Published OnlineFirst January 9, 2017; DOI: 10.1158/0008-5472.CAN-16-1106

Cancer Research

Genomic Profiling of a Large Set of Diverse Pediatric Cancers Identifies Known and Novel Mutations across Tumor Spectra

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Abstract

Pediatric cancers are generally characterized by low mutational burden and few recurrently mutated genes. Recent studies suggest that genomic alterations may help guide treatment decisions and clinical trial selection. Here, we describe genomic profiles from 1,215 pediatric tumors representing sarcomas, extracranial embryonal tumors, brain tumors, hematologic malignancies, carcinomas, and gonadal tumors. Comparable published datasets identified similar frequencies of clinically relevant alterations, validating this dataset as biologically relevant. We identified novel ALK fusions in a neuroblastoma (*BEND5–ALK*) and an astrocytoma (*PPP1CB–ALK*), novel *BRAF* fusions in an astrocytoma (*BCAS1–BRAF*) and a ganglioglioma (*TMEM106B–BRAF*), and a novel *PAX3–GLI2* fusion in a rhabdomyosarcoma. Previously characterized *ALK*, *NTRK1*, and *PAX3* fusions were observed in unexpected malignancies, challenging the "disease-specific" alterations paradigm. Finally, we identified recurrent variants of unknown significance in *MLL3* and *PRSS1* predicted to have functional impact. Data from these 1,215 tumors are publicly available for discovery and validation. *Cancer Res; 77(2); 1–11. ©2017 AACR.*

Introduction

Pediatric cancers are rare malignancies worldwide and represent ~1% of new cancer diagnoses in the United States (1). Unlike adult solid tumors that are predominantly carcinomas derived from epithelial cells, pediatric solid tumors are histologically diverse and include carcinomas from epithelial cells, embryonal tumors from developing tissues, gonadal tumors from sex-cord stromal cells, brain tumors from neural and glial cells, leukemias and lymphomas from hematopoietic cells, and sarcomas from mesenchymal cells (2). Advances in detection and treatment of childhood cancers have resulted in improved survival for many subtypes. However, over 1,900 pediatric patients in the United States succumb to disease each year, and survivors often face lifelong side effects from toxic chemo and/or radiotherapy treatments (1).

The genomic landscape of pediatric tumors is distinct from adult tumors due to low mutational burden, and relatively few, albeit highly recurrent, significantly mutated genes (3). Even within the same tumor type, mutation profiles in pediatric samples are distinct from their adult counterpart. For example, the *BCR–ABL1* fusion protein is observed in 2% of childhood acute lymphoid leukemia (ALL) but up to 25% of adult ALL cases (4). Certain tumors that occur primarily in children and young adults are marked by characteristic alterations, such as *BRAF* alterations in low-grade gliomas, *EWSR1* fusions in Ewing sarcoma, and *ALK* alterations in neuroblastoma (5–7). In some cases, the identification of genomic alterations has guided targeted therapy selection. Clinical efficacy has been demonstrated with crizotinib against *ALK*-driven neuroblastomas and anaplastic large cell lymphomas (8), and with imatinib against *BCR–ABL1*-driven ALL (9). Additionally, mutational signatures have assisted in the stratification of some disease, such as medulloblastoma, where alteration patterns can define clinically distinct subtypes within the same histology (10). Recent data suggest that the incorporation of genomic information can help inform therapeutic decisions in pediatric oncology (11–14).

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Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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doi: 10.1158/0008-5472.CAN-16-1106

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While improvements in detection and treatment have led to a decline in pediatric cancer mortality, many survivors face decreased quality of life and an increased risk of secondary cancer development as side effects from efficacious, but toxic, treatments (15). Therefore, the development of less toxic regimens is needed urgently. Improved therapeutic strategies are also needed in cancers with the highest mortality rates, including CNS malignancies, high-risk neuroblastoma, metastatic bone cancers, and soft tissue sarcomas, where standard-of-care treatment has limited efficacy. An enhanced understanding of the genomic alterations contributing to tumorigenesis in this population may identify new targets and strategies for improved therapeutic intervention across childhood cancers.

Here, we describe a dataset of 1,215 pediatric tumors (ages 0–18) comprised of sarcomas (26.7%), extracranial embryonal tumors (22.8%), brain tumors (20.8%), hematologic malignancies (19.3%), carcinomas (9.1%), and gonadal tumors (1.4%). This collection represents tumor specimens referred for sequence analysis of all classes of somatic variation in cancer-associated genes to guide clinical management. While many studies have focused on the genomic analysis of more common tumors from which specimens are easy to obtain, this collection contains multiple rare entities that have not been profiled previously in large numbers. We describe the discovery of novel fusions, point mutations, and the spectrum of therapeutic targets across multiple tumor types. Collectively, this dataset represents one of the largest groups of genomically profiled pediatric tumors to date and can be used as a resource for discovery of novel alterations and validation of findings from other studies.

Materials and Methods

Samples were submitted to a CLIA-certified, New York State-accredited, and CAP-accredited laboratory (Foundation Medicine) for next-generation sequencing (NGS)-based genomic profiling. The pathologic diagnosis of each case was confirmed by review of hematoxylin and eosin (H&E)–stained slides or Wright-Giemsa stained blood/aspirate smears and all samples that advanced to DNA and/or RNA extraction contained a minimum of 20% tumor cells. DNA and RNA were extracted from formalin-fixed paraffin-embedded (FFPE) 10-μm sections or from fresh blood or bone marrow aspirates. For samples assayed on FoundationOne, DNA was adaptor ligated, and hybrid capture was performed for all coding exons of 182 (v1), 287 (v2), 323 (v3), or 395 (v5) cancer-related genes plus select introns from 14 (v1), 19 (v2), 24 (v3), or 31 (v5) genes frequently rearranged in cancer (Supplementary Tables S1–S5); samples assayed on FoundationOne Heme (v4) underwent DNA-based hybrid capture for all coding regions of 465 genes plus select introns from 31 genes frequently rearranged in cancer. For samples in which RNA was available, targeted RNA-seq was performed for rearrangement analysis in 333 genes (Supplementary Table S4; refs. 16, 17). Captured libraries were sequenced to a median exon coverage depth of >600× using Illumina sequencing, and resultant sequences were analyzed for base substitutions, insertions, deletions, copy number alterations (focal amplifications and homozygous deletions) and select gene fusions, as previously described (16, 17). RNA sequences were analyzed for the presence of rearrangements only. Frequent germline variants from the 1000 Genomes Project (dbSNP142) were removed, and known confirmed somatic alterations deposited in the Catalog of Somatic Mutations in Cancer (COSMIC v62) were highlighted as biologically significant. Germline variants documented in the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/) and germline variants with two or more counts in the ExAC database (~0.0003% population frequency, http://exac.broadinstitute.org/) were removed, with the exception of known cancer driver germline events (e.g., documented hereditary *BRCA1/2* and *TP53* deleterious mutations). Additionally, recurrent variants of unknown significance that were predicted to be germline were removed using an internally developed algorithm (Sun and colleagues, in review 2016). In brief, a CGH-like profile was created based on coverage and allele frequencies (AF) of ~3,500 genome-wide SNPs. This profile incorporated tumor purity (p), copy number (C), and minor allele count (M). A variant's measured frequency was compared with the expected frequency: $AF_{germline} = (pM + 1 - p)/(pC + 2(1 - p))$ versus $AF_{somatic} = pM/(pC + 2(1 - p))$ and a prediction was made with statistical confidence based on read depth and local variability of SNP allele frequencies (18). All truncations and deletions in known tumor suppressor genes were also called as significant. To maximize mutation-detection accuracy (sensitivity and specificity) in impure clinical specimens, the test was previously optimized and validated to detect base substitutions at a ≥5% mutant allele frequency (MAF), indels with a ≥10% MAF, and focal copy number alterations at ≥20% tumor fraction with high accuracy (16, 17). This study was reviewed and approved by the Western Institutional Review Board (WIRB). Sequence analysis results are available publicly in a browsable web portal at https://pediatric-data.foundationmedicine.com.

