionability of the germline mutations in \textit{TSC1} and \textit{TSC2} (NCI0211; malignant melanoma) is controversial. In the setting of a congenital melanoma with a low somatic mutational burden and no other actionable mutations, combined with predicted damaging germline mutations in two \textit{TSC} genes, in which one (\textit{TSC2}), was reported as a causal mutation in HGMD (CM087814), we concluded this would be considered actionable.

Our study highlights the lack of drugs available for the majority of the somatic mutations that are detected in high confidence but are mutations of unknown significance. Nevertheless, 44\% of the gene-matches have a pediatric trial that is currently open, which could potentially enable precision therapy to be given to these identified patients. As many as 50\% of the matches had an FDA approved drug available with an adult indication, which can potentially be tested in future clinical trials.

On the basis of this pilot study, the CCR has established the ClinOmics program to provide a multi-dimensional genomics platform to enable precision therapy trials in
children and adults with cancer enrolled on NCI trials (Supplementary Fig. 5). The infrastructure under the ClinOmics program will provide an umbrella protocol to identify actionable germline and somatic alterations in a patient within a CLIA-certified environment, which will be evaluated in germline genetics and molecular tumor boards, and then reported into the electronic medical records. In our feasibility study, our multi-omics analysis was performed using fresh frozen tumors, which can cause potential problems due to normal tissue contamination. Ongoing clinical studies including the NCI-MATCH trial and the planned ClinOmics trial will utilize DNA and RNA macro-dissected from FFPE tissues verified by pathologists to contain high tumor content. This, together with deep sequencing, will allow for the detection of sub-clonal mutations as low as 5%.

Although the use of these comprehensive genomic analyses of germline and tumor will likely identify actionable genomic alterations, single agent targeted monotherapy may prolong survival, but is unlikely to be curative, as shown in our two clinical vignettes. This underlies the importance of combination gene-drug matching, paired with conventional therapy, both at diagnosis of high-risk metastatic cancers, as well in the refractory and relapsed setting. Nevertheless, our findings demonstrate that multi-dimensional omics profiling in cancer is not only feasible, but also will likely have high diagnostic, therapeutic, and scientific yield for both adults and children with cancer.

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Table and Figure Legends

Figure 1.

Total and expressed single nucleotide variants (SNVs) in tumor samples. Red bars indicate expressed somatic SNVs, turquoise bars denote gene not expressed, and green show the reference allele only was expressed. Horizontal dotted lines show the previously reported mean of total exonic SNVs by tumor diagnosis in literature. The black triangle represents a relapsed or refractory tumor sample. MM, malignant melanoma; TCC, transitional cell carcinoma; NET, neuroendocrine tumor; OS, osteosarcoma; FN-RMS, fusion-negative rhabdomyosarcoma; FP-RMS, fusion-positive rhabdomyosarcoma; US, undifferentiated sarcoma; DSRCT, desmoplastic small round cell tumor; MEC, myoepithelial carcinoma; CCS, clear cell sarcoma; SS, synovial sarcoma; MRT, malignant rhabdoid tumor; WT, Wilms tumor; ACC, adrenocortical carcinoma; ASPS, alveolar soft part sarcoma; MTC, medullary thyroid carcinoma; IMT, epithelioid inflammatory myofibroblastic sarcoma; RCC, renal cell carcinoma; PM, peritoneal mesothelioma.

Figure 2.

Landscape of Pediatric and Adolescent Young Adult Solid Tumors of Index Cases. At the top are the clinical characteristics, including change in diagnoses indicated by a vertical arrow; the diagnostic abbreviations are the same as in Figure 1 with the addition of EWLS for Ewing-like sarcoma. Fusion genes, somatic SNVs, copy number alterations...
in known cancer consensus genes, and germline alterations in cancer genes and American College of Medical Genetics (ACMG) genes are color coded as shown.

Figure 3.

Comparison of Somatic SNVs in relapsed tumors and across metastases in the exome. A minimum threshold of 10 total reads, 3 variant reads, and a variant allele frequency of ≥ 10% in the tumor DNA was used. Somatic SNVs common to the paired metastatic or relapsed tumors are shown in red bars, indicating a common origin. Unique SNVs seen in each sample are shown in light blue bars.

Table 1.

Germline reportable mutations in American College of Medical Genetics (ACMG) reportable genes and tumor suppressor genes identified in 7 patients. Mutations were confirmed by direct visualization on an IGV viewer, and by Sanger sequencing.

Table 2.

Summary of actionable mutations in relapsed and refractory pediatric solid tumors. Single nucleotide variants were confirmed by direct visualization on an IGV viewer, and validation by Sanger sequencing or confirmation CLIA-certified laboratories.
References


doi: 10.1038/nature11284. PubMed PMID: 22832583; PubMed Central PMCID: PMC3662966.


Figure 3

(A) Metastasis

(B) Relapse

Somatic SNV Counts

Legend:
- Unique
- Common

[Graph showing data for various categories and SNV counts]
<table>
<thead>
<tr>
<th>Sample</th>
<th>Diagnosis</th>
<th>Gene</th>
<th>Mutation</th>
<th>Disease</th>
<th>Hotspot</th>
<th>Notes</th>
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<td>NCI0072</td>
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<th>Drug</th>
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<th>FDA Approval in Adults</th>
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<th>Reference Preclinical Data for Level 3</th>
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**Table 2**
Clinical Cancer Research

Multi-Dimensional ClinOmics for Precision Therapy of Children and Adolescent Young Adults with Relapsed and Refractory Cancer: A report from the Center for Cancer Research

Wendy Chang, Andrew Brohl, Rajesh Patidar, et al.

Clin Cancer Res  Published OnlineFirst March 18, 2016.

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