Results

Characteristics of the pediatric dataset

This dataset was composed of 1,215 unique samples from patients age 18 or younger that underwent genomic profiling as part of clinical care (Supplementary Table S6). To facilitate classification, tumors were grouped into one of six major categories and assigned a detailed tumor subtype that more accurately described the diagnosis at the time of genomic testing (Fig. 1). The samples represented sarcomas (26.7%; 16 subtypes), extracranial embryonal tumors (22.8%; 3 subtypes), brain tumors (20.8%; 9 subtypes), hematologic malignancies (19.3%; 7 subtypes), carcinomas (9.1%; 13 subtypes), and gonadal tumors (1.4%; 1 subtypes). The most common sarcoma subtypes included rhabdomyosarcomas (20.4%), bone sarcomas, including both osteosarcomas and other rare bone cancers (19.4%), and Ewing sarcomas (12.0%) followed by 13 other subtypes of varying frequency (Fig. 1). Extracranial embryonal tumors included neuroblastomas (83%), Wilms tumors (10.5%), and hepatoblastomas (6.5%). The brain tumors were astrocytomas (26.5%), glioblastomas (23.3%), medulloblastomas (12.6%), gliomas (11.5%), and five additional subtypes (Fig. 1). Hematologic malignancies included acute lymphoblastic leukemias (ALL; 38.0%), acute myeloid leukemias (AML; 31.2%), lymphomas (8.5%), as well as four subcategories with frequencies <10% (Fig. 1). Of the 13 carcinoma subtypes, the most common were head and neck cancers (12.7%), neuroendocrine tumors (10.0%), lung cancers (10.0%), and kidney cancers (10.0%). Finally, gonadal tumors were comprised entirely of ovarian/testis tumors.

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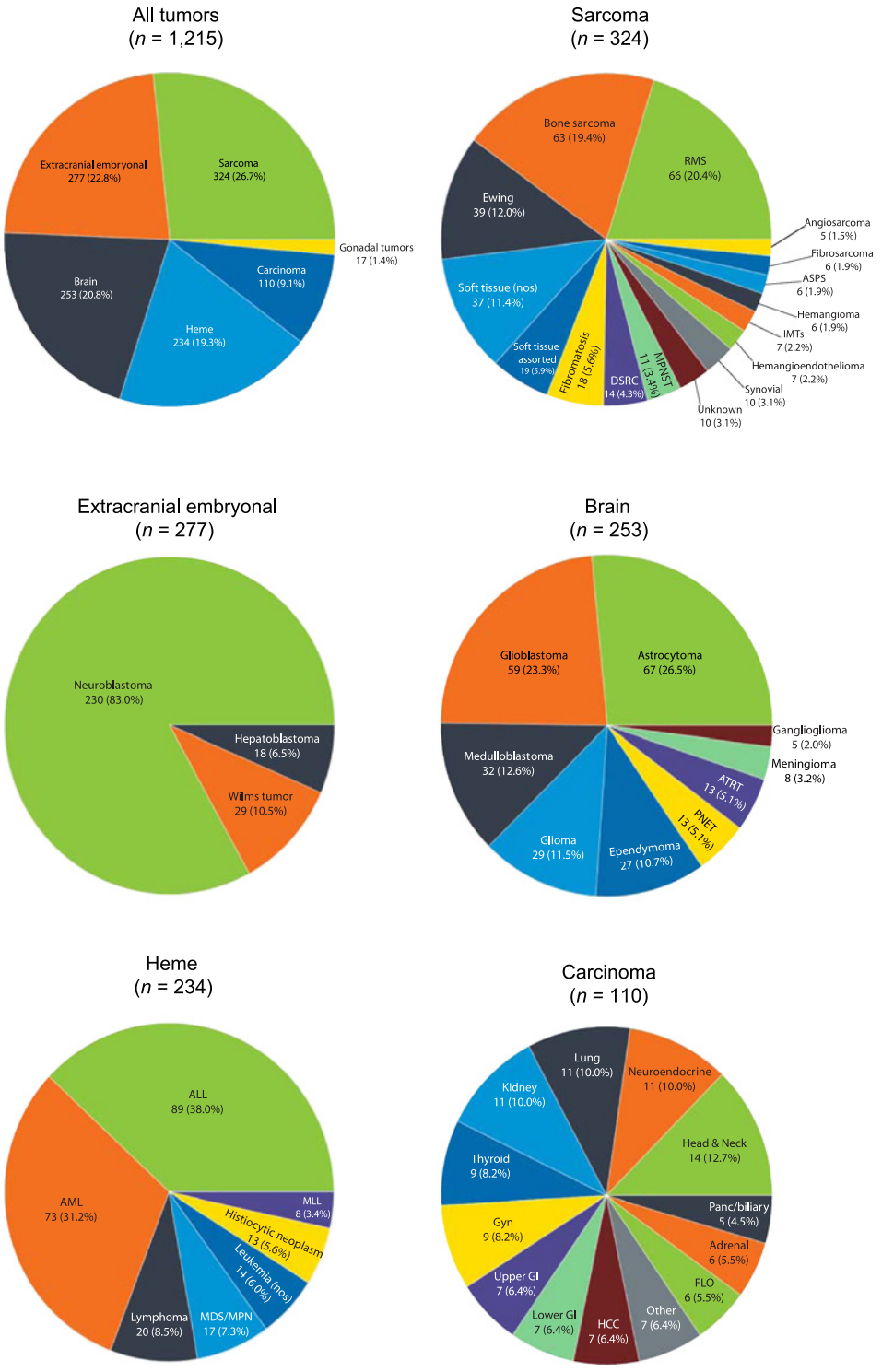

Figure 1.
Distribution of sample types within the pediatric data cohort. Samples were grouped into one of six major categories (top left). Each major category was subsequently divided into multiple subcategories that contained detailed information about the tumor diagnosis, with the exception of gonadal tumors. Sarcomas contained 16 subtypes (top right), extracranial embryonal tumors contained 3 subtypes (middle left), brain tumors contained 9 subtypes (middle right), heme malignancies contained 7 subtypes (bottom left), and carcinomas contained 13 subtypes (bottom right). Gonadal tumors were composed entirely of this tumor type (not shown).

All sample subcategories contained at least five tumors; the most common tumors in this dataset were neuroblastoma, ALL, AML, astrocytoma, and rhabdomyosarcoma (Fig. 2A). The gender distribution showed a slight predominance of male patients (Fig. 2B). Age at testing showed a bimodal distribution with one peak in young patients (ages 0–8) and a second peak in teenage patients (ages 14–18). Extracranial embryonal tumors, including neuroblastomas, Wilms tumors, and hepatoblastomas, were the most common tumors in very young patients (<8 years old), while sarcomas were predominant in teenage patients (ages 14–18; Fig. 2C). It is unknown if the samples submitted for genomic profiling represented primary or recurrent tumors. Disease stage and prior treatment history were also unavailable.

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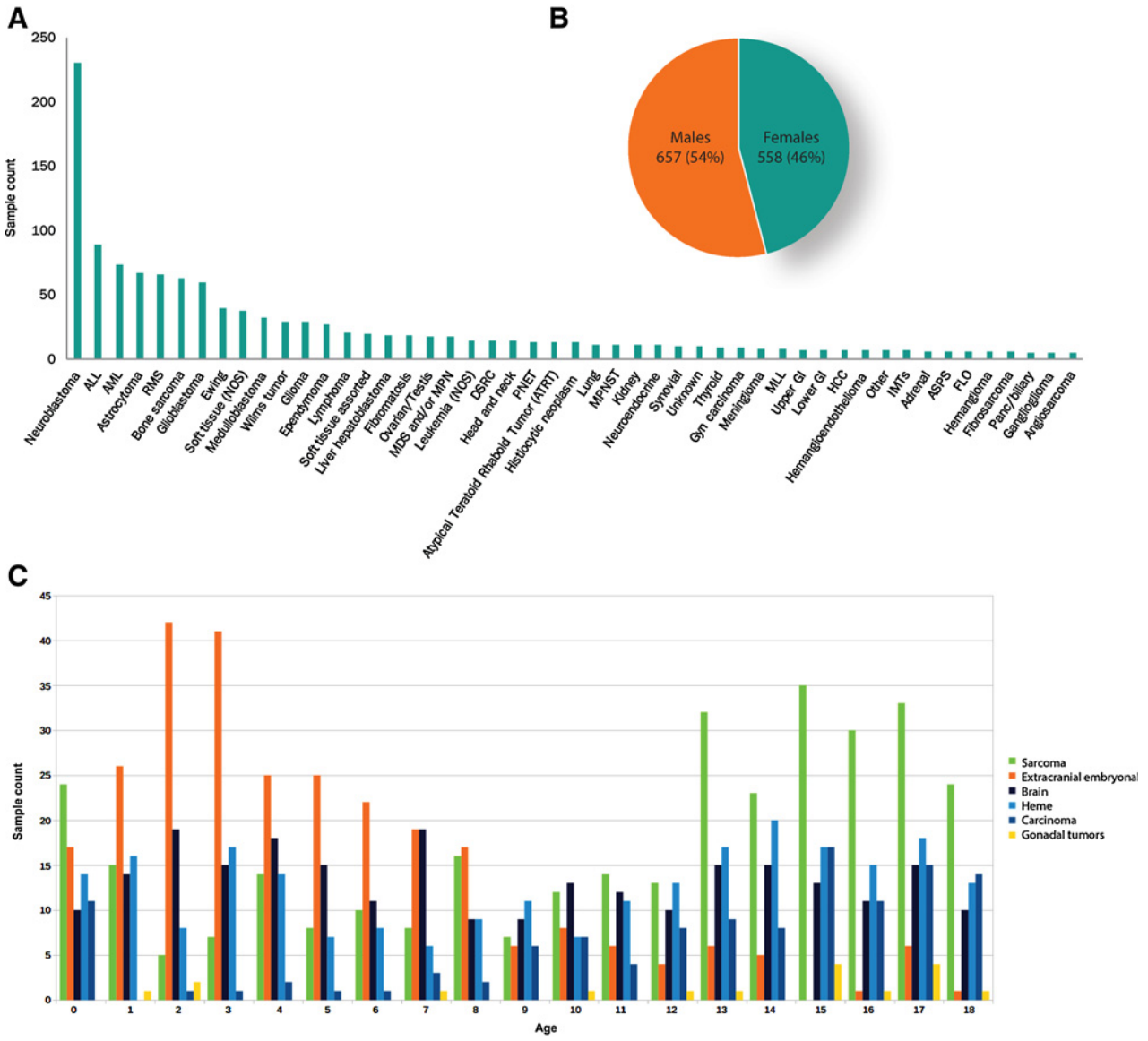

Figure 2.

Characteristic of the pediatric data cohort. **A,** The sample cohort contained 1,215 tumors from 49 unique subtypes. **B,** Each subtype contained at least 5 samples. Slightly more males were presented. **C,** Age at the time of testing showed a bimodal distribution with a predominance of extracranial embryonal tumors at younger ages (<8 years old) and a peak of sarcomas at older ages (13–18 years old).

We next analyzed the most commonly altered genes within each broad disease category. Across the dataset, we observed a median of 2.5 alterations per sample. The most commonly affected genes in sarcomas were *TP53* (18.8%), *EWSR1* (15.4%), *CDKN2A* (9.4%), *MYC* (7.5%), and *CDKN2B* (6.6%; Supplementary Fig. S1). Extracranial embryonal tumors showed a different distribution of alterations with frequent events in *MYCN* (23.9%), *ALK* (14.9%), *TP53* (5.4%), *ATRX* (5.4%), and *CTNNB1* (4.0%; Supplementary Fig. S2). Brain tumors had frequent disrupting events in *TP53* (25.3%), *BRAF* (19.4%), *CDKN2A* (12.6%), *NF1* (12.3%), and *H3F3A* (9.9%; Supplementary Fig. S3). Hematologic malignancies harbored alterations in *CDKN2A* (18.9%), *NRAS* (15.5%), *TP53* (14.7%), *CDKN2B* (12.9%), and *KRAS* (11.6%; Supplementary Fig. S4). Carcinomas were characterized by *TP53* (18.2%), *CTNNB1* (7.3%), *CDKN2A* (7.3%), *SMARCA4* (6.4%), and *KRAS* (5.5%; Supplementary Fig. S5). Finally, gonadal tumors contained alterations in *TP53* (29.4%), *KRAS* (17.6%), *CHD2* (11.8%), *ARID1A* (11.8%), and *DICER1* (11.8%; Supplementary Fig. S6).

Comparison of genomics with previously published pediatric tumors

We sought to compare how the genomic profiles in this clinical dataset compared with those from previously published studies. This analysis included tumors for which at least 30 samples were available in our dataset, and corresponding genomic landscape

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papers had been published for matched disease subtypes. Because our samples were analyzed for only focal copy number events, we omitted from this comparison disease subtypes that contained arm-level copy number events as distinct features (e.g., bone sarcomas). Samples within this dataset also lacked information about grade and stage, so we also excluded comparisons with datasets that were preselected for these features (e.g., low-grade glioma).

Neuroblastoma was the most common tumor in our database ($n = 230$). The most frequent alterations within this subtype were observed in *MYCN* (26.5%), *ALK* (17.8%), *ATRX* (6.5%), *CDKN2A* (4.8%), and *RPTOR* (4.8%; Fig. 3A). Previous integrative genome, exome, and transcriptome sequencing of a similarly sized high-risk neuroblastoma series ($n = 240$) identified five genes (*ALK*, *ATRX*, *PTPN11*, *MYCN*, *NRAS*) as biologically significant and statistically enriched in this disease (7). Comparing the frequency of alterations in these genes across the two datasets, no statistically significant differences were observed (Table 1).

We next investigated the frequency of common alterations in ALL. ALL is the most common childhood cancer (1), and the second most common tumor subtype in this series ($n = 89$). From our data, the most frequent alterations in this disease occurred in *CDKN2A* (28.1%), *NRAS* (21.3%), and *CDKN2B* (20.2%) (Fig. 3B). Multiple studies have previously investigated the genomic alterations underlying development of ALL and their association with treatment response or failure (19). Excluding chromosomal arm-level events, seven other genomic rearrangement events have been described as clinically significant in this disease (*ETV6–RUNX1*, *TCF3–PBX1*, *BCR–ABL1*, *P2RY8–CRLF2*, *PICALM–MLLT10*, and diverse rearrangements involving *MLL* and *PAX5*). Comparing the frequency of these alterations in our dataset to multiple published series (20–24), no significant differences were observed (Table 1).

Treatment of AML has incorporated mutations in three genes (*FLT3*, *NPM1*, and *CEBPA*) as markers of prognosis, therapeutic targets, and inclusion criteria for clinical trials. Our AML dataset contained 76 unique samples, and harbored frequent alterations in *NRAS* (20.5%), *RUNX1* (16.4%), *MLL* (13.7%), *FLT3* (12.3%), and *WT1* (12.3%; Fig. 3C). A significantly lower rate of *FLT3* ITD events was observed in our dataset (5.3% versus 16.5%, $P = 0.0276$; ref. 25). The frequencies of alterations in *NPM1* and *CEBPA* were not statistically different between the Foundation Medicine cohort and published studies (Table 1; refs. 26, 27).

We next investigated the genomic landscape of rhabdomyosarcomas (RMS) in our dataset. The dataset presented herein ($n = 66$) harbored common alterations in *TP53* (20.3%), *FOXO1* (17.4%), *NF1* (10.1%), *MDM2* (8.7%), and *MYC* (8.7%; Fig. 3D). Information about alveolar or embryonal characterization was not available for these tumors. Within our dataset, 45 RMS tumors (68%) were tested for the presence of a *PAX3/7* fusion by RNA-seq; because fusion information for the remaining 21 samples was unavailable, they were excluded from the subsequent analyses described below. Somatic alterations affecting the MAPK/PI3K signaling pathway, including point mutations in *NRAS*, *FGFR4*,

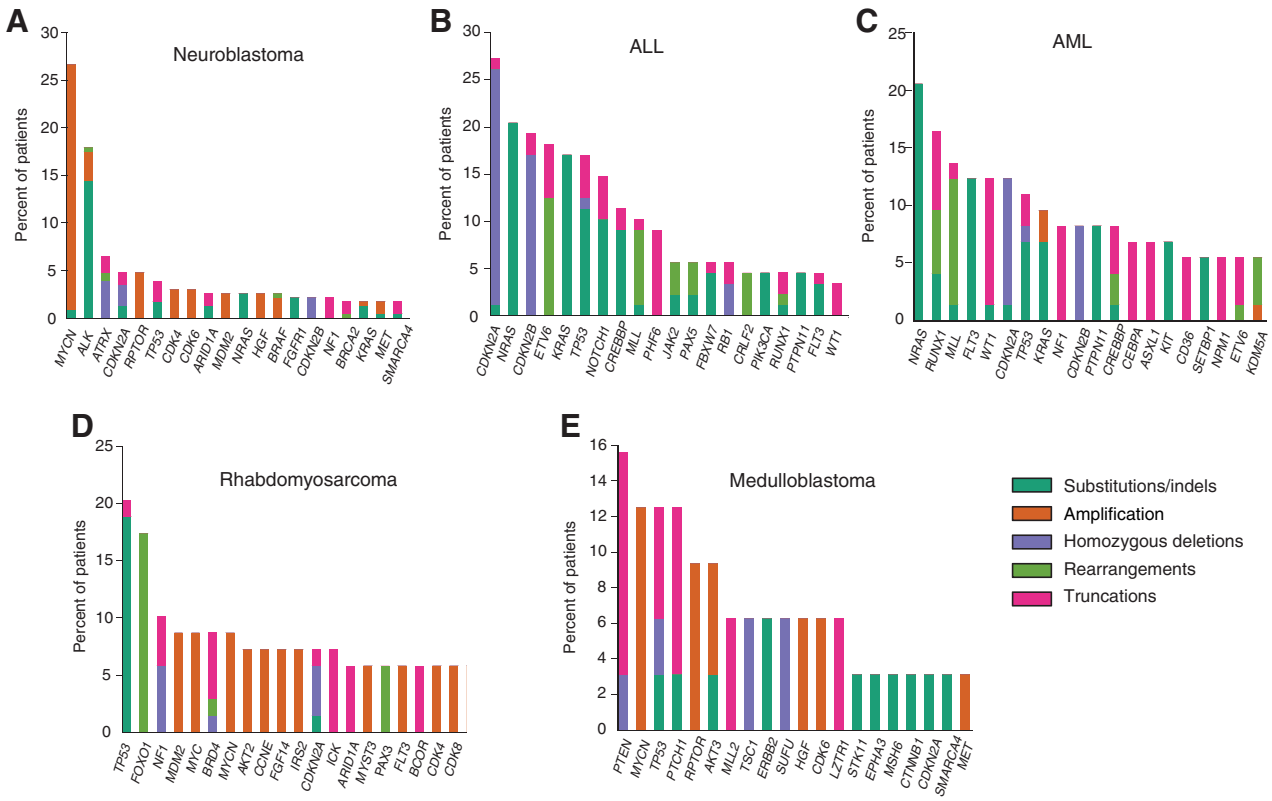

Figure 3.

Long tail distributions across the five most common diseases. The top 20 altered genes in neuroblastoma (**A**), ALL (**B**), AML (**C**), rhabdomyosarcoma (**D**), and medulloblastoma (**E**). Types of alterations are color coded using the key to the right.

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Table 1. Comparison of alteration frequencies

Tumor type and gene alteration	FM	Published frequency	Fisher exact test
Neuroblastoma			
ALK point mutation	14.3% (33/230)	9.2% (22/240)	$P = 0.0865$
PTPN11 point mutation	1.3% (3/230)	2.9% (7/240)	$P = 0.3396$
ATRX point mutation	1.8% (4/227)[a]	2.5% (6/240)	$P = 0.7522$
ATRX focal deletion	4.0% (9/227)[a]	7.1% (17/240)	$P = 0.1610$
MYCN point mutation	0.9% (2/230)	1.7% (4/240)	$P = 0.6859$
MYCN focal amplification	25.7% (59/230)	32.0% (77/240)	$P = 0.1284$
NRAS point mutation	2.6% (6/230)	0.8% (2/240)	$P = 0.1677$
ALL			
ETV6–RUNX1 rearrangement	12.0% (10/83)	19.4% (47/242)	$P = 0.1362$
TCF3–PBX1 rearrangement	2.4% (2/83)[a]	7.0% (17/242)	$P = 0.1749$
BCR–ABL1 rearrangement	1.1% (1/89)	3.7% (9/242)	$P = 0.2986$
MLL rearrangement	4.8% (4/83)[a]	11.4% (16/140)	$P = 0.1442$
P2RY8–CRLF2 rearrangement	4.8% (4/83)[a]	7% (19/272)	$P = 0.6151$
PAX5 rearrangement	6.0% (5/83)[a]	2.2% (10/446)	$P = 0.0697$
PICALM-MLLT10 rearrangement	2.4% (2/83)[a]	9.5% (4/42)	$P = 0.1778$
AML			
FLT3 internal tandem duplication	5.5% (4/73)	16.5% (15/91)	$P = 0.0472$
NPM1 point mutation	4.1% (3/73)	7.8% (23/295)	$P = 0.4422$
CEBPA point mutation	6.8% (5/73)	4.5% (38/847)	$P = 0.3785$
Fusion-negative RMS			
NRAS point mutation	6.7% (3/45)	11.7% (11/94)	$P = 0.5482$
FGFR4 point mutation	4.4% (2/45)	9.6% (9/94)	$P = 0.5030$
PIK3CA point mutation	2.2% (1/45)	7.4% (7/94)	$P = 0.4370$
BCOR point mutation	6.7% (3/45)	7.4% (7/94)	$P = 1.00$
FBXW7 point mutation	0% (0/45)	7.4% (7/94)	$P = 0.0960$
KRAS point mutation	2.2% (1/45)	6.4% (6/94)	$P = 0.4279$
TP53 point mutation	20% (9/45)	5.3% (5/94)	$P = 0.0132$
NF1 point mutation	4.4% (2/45)	5.3% (5/94)	$P = 1.00$
HRAS point mutation	2.2% (1/45)	4.3% (4/94)	$P = 1.00$
Medulloblastoma			
CTNNB1 point mutation	3.1% (1/32)	6.5% (6/92)	$P = 0.6760$
PTCH1 point mutation	12.5% (4/32)	7.6% (7/92)	$P = 0.4722$
MLL2 point mutation	6.3% (2/32)	8.7% (8/92)	$P = 1.00$
SMARCA4 point mutation	3.1% (1/32)	4.30% (4/92)	$P = 1.00$
TP53 point mutation	9.4% (3/32)	3.3% (3/92)	$P = 0.1775$
BCOR point mutation	0% (0/31)[a]	3.3% (3/92)	$P = 0.5712$
DDX3X point mutation	0% (0/5)[a]	7.6% (7/92)	$P = 1.00$
MYC amplification	3.1% (1/32)	1.1% (1/92)	$P = 0.4511$
MYCN amplification	12.5% (4/32)	4.30% (4/92)	$P = 0.2025$

Abbreviation: FM, Foundation Medicine dataset.
[a]Corrects for the gene not being assayed on all versions of the test.

PIK3CA, *BCOR*, *FBXW7*, *KRAS*, *TP53*, *NF1*, and *HRAS*, have been reported as potential driver alterations in tumors lacking *PAX3/7* fusion events (28). Consistent with these data, we observed alterations exclusively in our collection of fusion negative tumors. Alteration frequencies in these genes were not significantly different in our cohort versus the published study (Table 1), except for the frequency of *TP53* mutations, which was significantly higher in the Foundation Medicine dataset (20% versus 5.3%, $P = 0.0132$).

Finally, we undertook an analysis of medulloblastoma alterations. Within our series ($n = 32$), alterations were observed in *PTEN* (15.6%), *MYCN* (12.5%), *TP53* (12.5%), *PTCH1* (12.5%), and *RPTOR* (9.4%; Fig. 3E). We compared these data with a published series that interrogated the exomes of 92 primary medulloblastomas (10). Pugh and colleagues identified statistically significant rates of mutation in *CTNNB1*, *PTCH1*, *MLL2*, *SMARCA4*, and *TP53* as well as recurrent mutations in *DDX3X*, *GPS2*, *BCOR*, and *LDB1*. Additionally, amplification of *MYC* and *MYCN* was shown previously to be important in subgroups of medulloblastoma (29). *GPS2* and *LDB1* were not included on any of our gene lists, and were therefore excluded from our comparison. Similar to the previous tumor types, no statistically significant differences were observed in mutation rates between our cohort and the published frequencies (Table 1).

Discovery of novel fusions in pediatric tumors

Because canonical fusion proteins involving kinases (e.g., ALK fusions in IMT) and transcription factors (e.g., EWSR1 fusions in Ewing sarcoma) are observed in multiple pediatric tumors, we next investigated the dataset for new and potentially oncogenic fusion proteins involving genes within these families. In total, we identified seven novel, but nonrecurrent, kinase fusions. Two novel *ALK* fusions were identified in a neuroblastoma (*BEND5–ALK*) and an astrocytoma (*PPP1CB–ALK*; Fig. 4A). In both cases, the breakpoints in *ALK* (intron 19) were similar to known fusions, such as *EML4–ALK*, with established oncogenic activity and therapeutic potential. We also identified two novel *BRAF* fusions in an astrocytoma (*BCAS1–BRAF*) and a ganglioglioma (*TMEM106B–BRAF*; Fig. 4B). The breakpoints in *BRAF* (introns 9 and 7) were also similar to those in other known oncogenic fusions. An analogous *TMEM106B–ROS1* fusion that incorporated a similar region of *TMEM106B* was identified previously in an adult lung adenocarcinoma (30). The fusion protein

Published OnlineFirst January 9, 2017; DOI: 10.1158/0008-5472.CAN-16-1106

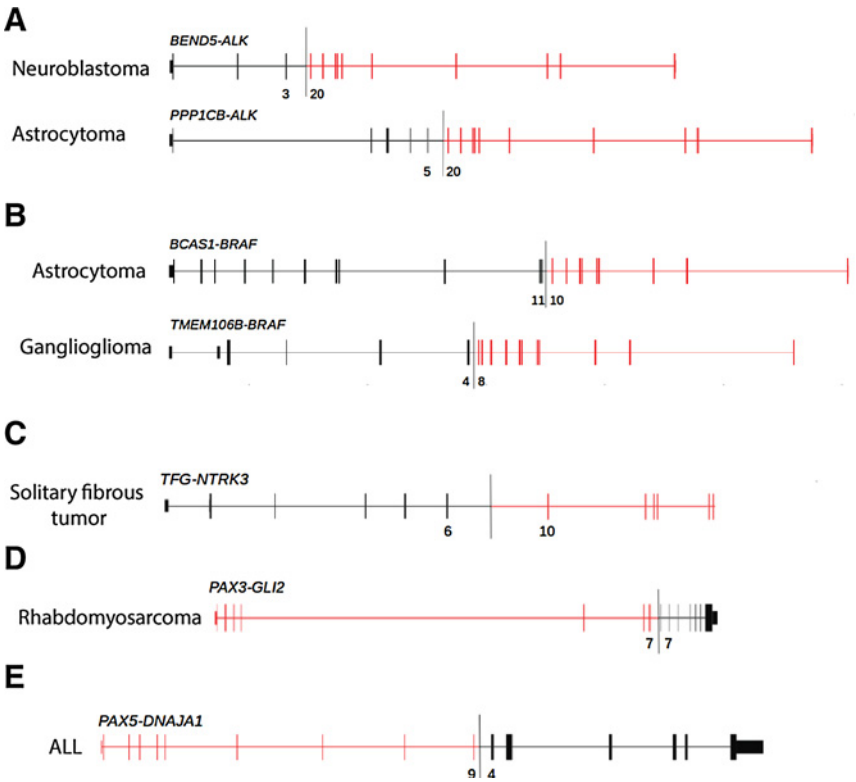

Figure 4.
Novel kinase and transcription factor fusions. Novel kinase fusions in *ALK* (**A**), *BRAF* (**B**), and *NTRK3* (**C**) have similar breakpoints to known fusions involving these genes. Novel transcription factor fusions involving *PAX3* (**D**) and *PAX5* (**E**) were also identified. Exon numbers at the fusion boundary are depicted below each diagram.

identified in the lung cancer sample involved exons 1–3 of *TMEM106B*, while this pediatric ganglioglioma fusion involved exons 1–4 of the same gene. Finally, a novel *TFG–NTRK3* fusion was identified in a solitary fibrous tumor (Fig. 4C); the breakpoint here kept the kinase domain intact and is predicted to produce a functional fusion protein. Interestingly, this solitary fibrous tumor lacked the canonical *NAB2–STAT6* fusion that is ubiquitous in this disease (31); this fusion involving *NTRK3* may suggest a differential diagnosis.

In addition to the novel kinase fusions, we also identified a previously characterized fusion involving *ALK* and *NTRK1* in different diseases from which they were originally reported. For example, an *SQSTM1–NTRK1* fusion was identified in a fibrosarcoma (Supplementary Fig. S7A). This fusion protein was recently reported in a 45-year-old male with lung adenocarcinoma and associated with clinical sensitivity to the NTRK1 (TrkA) inhibitor entrectinib (32). However, this is the first report of this fusion in a soft tissue tumor. We also identified an *STRN–ALK* fusion protein in a pediatric kidney carcinoma (Supplementary Fig. S7B). This *ALK* fusion was reported previously as a recurrent genomic event in aggressive thyroid cancers from adults; the fusion was sensitive to ALK inhibitors in preclinical studies (33). *EML4–ALK* rearrangements are best known for their role in ∼5% of lung adenocarcinomas and their clinical sensitivity to the ALK inhibitor crizotinib (34). Interestingly, we identified *EML4–ALK* fusion events in three non-lung cancers (thyroid cancer, histiocytic neoplasm, and a ganglioglioma). All three events were similar in structure to the variants that have been reported in lung adenocarcinoma and are predicted to be oncogenic (Supplementary Fig. S7C).

We next evaluated whether there were novel fusions involving transcription factors in the data. We focused specifically on *PAX3/7* fusions as they define distinct subsets of rhabdomyosarcomas and ALL (28). In addition to known fusion events involving these genes, we identified a novel *PAX3–GLI2* fusion in rhabdomyosarcoma (Fig. 4D) and confirmed a second occurrence of the rare *PAX3–NCOA1* fusion in also in rhabdoymosarcoma (Supplementary Fig. S7D; ref. 6). The related protein PAX5 is fused to various partners in ∼2.5% of ALL (23). This dataset adds to the growing list of *PAX5* fusion partners in ALL with the identification of a novel *PAX5-DNAJA1* rearrangement (Fig. 4E) that juxtaposes exons 1–9 of *PAX5* with exons 4–9 of *DNAJA1*. Expression of this alteration was confirmed in RNA sequencing analysis.

Analysis of genomic alterations associated with clinical sensitivity to targeted therapies

Given the long-term side effects associated with many conventional therapies in pediatric cancer, we mined our data for alterations associated with sensitivity to potentially less toxic targeted therapies. This analysis was restricted to agents with established dosing regimens and documented clinical efficacy against specific alterations in pediatric populations. With these filters, we included vemurafenib for *BRAF* V600E-mutant tumors (35, 36), crizotinib for *ALK* driven cancers (8), various experimental TRK inhibitors for *NTRK*-rearranged cancers (NCT02637687; ref. 37), and imatinib in *ABL1*-rearranged cancers (9).

The canonical *BRAF* V600E alteration was observed in 27 samples, including astrocytomas ($n = 8$, 11.9%), glioblastomas ($n = 5$, 8.5%), gliomas ($n = 4$, 13.8%), histiocytic neoplasms ($n = 3$, 23.1%), gangliogliomas ($n = 2$, 40%), thyroid cancers ($n = 2$, 22.2%), meningioma ($n = 1$, 12.5%), rhabdomyosarcoma ($n = 1$, 1.5%), and AML ($n = 1$, 1.3%). Rearrangements involving *ALK,* including those novel events described above, were identified in 11 disease types including lymphoma ($n = 6$, 30%), IMTs ($n = 3$, 42.9%), neuroblastoma ($n = 2$, 0.9%), assorted soft tissue

Published OnlineFirst January 9, 2017; DOI: 10.1158/0008-5472.CAN-16-1106

sarcomas ($n = 2$, 9.1%), and within a single specimen from lung (8.3%), unknown primary (7.1%), histiocytic neoplasms (7.7%), thyroid (11.1%), astrocytoma (1.5%), ganglioglioma (20%), and kidney cancer (8.3%). *NTRK* fusions were identified in fibrosarcoma ($n = 2$, 28.6%), assorted soft tissue sarcoma ($n = 1$, 4.5%), glioblastoma ($n = 1$, 1.7%), hemangioma ($n = 1$, 16.7%), and bone sarcoma ($n = 1$, 1.6%). Finally, *ABL1* rearrangements were identified in ALL ($n = 2$, 2.3%), lymphoma ($n = 1$, 5%), and myelodysplastic &/or MPN ($n = 1$, 5.9%).

Identification of potentially novel recurrent somatic variants with unknown clinical relevance

To identify potentially novel oncogenic alterations, we first investigated the list of single amino acid substitutions (i.e., point mutations) with unknown clinical relevance. These alterations were neither reported in COSMIC nor dbSNP databases and underwent additional filtering against the ExAc database (see Materials and Methods) to remove benign germline polymorphisms. Internal algorithms were also used to highlight those likely somatic alterations (see Materials and Methods). A complete list of variants meeting these criteria can be found in Supplementary Table S7. Recurrent point mutations ($n \geq 3$) were evaluated further using MutationAssessor, an *in silico* analysis tool that predicts functional impact of base substitutions based on evolutionary conservation (38). Using this approach, we identified three likely somatic variants in two genes with potential functional consequences.

Two variants in KMT2C (A293V and P309L) predicted to have functional impact were each identified in five samples (Supplementary Table S7). *KMT2C*, also known as *MLL3* (mixed-lineage leukemia protein 3), encodes a methyltransferase and is frequently rearranged in subsets of mixed lineage leukemias. Both A293V and P309L occur outside of annotated protein domains in KMT2C, but were conserved among species. KMT2C A293V was observed across multiple tumors types, including two sarcomas (synovial and DSRC tumors) and one each of PNET, ALL, and neuroblastoma. The alteration co-occurred with canonical fusions in synovial sarcoma (*SS18-SSX1*) and DSRC (*EWSR1-WT1*; Supplementary Fig. S8A). This mutation has been reported once in a gastric adenocarcinoma sequenced as part of The Cancer Genome Atlas (TCGA) project (39). In contrast, the P309L mutation occurred in brain tumors (2 glioblastomas and 1 astrocytoma) and neuroblastomas ($n = 2$). This alteration co-occurred with the canonical *KIAA1549-BRAF* in the astrocytoma sample (Supplementary Fig. S8B). A similar P309R mutation has been reported in a single clear-cell renal cell carcinoma (40).

We also identified four PRSS1 G191R mutations in 3 brain tumors (2 medulloblastomas and 1 glioblastoma) and a Wilms tumor. *PRSS1* encodes the trypsin-1 protein in humans. Germline variants in this gene are implicated in hereditary pancreatitis and an increased risk of pancreatic ductal adenocarcinoma (41). The G191R alteration occurs within the peptidase domain and has been reported in a single primary central nervous system lymphoma (42). Notably, no other known alterations were found in two of the four samples harboring the *PRSS1* mutation (Supplementary Fig. S9).

While *in silico* functional analysis of small insertions and deletions (indels) was not possible with available tools, we searched for recurrent indels ($n \geq 3$) with potential functional significance based on domain structure. Using this approach, we observed alterations around DKC1 K505, including small indels (Supplementary Fig. S10), in eight tumors from hematologic malignancies (2 ALL and 2 AML), neuroblastoma ($n = 2$), a bone sarcoma, and a single tumor classified as other. This event occurs within the nuclear and nucleolar localization region (uniprot.org). Interestingly, six additional events around this region have been observed in sarcoma ($n = 2$; TCGA provisional data), breast cancer ($n = 2$; ref. 43), gastric cancer ($n = 1$; ref. 39), and clear cell renal cell carcinoma ($n = 1$; ref. 44).

Discussion

Pediatric cancers are diverse histological entities that have a distinct clinical course and genomic landscape compared with adult tumors. Increasing evidence suggests that an enhanced understanding of genomic alterations in pediatric patients may help to guide clinical decisions and the design of clinical trials (11–14). We describe a collection of 1,215 samples that underwent genomic profiling. This dataset represents 49 tumor subtypes across sarcomas, extracranial embryonal tumors, brain tumors, hematologic malignancies, carcinomas, and gonadal tumors. To our knowledge, this cohort represents one of the largest sets of genomically characterized pediatric cancers published to date.

Compared with other large published datasets in neuroblastoma (7), ALL (20–24), AML (25–27), rhabdomyosarcoma (28), and medulloblastoma (10), few significant differences were observed in the frequencies of alterations in most genes that were deemed biologically significant in these disease types. The two exceptions were a decreased rate of *FLT3* ITD mutations (5.3% vs. 16.5%, $P = 0.0276$) in AML (Table 1) and an increased rate of *TP53* mutations (20% vs. 5.3%, $P = 0.0132$) in rhabdomyosarcomas (Table 1) within the Foundation Medicine samples. A lower frequency of *FLT3* ITD events (5%) has been observed in patients younger than 10 years of age (45). Interestingly, the mean age of pediatric AML patients within this dataset was 9.1 years old. It is also unknown if these samples represent specific subtypes of AML associated with a low frequency of *FLT3* ITD events, such as non-promyelocytic AML (6.6%–8.8%; ref. 46). Some reports have noted that a fraction of samples positive for *FLT3* ITD at diagnosis are negative for this alteration at the time of relapse (47), suggesting a possible enrichment of relapsed samples within this cohort. Finally, variable frequencies of *FLT3* ITD in pediatric patients have been reported in the literature and range from 15% (48) down to 4% (49) and highlight the bioinformatics challenges of correctly calling these events. The previous study in rhabdomyosarcoma (28) examined somatic events only and did not investigate germline alterations that may have contributed to disease development. However, germline *TP53* mutations have been implicated in this tumor (50). Although mutations were not distinguished as somatic or germline in our data due to tumor-only testing, the observed increase in *TP53* mutations may be explained in part by germline events that would have been reported here due to their established role in carcinogenesis. Earlier studies focusing on somatic events in this disease may have focused on somatic events only. Additionally, inadequate depth of coverage may have hindered calling of subclonal or rare events.

To demonstrate the discovery potential of this dataset, we mined for novel fusion and mutational events across pediatric cancers. This search resulted in the discovery of five novel kinase fusions involving *ALK*, *BRAF*, and *NTRK3* (Fig. 4A–C). Based on structural similarities to similar characterized fusions, we hypothesize that these events are oncogenic and contribute to the

Published OnlineFirst January 9, 2017; DOI: 10.1158/0008-5472.CAN-16-1106

expanding list of fusions observed in solid tumors. We also identified novel and rare transcription factor fusions involving *PAX3* and *PAX5* (Fig. 4D and E). While these events are not targetable directly, they have implications for diagnosis, prognosis, and risk stratification. *In silico* analysis of recurrent variants of unknown significance (VUSes) identified four alterations in three genes with potential functional significance. The MLL3 A293V mutation was observed across solid and heme tumors whereas the MLL3 P309L alteration was enriched in gliomas. A mutation within PRSS1 (G191R) was observed in medulloblastomas, a glioblastoma, and a Wilms tumor. Interestingly, two samples with this alteration lacked other known alterations in cancer genes. Finally, we report deletions around DKC1 K505 within the nucleolar localization region. Functional experiments are ultimately needed to confirm the role of these novel alterations in tumorigenesis.

A dataset this large challenges the paradigm of "disease-specific" alterations. For example, although *EML4–ALK* is observed primarily in lung cancer, we identified this fusion in three non-lung tumors. We also report other *ALK* and *NTRK1* fusions in diseases other than the tumor types in which they were originally reported. Although rare, these data support the notion that so-called "disease-specific" events can be promiscuous and occur outside of their primary tissues. Therapeutically relevant alterations, such as *BRAF* V600E alterations, are also observed across a wide variety of tumor types. Prospective identification of such alterations can have potentially significant impact on consideration of treatment options and clinical trial selection (11–14).

We also sought to identify genomic alterations that may be sensitive to targeted therapies. Specially, vemurafenib, imatinib, and experimental NTRK inhibitors have demonstrated promising results against molecularly matched pediatric tumors (8, 9, 35–37; NCT02637687). For example, *BRAF* V600E has been documented in small percentages of central nervous system tumors, and recent data have demonstrated anecdotal, but durable, clinical responses to vemurafenib (35, 36). We observed proven therapeutically actionable alterations, including *ALK*, *NTRK*, and *ABL1* fusions as well as oncogenic BRAF V600E mutations, across a variety of diseases, suggesting that targeted therapies may have a broader role in the treatment of some pediatric cancers than previously appreciated, and clinical trials investigating their efficacy outside of their approved indications are warranted. This and recently published datasets could be utilized for the rational design of biomarker-driven trials in pediatric oncology (11–14).

This tumor collection is not without limitations. Unfortunately, corresponding clinical data are unavailable for these specimens, and it is unknown from where in the clinical course the tumor tissue was obtained for genomic profiling. There is also no information about previous treatments or tumor grade/stage, making direct comparison with publicly available datasets challenging. Due to the design of the genomic assay, only focal copy-number events are reported and arm-level amplifications and deletions cannot be assessed. This is a significant void, especially for hematologic samples where such information is crucial for risk stratification and treatment selection. Despite these limitations, we believe that this dataset represents a valuable resource.

While many highly recurrent events have already been described in pediatric cancers, scientists and physicians are becoming increasingly aware of rare, yet equally important, clinically relevant genomic alterations in pediatric malignancies. Novel therapeutic strategies are needed to improve survival and spare patients from long-term side effects of toxic treatments. Large collections of genomically profiled tumors are ripe with discovery potential, and can be used to generate hypotheses, validate rare findings, and investigate the genomic landscape of rare tumors for which only small studies exist. To facilitate exploration of this dataset by the research community, we have made it available publicly (http://pediatric-data.foundationmedicine.com), with the goal that these data will be incorporated into future experiments that will ultimately improve the treatment and prognosis for children with cancer.

Disclosure of Potential Conflicts of Interest

J. Chmielecki, M. Bailey, and J.A. Elvin have ownership interest (including patents) in Foundation Medicine. S. Ramkissoon is a consultant/advisory board member for Bristol-Meyers Squibb. J. Suh has received speakers bureau honoraria from Genentech. G.M. Frampton is a scientist at and has ownership interest (including patents) in Foundation Medicine. S.M. Ali has ownership interest (including patents) in Foundation Medicine. L. Gay is a senior scientist at and has ownership interest (including patents) in Foundation Medicine. R. Erlich is Senior Director, at Biomedical Informatics and has ownership interest (including patents) in Foundation Medicine. J.S. Ross is Medical Director at and reports receiving a commercial research grant from Foundation Medicine. J. Buxhaku is client services representative at Joana Buxhaku. V. Miller is Chief Medical Officer at Foundation Medicine, Inc. P.J. Stephens is CSO at and has ownership interest (including patents) in Foundation Medicine. D. Lipson is vice president at and has ownership interest (including patents) in Foundation Medicine. No potential conflicts of interest were disclosed by the other authors.

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Acknowledgments

The authors wish to thank colleagues at Foundation Medicine, including Catherine Winfield, Pauline Glushko, Kate Cook, Mark Freeman, Benjamin Newell, Jared White, Susan Hager, and Allen Nunnally, for their contributions to this project. We also thank the team at Extension Engine, including Eduardo Cortejoso, Ofri Markus, Furqan Nazeeri, Scout Stevenson, Marin Bareta, Antonio Zemunik, Jessica Pang, and Roya Rakhshan, for their assistance with developing the data portal.

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Received April 27, 2016; revised September 1, 2016; accepted September 20, 2016; published OnlineFirst January 9, 2017.

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Published OnlineFirst January 9, 2017; DOI: 10.1158/0008-5472.CAN-16-1106

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Genomic Profiling of a Large Set of Diverse Pediatric Cancers Identifies Known and Novel Mutations across Tumor Spectra

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Cancer Res Published OnlineFirst January 9, 2017.